Development of a Low Glucosinolate, High Erucic Acid Rapeseed Breeding Program

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ABSTRACT

A breeding program to develop a low glucosinolate, high erucic acid rapeseed cultivar was begun in 1969 at Oregon State University. (This was preceded by the agronomic evaluation of rapeseed as a crop in the years 1966-68.) The breeding program initiated in 1969 utilized existing stock with previously established desirable agronomic traits. At the same time, increased plantings of seed stock with variable quantities of glucosinolates and erucic acid were established. Work was conducted during the 1969-71 period on 2 *Brassica* species, *B. napus* and *B. campestris*. It was known that erucic acid content was governed by 2 genes, displaying no dominance and acting in an additive manner in *B. napus*, whereas, in *B. campestris*, erucic acid synthesis was controlled by a single, non-dominant gene. The acid composition of the oil was controlled by the genotype of the developing embryo seed on F_1 plants which constitutes the F_2 population. The "half-seed" technique developed in Canada was found to be a useful tool to identify high erucic acid genotypes 1 generation earlier than if bulk samples were used. The 1969-71 work revolved around certain specific reciprocal crosses between high erucic, low glucosinolate parents within both *B. napus* and *B. campestris*. This method was successful in increasing or maintaining the erucic acid levels in both species, whereas, only limited success had been noted in lowering the glucosinolate levels. Results so far suggest backcrossing as a useful tool in maintaining a low glucosinolate level while increasing the general agronomic desirability of the crop. Several new valuable sources of germ plasm for high erucic acid and low glucosinolate level have been added to the program. Future plans for changes in the approach and breeding procedures to increase erucic acid and lower glucosinolates level are reviewed.

Development Breeding Program

There is a need in the U.S. for the production of a crop as a reliable source of vegetable oil that is high in erucic (cis-13-docosenoic) acid. Oils that are sufficiently high in erucic acid are now utilized as a raw material for many industrial purposes. Traditionally, the seeds of *Brassica napus* and *Brassica campestris* have been the major source of high erucic acid oils. Annually we import 10-12 million lb of rapeseed oil. Over 6 million lb of this imported rapeseed oil is fractionated annually for the erucic acid component needed for non-food purposes (A.G. Johnson, personal communication, 1971).

There is considerable opportunity for the expansion of the industrial uses of erucic acid. High erucic oils are utilized in several forms: a) the oil can be used for some purposes without further processing; b) erucic acid can be obtained from the oil and then transformed into derivatives; or c) erucic acid can be cleaved at its unsaturated linkage to yield 2 different acids (brassylic and pelargonic) that can be further reacted to give many useful chemical products (1).

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A breeding program was initiated in 1969 at Oregon State University to develop a domestic source of high erucic acid from the oil of *Brassica* species. The objectives of this breeding program are to develop cultivars with: a) high seed yielding ability; b) maximum oil content of the seed; c) high erucic acid content of the oil; d) minimum amount of glucosinolates of the seed; and e) cold tolerance in the winter annual types.

Prior to 1969, a program for evaluating the agronomic potential of a number of cultivars of various *Brassica* species was done. The results of seed yields of 21 cultivars for a 6 year period showed 'Dwarf Essex' to be the best yielding *Brassica napus* winter hardy cultivar and 'Duro' as the best yielding *Brassica campestris* winter hardy cultivar. These tests also showed that fall seeded cold tolerant winter annual *B. napus* had nearly twice the seed yielding ability compared to spring seeded summer annual *B. napus* and *B. campestris* at Corvallis, Oregon.

At the time the breeding program was initiated in Oregon, the existing available germ plasm with the best agronomic and chemical traits was utilized. Then as new sources of potential germ plasm became available, chemical analyses of this seed were made to determine the percentage of oil in the seed, the erucic acid in the oil, and the percentage of glucosinolates in the seed. Samples of seed found to have desirable chemical traits were then planted at Corvallis to increase the seed stock and to evaluate the agronomic characteristics of each seed source.

