OIL CROPS: BRASSICA SUBNETWORK

PROCEEDINGS OF THE THIRD WORKSHOP,QUALITY TRAINING, AND CHINESE PROJECT REPORTS, HELD IN SHANGHAI, PEOPLE'S REPUBLIC OF CHINA, 21–24 APRIL 1990

ABBAS OMRAN

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Oil Crops: Brassica Subnetwork

Proceedings of the Third Workshop, Quality Training, and Chinese Project Reports, held in Shanghai, People's Republic of China, 21–24 April 1990

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INTRODUCTION

The Brassica Sub-network was first suggested during the 3rd Oilcrops Network Workshop held in Ethiopia in 1986. The first Brassica Sub-network workshop was then held in Sweden in May 1987 (Publication IDRC MR168e). Nine members proceeded from Sweden to Poland to participate in the 7th Rapeseed Congress. A review on "Aphid Screening" was recommended and published.

The second Brassica Sub-network workshop was held in India in January 1989 (Publication IDRC MR252e). Reviews on *Alternaria*, white rust, drought resistance and a colored bulletin on insects and diseases were recommended, and are being prepared for publication.

Brassica in China has developed so fast in the last few years. They are holding annual meetings which proved very useful in the world understanding of rapeseed improvement. IDRC, among other international organizations, supported the Chinese Ministry of Agriculture in organizing **"Symposium of China International Rapeseed Sciences"** which was held April 24 - May 2 at Shanghai, attended by more than 80 Chinese brassica researchers and more than 30 international scientists. The Symposium offered 83 scientific papers on different aspects of rapeseed-mustard research. The most prominent results can be summarized as follows:

In breeding,

- A large number of foreign germplasm was subjected to selection and hybridization.
- A number of single- and double-low *Brassica napus* lines were developed in the last 10 years, covering about one third of a million hectares.
- The Polima system of male sterility was developed to produce low-erucic lines with yields up to 2200 kg/ha.
- Genetic male sterility in *B. napus* was found to be conditioned by interaction of genes at two loci, and was used to develop hybrid rape at Shanghai.
- Hybrid rapeseed was developed all over China. Recently, research on heterosis was initiated at Henan Province and some hybrids showed >20% increase in seed yield.
- Understanding and promoting efficient flower and bud differentiation was behind the high and stable seed yield.
- Low-erucic *B. juncea* lines were developed.
- High oil content studies were initiated with great progress.
- Inheritance studies of apetalous character were conducted and its breeding implications are being investigated.
- Erucic acid was reported to be controlled by two non-dominant genes with additive effects.

In agronomy,

- Yield of rapeseed under zero-tillage was >23% higher without puddling after rice harvest in south China. On the other hand, tillage provided more soil moisture

conservation during drought periods.

- Applying boron, especially inefficient soils of Yaatze Valley, increased yield by >29% and oil content by 3%.

This symposium was the main focus of this Brassica Sub-network third meeting. We wanted our participating Brassica workers in Africa and Asia to meet with their Chinese colleagues and exchange views on the development of the Brassicas.

The commonly used TMS method of glucosinolate determination in rapeseed-mustard cake needs expensive equipment and chemicals. A special training session (course) on Canadian, Australian and Chinese methods of glucosinolate determinations was offered to all Brassica Sub-Network participants prior to the Brassica Sub-Network workshop. There was a general consensus that the session had been very useful and that the methods presented and demonstrated by **Dr. McGregor** (Agriculture Canada) were well received and well understood by the participants. **Dr. Truscott** (University of Wollongong, Australia) was strained in Hong Kong but he was able to present his method on the second day of the training session. On behalf of all participants (trainees), I give them our sincere congratulation for a job well done. The manuals of this course are published as Part I of this publication.

Since 1983, IDRC was continually supporting a "Rapeseed Breeding Project" in four Chinese Academics of Agricultural Science. We spent a whole day listening and discussing their reports on their achievements in the last 7-8 years. Although they are going to publish their reports in a more comprehensive and completed form in 1991, I found it worthwhile publishing the scientific part of their reports up to 1990 as Part II of this publication.

