BREEDING FOR FATTY ACID COMPOSITION OF OIL IN RAPESEED, Brassica napus L.

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Introduction

Main targets of rapeseed breeding in Japan have been earlier maturation, shorter plant height, resistance for lodging, higher productivity, higher disease resistance, higher oil content and higher winter hardiness. Until recently the only measure of oil quality was the iodine number. For most of the oils this measure of the degree of unsaturation seems to be sufficient, but the iodine number is a crude measure when applied to rapeseed oil which may vary considerably in its content in unsaturated acids: oleic, linoleic, linolenic, eicosenoic and erucic acids.

The development of gas-liquid phase chromatography by James and Martin (1952) has provided a new and powerful tool for semi-micro and micro-analysis. The rapid extension of this method to fats and oils has made possible the separation of small quantities of fatty acid esters. Craig and Murty (1958, 1959) and Craig and Wetter (1959) investigated the variation in fatty acid composition or oil quality within and between rapeseed cultivars and close relatives using gas chromatography. A knowledge of the fatty acid composition of oil from different rapeseed cultivars is valuable in breeding programs for oil quality.

The suitability of vegetable oil for any specific purpose is determined by its content in certain fatty acids. Table 1 shows fatty acid composition of rapeseed and other oily crop seeds. Linseed and perilla oils rich in linolenic acid are valued for industrial purposes. Safflower, olive, peanut, corn, sunflower, sesame and cotton oils rich in linolenic acid are for edible use. Soybean and rapeseed oils containing 8 to 9 percent linolenic acid are poor quality edible oils. The

Crop	Pal	Ste	Ole	Lin	Lnl	Eic	Eru
	16:0	18:0	18:1	18:2	18:3	20:1	22:1
Brassica napus*	4.0	1.5	17.0	13.0	9.0	14.5	41.0
Brassica campestris*	2.9	1.1	33.6	17.9	9.4	11.5	23.5
Soybean *	11.5	3.9	24.6	52.0	8.0	р	0
Olive *	14.6	3.1	76.2	5.5	0.6	0	0
Peanut *	10.1	3.2	53.9	24.2	0	3.3	3.2***
Sesame **	7.5	4.8	39.4	44.9	1.8	0	0
Corn *	12.1	2.3	28.7	56.2	0.7	0	0
Sunflower *	7.2	4.1	16.2	72.5	0	0	0
Safflower *	7.2	2.1	9.7	81.0	0	0	0
Cotton **	17.5	2.8	17.9	61.9	0	0	0
Linseed *	6.1	3.8	15.5	15.3	59.3	0	0
Perilla **	6.1	1.5	18.8	20.3	53.3	0	0

Table 1.	Fatty acid	composition of oil in	rapeseed and	other oily crop seed.

* After Downey, R. K. (1963).

** After Lee and Shiga (1974).

*** Present as behenic and lignoceric acids.

Key. Pal: palmitic, Ste: stearic, Ole: oleic, Lin: linoleic, Lnl: linolenic, Eic: eicosenoic and Eru: erucic acid.

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presence of linolenic acid makes these oils susceptible to autoxidation resulting in off-flavor. Rapeseed oil is composed of a substantial amount of erucic acid, 10 to 14 percent eicosenoic acid, 15 percent linoleic acid and a low level of saturated acids, particularly palmitic acid. The linoleic acid content of rapeseed oil is lower than that of the other major edible oils.

Rapeseed oil given to experimental animals is said to produce growth retardation compared to other vegetable oils. Rapeseed oil in experimental diets is found to reduce food consumption in most animal species. The decrease in food consumption has been related to low appetite, low digestibility and slow absorption rate of rapeseed oil from the digestive tract, mainly because of high erucic acid content. Dietary rapeseed oil has been reported to increase the content of adrenal cholesterol and to interfere with reproductive ability. Some experiments with dietary rapeseed oil have indicated a tendency to interstitial inflammatory changes of the myocardium. Much work has been done to elucidate factors responsible for the nutritive effects and the pathological changes of dietary rapeseed oil. Now it is concluded that the excess of erucic acid is the causal agent (Aaes-Jorgensen 1972)

Rapeseed oil is very important for edible use in India, China, Korea and Japan, for a few oil crops are cultivated in these areas. Indian rapeseed production has attained a record 1,800,000 tons in 1972-73 season, but the increments in harvests have all gone towards meeting increase in domestic requirements. Little is known of the production in China in recent years, although this country is apparently the world's third largest producer. Rapeseed production in Korea has increased suddenly in recent years. Japanese production has shown a steep decline but consumption of rapeseed rose to 600,000 tons in 1976.