Our efforts in the breeding of rapeseed so far have been done primarily on the 2 *Brassica* species, *napus* and *campestris*. But *B. carinata* is also a potential high erucic oilseed crop for the U.S. in those geographic areas of adaptation (2). *B. napus* is an amphidiploid ($2n = 38$), resulting from natural crossing between *B. oleracea* ($2n = 18$) and *B. campestris* ($2n = 20$), followed by doubling of the chromosome complement. *B. napus* is highly self fertile and is largely self pollinated in the field (3). Fertility in *B. campestris* ranges from highly self sterile to complete self fertility in a few cultivars.

It was known prior to the start of the breeding program that erucic acid content in seed oil of plants of *B. napus* was governed by 2 genes displaying no dominance and acting in an additive manner (4). The erucic acid synthesis in *B. campestris* is controlled by a single nondominant gene (5). Because the fatty acid composition of the oil is controlled by the genotype of the developing embryo, the seed on F_1 plants constitutes the F_2 population (6).

It was not reported until late in 1970 how glucosinolate levels were genetically controlled (7). However, it was reported in 1968 in Sweden that seeds of a Polish *B. napus* cultivar, 'Bronowski,' were very low in glucosinolate content (8). Individual plants of *B. campestris* with low glucosinolates were isolated in Canada and reported in 1969 (9).

Workers in Canada have developed a method to determine the fatty acid composition of oil from 1 cotyledon of an embryo from a single seed (6). This "half-seed" technique allows the genotypes to be determined 1 generation earlier than if bulk samples are used. This method was incorporated early in our breeding program to identify high erucic acid F_2 plants from F_1 seed for the many different crosses that were made.

An example of this method was a cross made between a

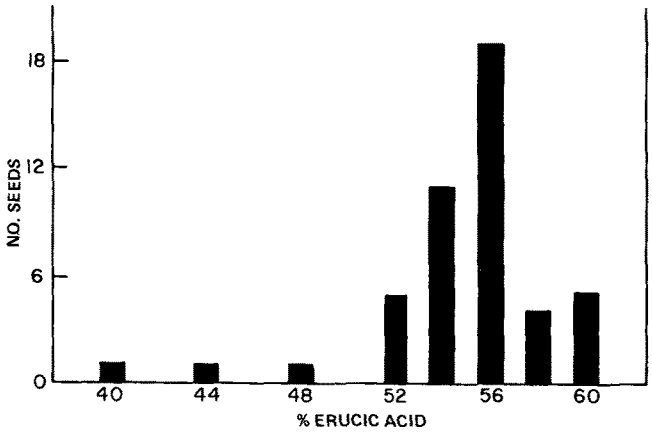

FIG. 1. Distribution of the % erucic acid in oil of 47 individual half-seeds from F_1 seed of the cross low x high erucic acid winter rapeseed.

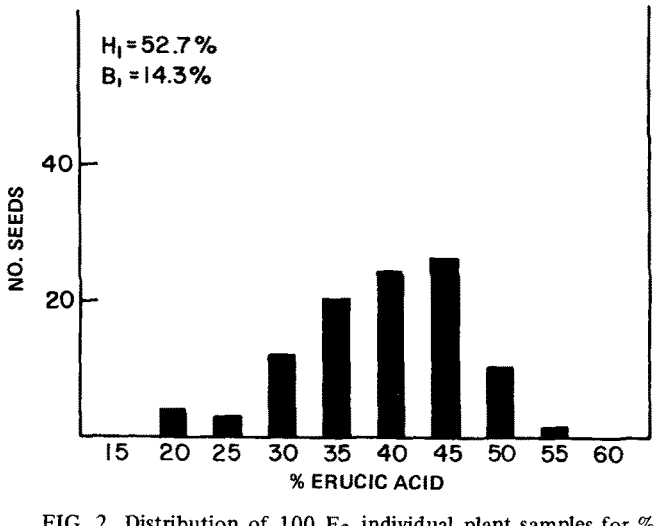

FIG. 2. Distribution of 100 F_2 individual plant samples for % erucic acid in oil of winter rapeseed from the cross H_1 x B_1.