This third Brassica Sub-network workshop was held in China in April 1990 with the following objectives :

1. To follow-up on germplasm exchange and possible cataloguing of Brassica collections.
2. To update processing/marketing problems.
3. To follow-up on the sub-network activities from January 1989 to April 1990, especially: training, nurseries, reviews, booklets, final aphid screening publication, contact persons, and information exchange.
4. To report on the training course on breeding/agronomy (Pantnagar, India, December 1989).
5. To elect new steering committee members.
6. To participate in the Chinese International Rapeseed Symposium (24-30 April 1990).
7. To organize a training session on glucosinolates.

The proceedings of the workshop and the valuable discussions which followed are published as parts III and IV of this publication. To keep the continuity, this workshop is

recommending to hold the fourth Brassica workshop along with the 8th Rapeseed Congress in Canada, July 1991.

It appears that the Brassica Sub-Network has made the most progress, and is well on the way towards a self-sustaining status, though limited continued support from IDRC will probably be needed for some time. It was clear at the China Symposium and this Sub-Network meeting that the strong research programs in China and India, linked closely with the Canadian program, and with other programs in countries such as Sweden, are fully capable of providing leadership and research backup for other member countries, and for the Sub-Network as a whole. Scientists from these programs have already provided valuable consultancy and training services to other Sub-Network member programs on request, and these activities are expected to increase in the future.

The Brassica Sub-Network member countries have contributed 64 germplasm lines/varieties which had already been dispatched to the member countries. This Sub-Network is also arranging collaboration with many other scientists around the world who are interested in Brassica research problems, on an equal partnership basis. The Sub-Network should also be able to attract support from other donors when necessary. The special collaborative program between India and China and involving Canada is one of the best achievements of this workshop.

In conclusion, this interesting publication offers the experience of world scientists in all aspects of Brassica improvements and quality training.

The Oilcrops Network Adviser is grateful to all the Chinese Scientists especially **Dr. Qu Ninjkang** (Rapeseed Breeding Project Leader), **Dr. Zhang Yan** (Commissioner of Agriculture, Shanghai Municipality), **Mr. Ma Jiuhui** (Ministry of Agriculture, Beijing), and **Dr. Wang Shu Jun** (President of Shanghai Academy of Agricultural Sciences) who were the vital force behind the success of the workshop.

Special thanks go to **Dr. Keith Downey** (Agriculture Canada) who offered to assist the collaborative programs among member countries and to host our fourth meeting in Canada.

Mr. Seid Ahmed (Network Assistant) continued his appreciated efforts to redesign all the figures and graphs in the proceedings and to proof-read all drafts. Three temporary typists worked in succession to make a better manuscript: **Miss Raei Melesse, Miss Zenaye Amare and Miss Ehite Hailemeskel.**

Abbas Omran
Coordinator / Editor

PART I

QUALITY TRAINING

SECTION 1
MANUAL OF SELECTED METHODS FOR GLUCOSINOLATE ANALYSIS

By

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These notes include procedures for five methods for glucosinolate analysis presented at this workshop. The procedures are for methods as developed, or adapted, and currently used at the Agriculture Canada Research Station, Saskatoon. Table 1 shows the comparison of all seven procedures (five presented in this workshop and two published somewhere else as mentioned under the Table.

Table 1. Comparison of seven methods for determining the total glucosinolate content of rapeseed.