Breeding for rapeseed oil free from erucic acid was started in Canada. Stefansson *et al.* (1961) isolated a rape plant, *Brassica napus* L., with seed oil free from erucic and low eicosenoic acids. Downey (1964) selected a rape plant, *Brassica campestris* L., containing no erucic acid in its seed. A breeding program for fatty acid composition in rapeseed oil in Japan was initiated in 1969 at the Rapeseed Breeding Center, Fukushima Agricultural Experiment Station (Rapeseed Breeding Center, Japan) by Mr. M. Saito and Dr. S. Sugiyama. Fatty acid was analyzed at the Division of Genetics, National Institute of Agricultural Sciences by Dr. J. I. Lee, Dr. K. Takayanagi, Mr. M. Saito and the author, and actual breeding was conducted at the Rapeseed Breeding Center, Japan, by Mr. M. Saito and the Mokpo Branch, Crop Experimental Station in Korea (Mokpo Branch Station, Korea) by Dr. J. I. Lee and the author.

Fatty acid composition of rapeseed oil in Asian cultivars.

To select cultivars with low erucic acid content among Asian cultivars, seed samples of 24 Japanese, 8 Korean, 7 Formosan and 21 European cultivars (used as comparison) were analyzed by gas chromatography. Oil was extracted from self pollinated seeds obtained from the Mokpo Branch Station, Korea.

Twenty mg seed sample was crushed and homogenized in mortar and pestle with 2 ml n-hexane. Oil was extracted and fatty acid was directly transmethylated in 1 ml of sodium methanolate containing 10 g sodium in one liter of methanol for half an hour and cooled to room temperature. The mixture was neutralized by 1 N H₂SO₄ and washed with distilled water three times in separating funnel. Hexane layer was dehydrated by Na₂SO₄ (sodium sulfate, anhydrous) over night. After filtration of the sample, the solvent was dried up in vacuum, the methylester sample of fatty acid was dissolved again with 1 ml ether and loaded in gas chromatograph.

The fatty acid esters were separated by Shimazu GC-4BPF gas chromatograph equipped with flame ionization detector. Gas chromatograph operated at 200° C with 60 ml/min nitrogen gas flow rate as carrier using stainless steel column (3 mm x 3 m) packed with 10% poly-diethylene glycol succinate (DEGS) on 60-80 mesh Chromosorb AW. The peak area was calculated from the product of the peak height and width of the half height of the peak on the recorder chart. The peaks of palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids were clearly separated.

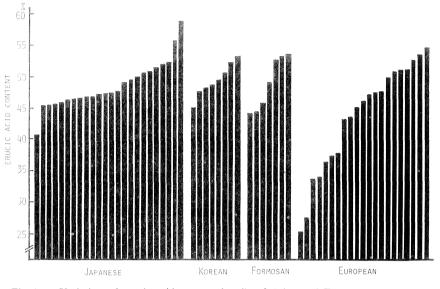


Fig. 1 Variation of erucic acid content in oils of Asian and European rapeseed cultivars. Names of cultivars were shown in Lee et al. (1974a).

Fig. 1 shows erucic acid content of rapeseed oil from Asian and European cultivars. The rapeseed oil from Japanese, Korean and Formosan cultivars was rich in erucic and low in oleic acid, while the oil from several European cultivars was low in erucic and high in oleic acid, but no difference was seen in the linoleic acid content of oil from Asian and European cultivars.

Although the Asian rapeseed cultivars have a high content in erucic acid, they have adapted to the Asian weather conditions, showing high productivity, early maturity and resistance to lodging. Consequently, Asian cultivars seem to be good breeding materials for improving oil quality. If the fatty acid composition of oil of Asian rapeseed cultivars were improved, they could become a highly desirable oil for edible use. Lower erucic acid rapeseed cultivars have been looked for among Asian cultivars which may be used for breeding materials, but they have not yet been found. Therefore, one may conclude that the use of low erucic acid lines introduced from Canada could contribute to the breeding program in Japan.