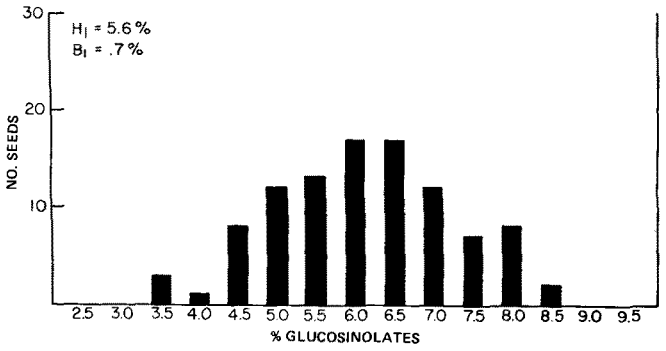

FIG. 3. Distribution of 100 F_2 individual plant samples for % total glucosinolates of the seed from the cross H_1 x B_1.

low erucic acid parent and a high erucic acid parent within the *B. napus* species. The maternal parent selected for this cross was 'Bronowski' (B_1), the Polish cultivar, which has average seed yielding ability, limited winter hardiness, oil content of seed 34.4%, 14.3% erucic acid, and 0.8% glucosinolates. The paternal parent of this cross was 'Heimer' (H_1), a Swedish cultivar with very good seed yielding ability, good winter hardiness, seed oil content 34.2%, 52.7% erucic acid, and 5.6% glucosinolates. In the cross B_1 X H_1 the oil from 47 F_1 half-seeds averaged 54.9% erucic acid with a range of 39.2-60.6% (Fig. 1).

The breeding work started at Corvallis in 1969 was with

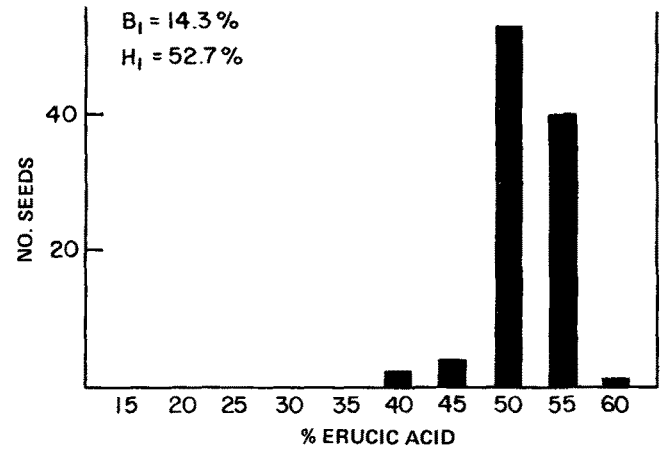

FIG. 4. Distribution of 100 F_2 individual plant samples for % erucic acid in oil of winter rapeseed from the reciprocal cross B_1 x H_1.

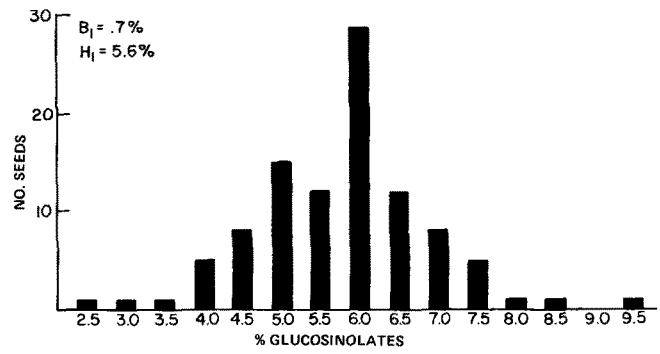

FIG. 5. Distribution of 100 F_2 individual plant samples for % total glucosinolates of seed of winter rapeseed from the reciprocal cross B_1 x H_1.

certain specific reciprocal crosses between high erucic acid and low glucosinolate parents within both *B. napus* and *B. campestris* species. This method has been successful in increasing or maintaining the high erucic acid levels in both species, but not so successful in reducing the total glucosinolates to a stable level.