	METHODS*						
Sample	Canadian Grain Commission GLC-TMS[1)]	Agriculture Canada Saskatoon GLC-TMS[2)]	TMS glucose[3)]	Glucose Analyzer[4)]	Glucose oxidase/ ion exchange[5)]	Thymol[6)]	HPLC[7)]
BC86-18	4.70	5.30	5.50	4.80	7.40	4.20	5.10
Tobin (S)	14.10	14.80	15.80	13.40	18.00	14.70	14.00
Westar	28.20	28.50	28.10	25.30	31.50	25.20	24.20
Tobin (Cert.)	25.30	25.50	27.50	23.40	30.10	27.60	23.10
BL802	47.90	47.50	51.50	42.20	54.10	51.20	42.40
226-1	16.00	16.20	14.30	13.90	19.90	14.10	14.50
226-2	34.50	36.50	31.40	32.40	39.80	34.50	31.90
BC:BL 3:1	14.70	15.00	16.40	15.00	20.00	14.90	14.20
BC:BL 1:1	26.10	30.00	26.50	24.40	31.40	28.20	23.50
BC:BL 1:3	36.70	37.50	39.90	33.60	42.80	39.60	33.00
S.E.**	0.34	0.48	0.33	0.45	0.84	0.49	0.67
Overall mean	24.80	25.40	25.70	22.80	29.50	25.40	22.60

*1) Daun, DeClerq and McGregor. 1989. (page 8 of this proceedings).
2) Raney and McGregor. 1990. (page 14 of this proceedings).
3) McGregor. 1990a. (page 20 of this proceedings).
4) McGregor. 1990b. (page 24 of this proceedings).
5) Wang, Yuan and McGregor. 1990. (page 33 of this proceedings).
6) McGregor, D.I. and R.K. Downey. 1986. Determination of total glucosinolate content in seed meal of rapeseed using thymol. In:Proceedings of the Third Oil Crops Network Workshop held in Addis Ababa, Ethiopia, October 6-10 (A.Omran, Editor) :242-250.
7) McGregor, D.I. 1985. Determination of glucosinolates in Brassica seed. In:Cruciferae Eucarpia Newsletter No. 10. :132-136.
** Pooled standard error of the means of three determinations.

Samples were selected to bracket the canola range of glucosinolate content (<30 micromoles glucosinolate excluding the indole glucosinolates per gram oil-extracted air-dried meal). All methods had good precision. The somewhat higher values obtained with the glucose oxidase/ion exchange method are under investigation. Somewhat lower values for the glucose analyzer methods are also being further investigated. Studies to date indicate that reducing the sample size from 250 mg of gram oil-extracted air-dried meal, as originally called for in the procedure of the glucose analyzer method, to 100 mg yields means comparable with the other methods of analysis.

The details of the five methods presented today are included in the next five papers:

- Daun, J.K., D.R. DeClerq and D.I McGregor. 1989. Analysis of Glucosinolates in canola and rapeseed, Method of the Canadian Grain Commission Grain Research Laboratory. December 15, 1981, revised September 30, 1983, September 1, 1989.
  (pp. 8-13 of this proceedings).

- Raney, J.P. and D.I. McGregor. 1990. Determination of glucosinolate content by gas chromatography of trimethylsilyl derivatives of desulfated glucosinolates.
  (pp. 14-19 of this proceedings).

- McGregor, D.I. 1990. Determination of glucosinolate content by gas chromatography of trimethylsilyl derivatives of glucose.
  (pp. 20-23 of this proceedings).

- McGregor, D.I. 1990. Determination of total glucosinolate and total indole glucosinolate content of rapeseed/canola using glucose oxidase to measure glucose and ferric nitrate to measure free thiocyanate ion.
  (pp. 24-32 of this proceedings).

- Wang, X., Z.Y. Yuan and D.I. McGregor. 1990. Determination of total glucosinolate content of rapeseed/canola using immobilized myrosinase and glucose oxidase.
  (pp. 33-39 of this proceedings).

ANALYSIS OF GLUCOSINOLATE IN CANOLA AND RAPESEED: DETERMINATION OF GLUCOSINOLATES BY GAS LIQUID CHROMATOGRAPHY OF THE TRIMETHYLSILYLETHERS

(Method of the Canadian Grain Commission, Grain Research Lab.)

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1. SCOPE AND FIELD OF APPLICATION

This test describes a method for the determination of the glucosinolate content in canola or rapeseed and their residues by gas liquid chromatography of the trimethyl-silylethers.