For improving fatty acid composition it is necessary to check the influence of climate on fatty acid composition. Fatty acids of oil from identical cultivars grown at different climatic locations were analyzed by gas chromatography. Seed samples were obtained from 10 open pollinated rapeseed cultivars grown in Koriyama and Mokpo, 7 cultivars grown in Cheju and

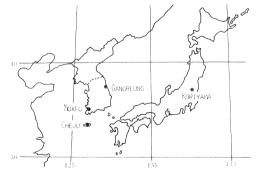


Fig. 2. Location of four stations in Japan and Korea.

Location	Pal 16:0	Ste 18:0	Ole 18:1	Lin 18:2	Lnl 18:3	Eic 20:1	Eru 22:1
Koriyama	3.4	1.0	14.3**	15.4**	9.0	9.3	46.1
Mokpo	3.0	1.0	11.6	14.0	10.4**	8.7	50.1**
Cheju	3.3	0.9	13.3	15.2*	11.2*	9.2	45.7
Mokpo	2.8	1.0	11.9	12.6	8.6	9.0	52.8**
Gangreung	3.4	0.9	15.7*	16.2*	9.0	9.7	42.8
Mokpo	3.1	1.1	12.2	14.2	9.4	8.8	50.0**

Table 2. The average fatty acid content of oil in 10 cultivars grown in Koriyama and Mokpo, 7 cultivars grown in Cheju and Mokpo and 7 cultivars grown in Gangreung and Mokpo.

Name of cultivars analyzed was shown in Lee et al. (1975a)

**: significant at the 1% level. *: significant at the 5% level.

Mokpo and 7 cultivars grown in Gangreung and Mokpo (Fig. 2). Table 2 shows the average values of fatty acid content in oil from rapeseed cultivars grown in four different locations. These results indicated that the oil of rapeseed grown in Mokpo had a higher erucic acid content compared with the oil of rapeseed grown in other locations.

In order to elucidate the cause of higher erucic acid content of oil from rapeseed grown in Mokpo, the relationship between accumulated average air temperature during ripening period and erucic acid content of oil from rapeseed grown in the four locations was examined, as shown in Fig. 3. A negative correlation was observed between accumulated average temperature and erucic acid content. It was considered that the reason for high erucic acid content in the Mokpo plants was that Mokpo had small amount of accumulated average air temperature during the ripening period compared with three other locations. These findings indicated that Mokpo is a good location for the selection of low erucic acid rapeseed cultivars.

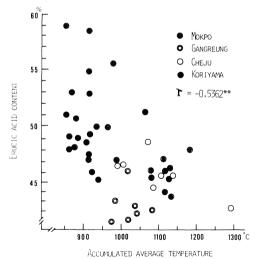


Fig. 3. Relationship between accumulated average temperature during ripening period and erucic acid content in oil from 10 cultivars grown in Koriyama and Mokpo, 7 cultivars grown in Cheju and Mokpo and 7 cultivars grown in Gangreung and Mokpo. Name of cultivars analyzed was shown in Lee et al. (1975a). **; significant at the 1% level.

Breeding procedure for fatty acid composition in rapeseed oil in Japan and Korea.

A zero-erucic acid line introduced from Canada through Dr. R. K. Downey, Canada Agriculture Research Station, Saskatoon, Saskatchewan, was used, as it was difficult to find low erucic acid cultivars in Asian cultivars. Breeding program for fatty acid composition in rapeseed oil was started in 1969 at the Rapeseed Breeding Center, Japan. Three Japanese cultivars, Chisaya-natane, Norin 16 and Asahi-natane were crossed reciprocally with the zero-erucic acid line introduced from Canada. Fig. 4 shows this breeding procedure.

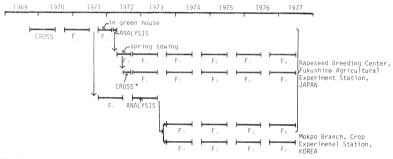


Fig. 4. Breeding procedure for fatty acid composition in rapeseed oil in Japan and Korea. *: intercross between zero-erucic acid F₃ plants.