The analysis of seed from individual F_2 plants of *B. napus* showed a full range of erucic acid value between a parent with low erucic acid value and a parent with high erucic acid value. On the other hand, the glucosinolate level of defatted meal remained at high level in crosses between a parent with low glucosinolate level and a parent with high glucosinolate level.

In the cross of H_1 X B_1, the oil from F_2 seed averaged 39.1% erucic acid with a range of 17.6-53.8% (Fig. 2). The defatted mean from F_2 seed of this cross averaged 6.1% glucosinolates with a range of 3.5-8.6% (Fig. 3).

The oil from F_2 seed of the reciprocal of this cross B_1 X H_1 averaged 51.6% erucic acid with a range of 38.6-57.6% (Fig. 4). The defatted meal from the F_2 seeds of this reciprocal cross averaged 5.8% glucosinolates with a range of 2.4-9.4% (Fig. 5). The best individual F_2 plant of the B_1 X H_1 cross and reciprocal cross was a B_1 X H_1 cross with 52.7% erucic acid, 2.4% glucosinolates, and 46% oil.

It has been found that the inheritance of glucosinolates in seed meal of *B. napus* is controlled by 11 or 12 recessive genes and is determined by the genotype of the mother plant rather than the embryo genotypes (7). The complexity of the inheritance of glucosinolate levels suggest backcrossing as a useful tool in maintaining a low glucosinolate level while increasing the general agronomic desirability of the crop.

A promising cross that illustrates the effects of back-

crossing was that made between the cultivars 'Dwarf Essex' and 'Bronowski,' both of *B. napus*. The 'Dwarf Essex' (DE) parent has 44.3% erucic, 6.1% glucosinolates, and 39.2% oil. The analysis of 'Bronowski' (B), the other parent, was previously given. The cross DE X B was made in 1969. The analysis of seed from 25 F_2 plants showed that the plant with the best combination of desirable traits had 44.0% erucic acid, 3.0% glucosinolates, and 38.2% oil. Then the DE X B F_1 plant was backcrossed to B in 1970. An analysis of F_2 seeds showed there were plants capable of producing 41.8% erucic acid, 1.5% glucosinolates, and 44.7% oil (Fig. 6). Thus, backcrossing was effective in reducing the glucosinolate content to a much lower level. Further backcrossing appears to be necessary to reduce glucosinolate to a stable level below 1%. Stable level means the 11 or 12 pairs of genes that control the inheritance of glucosinolates in a homozygous recessive condition.

The results of our breeding efforts on *B. campestris* species are similar to that as reported on *B. napus* (4). One of the promising crosses in *B. campestris* was a cross between a high seed yielding plant collected as an escaped cultivar in Western Oregon and a very high erucic cultivar, 'Yellow Sarson' from India. The maternal parent B-12-68 (B-12) has 53.6% erucic acid, 6.1% glucosinolate, and 39.2% oil. The paternal parent 'P.I. 352808' (808) had 65.8% erucic acid, 8.6% glucosinolate, and 47.8% oil. The analysis of F_2 seed from the cross B-12 X 808 showed 62.5% erucic acid, 6.1% glucosinolate, and 34.8% oil (Fig. 6).

Under normal circumstances, there should be greater progress made towards the objectives of this breeding program than has been reported. However, in the spring of 1973 over 95% of the F_2 and F_3 populations of the rapeseed breeding material were accidentally destroyed by 2,4-D spray drift. The field of *B. species* breeding material happened to be adjacent to a neighbor's grass seed field that was sprayed by air to control broadleaf weeds. Further losses were sustained during Spring, 1974, due to a heavy cabbage maggot (*Hylemya brassicae*) infestation. Losses of over 30% of some critical F_2 and F_3 rapeseed were experienced. This infestation in spite of repeated spraying with Diazinon, an insecticide which normally keeps this insect under control. Nevertheless, we are still optimistic about achieving our previously stated goals in spite of the crippling setbacks to the program.