2. SAFETY AND HANDLING

2.1 Hazards:

2.1.1 Acetic acid, glacial: corrosive; causes skin burns; vapor irritates the respiratory tract.

2.1.2 Pyridine: toxic; flammable; vapor forms explosive mixtures with air; irritates skin and respiratory tract; vapor may cause headache, giddiness, nausea,and vomiting; TLV 5 ppm.

2.1.3 Barium Acetate: toxic; irritates eyes, skin, respiratory tract and mucous membranes.

2.1.4 Lead Acetate: highly toxic.

2.1.5 Sodium Hydroxide: corrosive, can cause severe skin burns; irritant to skin, eyes, respiratory tract and mucous membranes.

2.1.6 MSTFA: flammable; may be irritant to skin, eyes, and mucous membranes; toxicological properties not fully known, but assume to have some degree of toxicity.

2.1.7 TMCS; flammable; corrosive; irritant.

2.2 Handling and Storage:

2.2.1 Gloves, goggles and lab coat should be worn when handling liquid acids or corrosives. Acids are always diluted by being poured into water. Check compatibility of storing acids with other chemicals (e.g. do not store acetic acid with oxidizers). Transport acids in acid transport buckets and store large quantities in acid-storage cabinets.

2.2.2 Handle the pyridine-acetate buffer and the derivatization reagents in a well-ventilated area (preferably in a fume hood) away from heat, sparks and open flame. When handling these liquids use protective eye wear, gloves and a non-polyester lab coat.

2.3 Waste disposal:

2.3.1 Discarded column washes containing the pyridine-acetate solution can be disposad of down the special solvent drain, sealing the trap with excess water.

2.3.2 Used TMS ether samples can be disposed of down the solvent drain, sealing the trap with excess water.

3. REFERENCES

-DAUN, J.K., AND D.I. MCGREGOR, Glucosinolates in seeds and residues. In Analysis of Oilseeds, Fats and Fatty Foods edited by J.B. Rossell and J.L.R. Pritchard, Elsever Publishing Company.

4. PRINCIPLE

Aqueous extraction of glucosinolates followed by purification and dessulfation on micro ion-exchange columns. Isolated desulpho-glucosinolates are converted to TMS ethers and analyzed by gas-liquid chromatography.

5. REAGENTS

5.1 Pyridine-acetate(pyridyl-acetate) buffers (Reagent grade pyridine and reagent grade acetic acid.):

5.1.1 Pyridine acetate, 0.5 M: Place 930 mL water in a 1 L flask and add 30 mL glacial acetic acid and 40 mL pyridine. [Do not add glacial acetic acid directly to the pyridine as a violent reaction may occur.]

5.1.2 Pyridine acetate, 0.02 M: Place 4 mL of 0.5 M pyridine acetate into a 100 mL flask and dilute to 100 mL water.

5.2 Sodium Acetate, 1 M:

Weigh 20.5 g sodium acetate into a 250 Ml flask and dilute to 250 mL with water.

5.3 Barium acetate and lead acetate, 0.5 M solution:

Weigh 63.9 g of barium acetate and 94.9 g of leadacetate in a 500 mL flash and dilute to 500 Ml with water.

5.4 Sodium Hydroxide, 0.5 N solution:

Weigh 2 g sodium hydroxide into a 100 mL flask and dilute to 100 mL with water.

5.5 Internal Standards:

5.5.1 Allylglucosinolate (sinigrin, monohydrate potassium salt), 1 millimolar: Weigh 0.415 g allylglucosinolate into a 100 mL flask and dilute to 100 mL with water.

5.5.2 Benzylglucosinolate (glucotropaeolium tetramethylammonium salt), 1 millimolar. Weigh 0.241g benzylglucosinolate into a 100 mL flask and dilute to 100 mL with water.