Owing to overlap of flowering period, the crosses between summer type Canadian line and winter type Japanese cultivars were carried out easily and many hybrid seeds were obtained. In the next season F_1 hybrids were grown in the field and F_2 seeds were obtained by selfing. All F_1 hybrids expressed heterosis in yielding performance. There were differences between characteristics of reciprocal hybrids. Using Canadian line as female parent the hybrids had higher plant height and longer ear length but they were lodging prone. On the basis of these results, it was considered that many Japanese types with shorter plant height and resistance to lodging would appear in the progeny of crosses using Japanese cultivar as female parent.

 F_2 seeds were divided into two groups. The first group which consisted of F_2 seeds resulting from the crossing between Chisaya-natane and zero-erucic acid Canadian line was sown in paper pot on August 23, 1971. The other group was sown in the field in September, as mentioned later (see Fig. 4). The former was vernalized at low temperature and grown in the green house. The seed samples from 70 F_2 plants were obtained early in March next year and their fatty acids were analyzed by gas chromatography. Five zero-erucic acid seed samples were found and immediately sown in the nursery on March 29. After transplantation in the field they were maintained by selfing and also intercrossed in order to get as soon as possible the Japanese type with seed oil free from erucic acid. F_4 plants resulting from selfing of F_3 as well as subsequent generations were grown during the usual season in the field. By proceeding to a selection in each generation, the high yielding plants with desirable agronomic characteristics were selected from the progenies of these crosses.

As previously mentioned (see Fig. 4), the F_1 hybrid plants resulting from intercrosses between zero-erucic acid plants were grown in the field and F_2 seeds were obtained by selfing. According to the breeding program, F_2 generation and subsequent generations were grown in the field. Many Japanese type plants with oil free from erucic acid were easily selected from the progenies of these crosses.

The remaining F₂ seeds resulting from reciprocal crosses, zero-erucic acid line X Chisaya-

natane, and F_2 seeds from reciprocal crosses, zero-erucic acid line X Norin 16 and zero-erucic acid line X Asahi-natane, were sown in the field in autumn 1971. Four hundred F_2 plants per each cross were grown under natural winter conditions in the field. Summer type plants resembling Canadian rapeseed did not winter under these conditions. Approximately 50 plants per cross which had desirable agronomic characteristics were selected from the winter type plants which survived in F_2 population and their slef-pollinated seed samples were harvested. These 293 seed samples were analyzed by gas chromatography and eighteen seed samples free from erucic acid were found.

Table 3 shows segregation for zero-erucic acid plants in the seeds (bulk sample of F_3) from selected plants of F_2 generation resulting from six reciprocal crosses. The plants with 0 to 4% erucic acid content represented approximately 1/16 of the total F_2 population. Segregation ratios of all families of the crosses fitted to 1:15 (low vs. high erucic acid). This result indicated that the erucic acid content was governed by two genes (E_1 and E_2) acting additively, as previously reported (Harvey and Downey 1964).

F ₂ population	Analyzed plants		ed frequency of with erucic %	χ^2 value for fit	P value for fit
- 2 F - F - F - F		0 - 4%	more than 5%	to 1:15 ratio	to 1:15 ratio
in the green house					
Chisaya X 0-erucic	70	5	65	0.0953	0.80-0.70
in the field					
0-erucic X Chisaya	49	4	45	0.3061	0.70-0.50
Chisaya X 0-erucic	50	3	47	0.0053	0.95-0.90
Total	99	7	92	0.1138	0.80-0.70
0-erucic X Norin 16	50	· 1	49	1.5413	0.30-0.20
Norin 16 X 0-erucic	47	5	42	1.5446	0.30-0.20
Total	97	6	91	0.0006	0.99-0.98
0-erucic X Asahi	59	3	56	0.1367	0.80-0.70
Asahi X 0-erucic	48	2	46	0.3555	0.70-0.50
Total	107	5	102	0.4542	0.70-0.50
Total	363	23	340	0.0046	0.95-0.90

Table 3. Segregation for erucic acid content (%) in the seed (bulk samples of F_3) from selected plants of F_2 generation from crosses, zero-erucic acid Canadian rapeseed cultivar X high erucic acid Japanese rapeseed cultivar.