Since our rape seed breeding program was started, several new valuable sources of germ plasm have become available. All of these new plant materials have been incorporated into this program. The *B. napus* 'S-69-914' obtained from Canada is as good a source of low glucosinolates as 'Bronowski,' yet contains a much higher erucic level. Both of these cultivars have a glucosinolate level of $< 1\%$, but 'S-69-914' has an erucic acid level of 43.3% compared to 14% erucic acid of 'Bronowski.' 'Hector,' a *B. napus* cultivar received from Sweden, is a good source of high erucic acid, and has good cold tolerance and high seed yielding potential. 'Hector' has 57.2% erucic acid compared to 52.7% for 'Heimer,' the next highest erucic acid source in *B. napus*. There are 4 different yellow Sarson *B. campestris* accessions from India which were found to have 63.5, 64.0, 64.8, and 65.8% erucic acid. These are important sources of germ plasm for high erucic acid characteristics in *B.* species.

At present, the biggest difficiency of germ plasm is a lack of low glucosinolates source in *B. campestris* species. So far a yellow Sarson accession 'P.I. 347,846' from India with 2.9% glucosinolate is the only source of consistently lower glucosinolate level in *B. campestris* in our program.

There are some changes being made in our rapeseed breeding program. As projected, we plan to try a little different approach and make some modifications in the breeding procedures. Thse changes have come as a result of our increased experience, more knowledge, and the re-evaluation of our breeding program.

CULTIVARS AND CROSSES	% ERUCIC ACID	% GLUCOSINOLATES	% OIL
BRASSICA NAPUS			
DWARF ESSEX (DE) MATERNAL	44.3	6.1	39.2
BRONOWSKI (B) PATERNAL	14.3	.7	34.4
DE X B F_2	44.0	3.0	38.2
(DE X B) X B F_2	41.8	1.5	44.7
BRASSICA CAMPESTRIS			
B-12-68 (B-12) MATERNAL	53.6	6.1	39.2
PI352808 (808) PATERNAL	65.8	8.6	47.8
B-12 X 808 F_2	62.5	6.1	34.8

FIG. 6. Effects of back crossing on levels of erucic acid, glucosinolate, and oil of *Brassica napus* and *Brassica campestris* cultivars.

The crosses will continue to be made as in the past, usually with high erucic acid and glucosinolates in one parent and low glucosinolates and erucic acid in the other parent. Backcrosses will be made in the second year to the low glucosinolate parent. Then, in the third year, the "half-seed" technique will be used to isolate high erucic acid individual plants to backcross to the low glucosinolate parent again. This technique of using only half of a seed for analysis of erucic acid allows the other half of the seed with attached embryo to be germinated for the production of a thriving plant. Only those individual plants that analysis shows have a high erucic acid level will be backcrossed to the low glucosinolate parent. In the fourth year, it will be necessary to grow plants for the production of F_1 seed. For *B. campestris* breeding, it will be necessary to provide isolation to prevent outcrossing. The F_2 population will be segregating in the fifth year. Individual plant selections will be made based on desired phenotypic characters and the analysis of the F_2 seed for each plant. It is expected that plants with high erucic acid, low glucosinolates, high oil content with high yielding ability (and cold tolerance in *B. napus*) will be identified from this population.

The improvement of crops is a continuous process of change. There has been considerable work done to improve rapeseed but there is still more work to be done. The cooperation of research workers in different fields is necessary to accomplish the task of improvement in rapeseed.

ACKNOWLEDGMENTS

Chemists at the Northern Regional Research Laboratory, ARS-USDA, Peoria, Illinois, performed the analysis of seed oil content, erucic acid, and glucosinolate determinations.

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