5.6 DEAE Sephadex A-25 and SP Sephadex C-25:

5.6.1 DEAE Sephadex A-25: Weigh 25 mg into a micro ion exchange column (6.8). Add 10 mL water and allow to swell overnight. Pass 5 Ml 0.5 N sodium hydroxide (5.4) through the column and then wash with 5 Ml water to remove excess sodium hydroxide. (Check to ensure the Ph of the effluent is neutral.) Pass 5 Ml of 1 M sodium acetate (5.2) through the column followed by 7 Ml water. (Check to ensure the Ph of the effluent is neutral.)

5.6.2 SP Sephadex C-25: Weigh 25 mg into a micro ion exchange column (6.8). Add 10 Ml water and allow to swell overnight.

5.7 Sulfatase (Aryl sulphatase, type H1 from *Helix pomatia*):

5.7.1 Purification of sulfatase: Weigh about 70 mg of sulphatase into a 16 x 150 mm test tube. Add 3 mL water to dissolve the sulphatase and dilute with an equal volume of ethanol. Centrifuge for 10 minutes at 2000 x g. Decant the supernatant fluid into a second test tube and discard the precipitate. Add 9 mL of ethanol to the supernatant fluid and dissolve the precipitate in 2 mL of water. Pass the enzyme solution through the DEAE Sephadex C-25 column (for convenience, the DEAE column way elute directly into the SP column). Wash the columns with 10 mL water and dilutE the combined eluted enzyme solution to 35 mL with water. (It may be more convenient to carry out this procedure in triplicate and dilute the combined eluted enzyme solutions to 100 mL). The purified enzyme should be stored at -20° C. and thawed immediately before use. It is stable for about 2 weeks if stored in a refrigerator.

5.8 Pyridine (Silylation/ Derivatization Grade):

The pyridine used for preparing trimethylsilyl ethers must be moisture free.

5.9 MSTFA (or MSHFBA) N-Methyl-N-trimethysilyl-trifluoroacetamide (or N-Methyl-N-trimethylsilyl-heptafluorobutyramide):

MSHFBA, although more expensive, does not produce deposits in the combustion chamber of the flame ionization detector.

5.10 TMCS, Trimethylchorosilane (Silylation Grade).

5.11 Ethanol, Reagent Grade.

6. APPARATUS

Usual laboratory apparatus and in particular:

6.1 Micro-grinder, e.g. coffee mill.

6.2 Desiccator.

6.3 AnalyticaL Balance.

6.4 Water Bath, boiling, or block-type heater at 95 +/-2 °C.

6.5 Oven, at 120 +/-2 °C.

6.6 Centrifuge, reaching a centri-fugal acceleration of 5000 x g.

6.7 Stirrer, Vortex type, for test tubes.

6.8 Micro ion exchange columns:

May be purchased commercially or prepared from pasteur pipettes or disposable pipette tips. The details given here are for a column containing about 100 mg (dry weight) of DEAE-Sephadex A-25 ion exchange resin which has been swelled with water, swirled to remove air bubbles and allowed to settle giving an ion exchange bed of 15 mm x 8 mm (dia.). Smaller columns or columns of different dimensions may be prepared but these should be tested for capacity, for the amount of enzyme required, and for the amount of solvent required to completely elute the glucosinolates or desulpho-glucosinolates.

6.9 Tube, borosilicate glass, 8 mL capacity, with PTFE lined caps.

6.10 Tube, borosilicate glass, 2 to 4 mL capacity, with PTFE lined caps which may be screw-type or crimp type for use with auto-samplers.

6.11 Gas Chromatography:

With temperature programming capability and flame ionization detector. If a packed column instrument, injector and detector connections should be glass-lined. Packed column instruments should be equipped with a 1.2 meter x 6 mm o.d., 2 mm i.d. glass column containing 2% OV-7 on Chromosorb AW DMCS 100/120 mesh. The column should be conditioned by attached by attaching to the injection part of the chromatograph and purging with helium at room temperature for 1 hour before programming at 1 °C. per minute from 100 °C. to 280 °C. then holding overnight. Capillary column instruments may be equipped with a 25 meter x 0.20 mm i.d. fused silica column coated with cross linked methyl silicone (.11 um). The column should be conditioned in a similar manner to the packed column (above). A precolumn (1 cm) packed with 1% silicone gum rubber SE 30) on Chromosorb W high performance 80/100 mesh may be used. After attaching to the detector end, initial operating conditions are:

	Packed Column	Capillary Column
Injector Temp. (°C)	280	220
Detector Temp. (°C)	280	300
Initial Column Temp.(°C)	180	200
Initial Time (min)	4	0
Program Rate (°C/min	8	4
Final Temperature (°C)	280	280
Final Time (min)	4	0
Carrier Gas-Helium(mL/min)	30	30
Air (FID) (mL/min)	500	500
Hydrogen (FID) (mL/min)	50	50
Detector Range	1	1
Detector Attenuation	64	16
Split Ratio	N/A	100:1

[Settings may vary somewhat from instrument to instrument]

7. PROCEDURE

7.1 Preparation of the test sample:

This method assumes that the sample has been selected according to appropriate sampling procedures. Grind the sample using the micro-grinder (6.1). If the oilseeds have a water content greater than 10% (m/m), they must be dried beforehand to ca. 8% moisture with an air flow at about 45 °C.

NOTE: for the analysis of seeds treated with pesticide, wash with dichloromethane prior to grinding.

7.2 Determination of moisture and volatile matter content:

Determine the moisture and volatile matter content of a portion of the ground test sample with an appropriate procedure (e.g. 5g of the ground test sample at 103 +/-2 °C. for 3 hours or to a constant weight in a forced-air oven).

7.3 Determination of oil content (if the results are desired on an oil-free basis):

Determine the oil content in accordance with the appropriate standard procedure (see Method OIL1).

7.4 Test Portion:

Weigh into a tube (6.9) 200 mg of the ground test sample of oilseeds or 100 mg of the test sample of ground residue or flour.

7.5 Extraction of glucosinolates:

7.5.1 Extraction: Place the tube containing the test portion into the water bath (6.4) at 95 °C. and leave for 15 min. Then add 1 mL of boiling water. Wait 2 min. and withdraw the tube, add 1 mL internal standard, (5.5.1 or 5.5.2) and stir with the stirrer (6.7). Cap the tube with the lined cap and replace the tube in the water bath at 95 °C for a further 1 minute. Remove from the water bath and leave to cool to room tempera-ture. Add 100 μL of the lead acetate and barium acetates solution (5.3), then centrifuge for 15 min. at an acceleration of 5000 x g.

7.5.2 Calibration mixture: If using benzylglucosinolate as the internal standard, prepare one or more samples containing 1 mL of internal standard (5.5.2) and 1 mL of sinigrin solution (5.5.1). and carry this mixture through the extraction procedure (7.5.1).

7.6 Preparation of ion exchange columns: Pass 5 mL of 0.5 N sodium hydroxide (5.4) through the hydrated column (6.8):

Pass 10 mL distilled water through the column and continue washing with water until the eluant is neutral to test paper. Pass 5 mL of the 0.5 M pyridine acetate (5.1.1) through the column followed by 10 mL of water. Leave sufficient water to form a meniscus for ease of sample application.

7.7 Isolation of glucosinolates:

Transfer 1 mL of the extracted glucosinolates containing internal standard (7.5.1) to the ion exchange column (6.8):

When the solution has run onto the column, wash with 3 mL of 0.02 M pyridyl acetate (5.1.2). Add 0.5 mL of the purified sulphatase solution (5.7.1) onto the column. When the enzyme solution has completely entered the column, stop the flow and cover the column to reduce evaporation. Allow to stand at room temperature overnight (a minimum of 4 hours at 20 °C.). Elute the desulpho-glucosinolates with 2.0 mL water into a small tube (6.10).