Each zero-erucic acid F_3 seed sample was divided into two groups. One group was grown at the Rapeseed Breeding Center, Japan, and the other group was grown at the Mokpo Branch Station, Korea. In both Breeding Stations the excellent plants with desirable agronomic characteristics were selected in the progenies of those seeds. Table 4 shows maturity date, plant height, ear length, total number of branches, 1,000 seed weight and seed yield of selected lines in both Stations.

Line	Maturity date	Plant height	Ear length	Total number of branches	1,000 seed weight	Seed yield kg/10a
		cm	cm		g	0,
Fransplanting culture i	in the field at Rap	eseed Breeding	Center, Japar	1.		
Chisaya-natane X Zero-erucic F ₇ -1	June 19	110	38	65	3.2	226
Chisaya-natane X Zero-erucic F ₇ -3	June 20	112	36	60	3.4	221
Chisaya-natane X Zero-erucic F ₇ -4	June 21	103	44	58	3.2	243
Chisaya-natane X Zero-erucic F ₇ -5	June 22	111	40	55	3.8	199
Chisaya-natane X Zero-erucic F ₇ -10	June 22	115	41	48	3.8	229
Chisaya-natane	June 20	111	38	62	3.6	247
Zero-erucic cult.	June 22	121	45	35	2.9	150
Direct sowing culture i	in the field at Mol	po Branch Star	tion, Korea.			
Norin 16 X Zero-erucic F ₅ -37	June 6	153	42	16	2.6	266
Chisaya natane X Zero-erucic F ₅ -11	June 5	164	42	15	2.6	227
Chisaya-natane X Zero-erucic F ₅ -36	June 11	165	39	15	2.5	236
rudal	June 12	136	34	12	2.6	232

Table 4. Maturity date, ear length, total number of branches, 1,000 seed weight and seed yield per 10a of selected lines at the Rapeseed Breeding Center, Fukushima Agricultural Experiment Station, Japan, and Mokpo Branch, Crop Experiment Station, Korea.

In this breeding program the most important target was the resistance to lodging, because the progeny from hybrids resulting from the crossing of zero-erucic acid line and Japanese cultivars was taller than Japanese cultivars under the climate conditions of Japan and Korea. The plants with shorter stem, early maturity, multiple branches, winter hardiness and high yield were selected in both Stations. The F_7 lines bred by the method of shortening of the breeding cycle at the Rapeseed Breeding Center, Japan, were resistant to lodging and showed plant height, maturity and seed yield identical to those of Japanese leading cultivar Chisaya-natane. The F_5 lines bred by standard breeding method at Mokpo Branch Station, Korea, possessed high seed yield but were sensitive to lodging. The plant height should be improved in future, so as to get shorter plants.

Fatty acid composition of these F_5 lines bred at the Mokpo Branch Station, Korea, is shown in Table 5. These lines are free from erucic and eicosenoic acids, while they contain 65 to 70% oleic acid, 19 to 21% linoleic acid and 3 to 8% linolenic acid. Oil of these lines has been transformed into a much better oil than before. Its fatty acid composition is similar to that of peanut oil which is rich in oleic acid, except for 3 to 8% linolenic acid. The last objective of this program is to develop an oil rich in linoleic acid and low in linolenic acid. One of the striking characteristics of Norin 16 X zero-erucic acid F_5 -37 line is that it contains only 3% linolenic acid whereas common rapeseed oil contains 9% of that acid.

Line	Fatty acid composition								
	Pal 16:0	Ste 18:0	Ole 18:1	Lin 18:2	Lnl 18:3	Eic 20:1	Eru 22:1		
Norin 16 X 0-erucic F ₅ -37	2.02	1.60	70.10	20.81	3.26	0.21	0.00		
Chisaya X 0-erucic F5-11	3.13	1.97	65.71	20.43	7.54	1.07	0.15		
Chisaya X 0-erucic F ₅ -36	3.41	2.09	64.26	19.02	8.06	2.12	1.04		
Yudal	1.04	1.27	10.43	10.44	10.06	8.14	58.62		

Table 5. Fatty acid composition of oil in selected rapeseed lines at Mokpo Branch, Crop Experiment Station, Korea.

Problems encountered in the course of the breeding program.