7.8 Preparation of pertrimethylsilyl ésters:

Evaporate the moisture from the desulphoglucosinolates under a stream of nitrogen at 60 °C. To the dried desulphogluco-sinolates add 100 μL pyridine (5.8), 100 μL MSTFA or MSHFBA (5.9) and 10 μL TMCS (5.10) and securely cap the tube. Auto-sampler vials may be capped after addition of pyridine and the MSTFA and TMCS added through the septum [Note: It is essential that all reagents be dry. It is advisable to add the reagents to one sample at a time to minimize exposure to the atmosphere.] Heat at 120 °C. for 20 minutes.

7.9 Separation and determination by GLC:

Inject about 2 μL of the pertrimethylsilylated desulphoglucosinolates (7.8) onto the OV-7 column (6.11). Run the temperature program according to 6.11. If the internal standard used is benzylgluco-sinolate, the first sample run should be the calibration mixture containing equimolar benzyl-and allyl-glucosinolates.

7.9.1 **NOTE:** Samples silylated with MSTFA produce a small peak with a retention time similar to silylated 2-hydroxy-4-pentenyl desulphoglucosinolate. If this peak is inseparable, it may be necessary to adjust results for this glucosinolate downwards. When samples are silylated with MSHFBA, the peak occurs between 4-hydroxybutenyl- and 2-hydroxy-4-pentenyl desulphoglucosinolate and is separated.

7.9.2 **NOTE:** When analyzing samples with a wide range of glucosinolate content, some "memory" of the glucosinolate content of the preceding sample may occur (for example, some residue may remain in the injection syringe). For example, when analyzing with and without allylgucosinolate internal standard, analysis of a sample which does not contain allylglucosinolate immediately after a sample which does contain allylglucosinolate may result in an overestimate of the allyl-glucosinolate may result in an overestimate of the allylglucosinolate in the second sample. The reverse situation may also occur. Repeated analysis of the same sample until stable results are obtained may be necessary. It is also good practice to carry out several injections of the first sample prior to beginning an analytical session when the system has had a period of inactivity (e.g. overnight).

8. QUANTIFICATION OF RESULTS

8.1 Collect the peak areas for:

3-butenylglucosinolate
4-pentenylglucosinolate
2-hydroxy-4-butenylglucosinolate
2-hydroxy-4-pentenyl-glucosinolate allylglucosinolate (if present)
4-hydroxybenzylglucosinolate (if present) indolyl-3-methylglucosinolate
4-hydroxy-indolyl-3-methylglucosinolate benzylglucosinolate.

8.1.1 <u>For GLC with Benzyl-glucosinolate Internal Standard</u>: Since aromatic glucosinolates give different responses in the flame ionization detector to aliphatic glucosinolates, it is necessary to correct the benzyl-gluco-sinolate response factor. The theoretical value for benzyl-glucosinolate relative to allylglucosinolate is 0.86 but because of day to day variation in columns and equipment, the equimolar mixture of allyl- and benzylglucosinolates (7.5.2) is used for this purpose. Divide the area obtained for benzyl-gluco-sinolate by the area obtained for allylglucosinolate to obtain the relative response factor Rr for the particular conditions of analysis.

8.1.2 <u>For GLC with Allyl-glucosinolate Internal Standard</u>: Small amounts of allyl-glucosinolate may be present in the sample either as a minor component of the rapeseed glucosinolates or as a result of contamination from weed seeds (especially *Thlaspi arvense* (stinkweed)). In order to correct for naturally occurring allylglucosinolate, samples must be run with and without allyl-glucosinolate internal standard. The true peak area (A) of allyl-glucosinolate in the internal standard may then be calculated as:

$$A(true) = A(found) - A(contam)$$

where:
-**A(true)** is the area due to added allylglucosinolate (internal standard).
-**A(found)** is the total area for allylglucosinolate in the chrmatogram with added internal standard.
-**A(contam)** is the area due to natural contamination and is calculated as:

A(contam)= A(allyl-) x A(2-OH-3-Butenyl+)/A(2-OH-3-Butenyl-)

where:
-**A(allyl-)** is the area of the allylglucosinolate peak in the chromatogram without added internal standard.
-**A(2-OH-3-Butenyl+)** is the area of the 2-OH-3-butenyl glucosinolate peak in the chromatogram with added internal standard.
-**A(2-OH-3-Butenyl-)** is the area of the 2-OH-3-butenyl-glucosinolate peak in the chromatogram without added internal standard.