1. Shortening of the breeding cycle may be an effective breeding method for improving oil quality as well as for other breeding purposes. In the season of 1971 to 1972, two generations were experimented through summer sowing in the green house after vernalization and through spring sowing in the field. Mukade (1974) succeeded in growing three generations of rapeseed plant in a year. In the future this method which shortens the breeding cycle will be applied widely in breeding programs for fatty acid composition of oil, because the program is time consuming.

2. Intercrosses between Japanese type F_3 plants whose oil is free from erucic acid should be used in the breeding of cultivars fit to Japanese type rapeseed. In the spring of 1972, five zero-erucic acid F_3 plant originated from F_2 generation which was crossed between Chisayanatane and zero-erucic acid Canadian line and selected for oil quality were intercrossed. Many Japanese type plants appeared in the progeny of subsequent generations from these crosses. These crosses will be used in breeding programs introducing a gene from foreign cultivar to Japanese cultivar.

3. In the progenies of crosses using Japanese cultivar as female parent, many short stem plants appeared. When the Japanese cultivar was used as female parent the F_1 hybrid plants possessed a short stem compared to that of the F_1 hybrid plants using Canadian line as female parent. The same results were also observed in F_2 and subsequent generations. The selected superior lines were also derived from these crosses. As resistance to lodging is an important problem in this breeding program, this characteristic is needed to consider the progress of the program.

4. The bulk seed sample method after selection for winter hardiness was more efficient than the half seed method (Downey and Harvey 1963). In the season of 1971 to 1972, F_2 plants were grown under natural winter conditions in the field, and the summer type plants resembling the Canadian line did not survive under these conditions. Most of the selected plants stemming from the F_2 plants which survived were of the winter type. Therefore many winter type plants appeared in their progenies, while zero-erucic acid F_2 plant selected by half seed method did not always belong to the winter type. In a breeding program introducing a gene from summer type plant to winter type plant, the bulk seed sample method after selection for winter hardiness is more efficient than the half seed method.

5. Future problems to solve for improving oil quality should aim at increasing the content of linoleic acid and at reducing the content of linolenic acid. In zero-erucic acid F_2 plant populations, negative correlations between oleic and linoleic acids, and oleic and linolenic acids were observed while a positive correlation between linoleic and linolenic acids was

noted (Shiga *et al.* 1974). Negative correlation between oleic and linoleic acids indicates that a high linoleic acid plant may easily be found in a zero-erucic acid plant population, but that these plants may show a high linolenic acid content, because there is a positive correlation between linoleic and linolenic acids, namely linolenic acid content increase being related to an increase in the linoleic acid content.

One of the most urgent tasks in breeding for oil quality will be to increase the linoleic acid content at the same time as decreasing the linolenic acid content. It will be difficult to select lines with a high linoleic acid content without increasing the linolenic acid content. For this reason an attempt must be made to induce a desired genotype by mutagenic treatment with X ray or ethylmethane sulphonate (EMS). The fact that the Norin 16 X zero-erucic acid F_5 -37 line which has been bred at the Mokpo Branch Station, Korea, contains only 3% linolenic acid is an encouraging result.

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Discussion

N. Murata, Japan: If erucic acid content and accumulated average temperature are always negatively correlated, it may sound difficult to get short maturing low erucic acid lines. However, judging from fig. 3 you presented, I feel we could get short maturing low erucic acid lines as it appears that erucic acid content is not necessarily correlated with accumulated temperature when tested at a location.

Answer: I agree with you. We do hope to be able to obtain such lines. Also, the extent of variation of erucic content among cultivars depends on locations along with the efficiency of selection for fatty acid composition. Mokpo in Korea is the place where we can select low erucic acid lines most effectively.

H. Fujimaki, Japan: What are the reasons for quite different responses in Japan and in Korea?

Answer: In populations derived from identical F_3 seeds, we get several lines with shorter stem, early maturity, multiple branches and winter hardiness in Japan while in Korea we obtain some lines with high yield performance and long stem. These differences seem attributable to differences in growth response to environmental conditions as well as differences in breeding objectives.

S. Samoto, Japan: Is there any genetic correlation between resistance to lodging and zero-erucic acid content?

Answer: There is no correlation between these parameters. Besides, no correlation was observed between winter hardiness and zero-erucic acid content. We have succeeded in breeding Japanese type zero-erucic acid lines showing resistance to lodging and winter hardiness at the Rapeseed Breeding Center in Japan.