8.2 Calculation of glucosinolate content:

The content of each individual glucosinolate may be calculated from the following appropriate equations:

For GLC with benzylgucosinolate internal standard:
μM Gluco-sinolate/g sample =A(gluc)/A(IS) x Rf/Rr X μM(IS)/g(sample).

For GLC with allylglucosinolate internal standard:
μM Gluco-sinolate/g sample = A(gluc)/A(IS) x Rf x μM(IS)/g(sample)

where:
-**g(sample)=** Grams of sample extracted.
-**A(gluc)=** Peak area for glucosinolate in consideration.
-**A(IS)=** Peak area of internal standard (may be corrected if allylglucosinolate).
-**Rf=** Response factor from table below.
-**Rr=** Determined response factor for benzylglucosinolate relative to allylglucosinolate (7.5.2).
-**μM(IS)=** Micromoles of internal standard added.

Glucosinolate	GLCResponse Factor (Rf)
Allyl-	1.0000
3-Butenyl-	0.9615
4-Pentenyl-	0.9259
2-Hydroxy-3-butenyl-	0.8621
2-Hydroxy-4-pentenyl-	0.8333
Benzyl-	Rr (8.1.1)
4-Hydroxybenzyl-	0.7813
Indolyl-3-methyl-	1.55
4-Hydroxy-indolyl-3-methyl-	1.55

9. EXPRESSION ON RESULTS.

Total glucosinolates may be reported as the sum of all the individual glucosinolates found. The moisture, oil and seed purity of the reporting basis should be stated in the report. For canola, the basis for reporting should be oil-free with 8.5% moisture in the flour unless the analysis is carried out on oil-free flour in which case actual moisture basis of the oil-free flour is determined. For canola, the sum of only the four aliphatic glucosinolates, (3-Butenyl-, 4-Pentenyl-, 2-Hydroxy-3-butenyl-and 2-Hydroxy-4-pentenylglucosinolates) are reported.

If so desired, calculate the concentration of the glucosinolate in the oil free sample from the formula:

(μM/g wf) x 100/(100-OC)= μM/g oil-free

where:

-**μM/g wf=** glucosinolate content in whole fat material,
-**OC** = oil content of material

Similarly, expression of results on different moisture bases may be calculated from:

(μM/g dry) x 100/(100-MB)= μM/g MB

where:

-**μM/g dry=** glucosinolate content on dry basis,
-**MB** = desired moisture basis.

10. TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention all operating details not specified regarded as optional, as

well as any incidents which may have influenced the result. The test report shall include all details required for the complete identification of the sample.

D. DeClercq
August 25, 1989

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DETERMINATION OF GLUCOSINOLATE CONTENT BY GAS LIQUID CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF DESULFATED GLUCOSINOLATES

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1. APPLICATION

The method is intended for determining the glucosinolate composition and content (1 to 200 μ moles per gram oil-extracted meal) of seed or meal samples of rapeseed/canola (*Brassica campestris* L. and *B. napus* L.). It may also be applied to the analysis of seed or meal samples of brown or Oriental (yellow seeded) mustard, *B. juncea* (L.) Coss., and white mustard, *Sinapsis alba* L.

2. PRINCIPLE

Glucosinolates are extracted from oil-extracted meal using aqueous methanol and purified and desulphated on an ion-exchange column, the desulfated glucosinolates are derivatized to form volatile trimethylsilyl (TMS) ethers, and separated and quanitified by gas liquid chromatography.

3. BACKGROUND

Gas chromatography of trimethylsilyl derivatives of desulfated glucosinolates is one of the most useful approaches to date for determining the glucosinolate content of rapeseed. The TMS method is used to define 'canola' rapeseed and has been adopted by the Canadian Grain Commission as its standard method of analysis (Daun and McGregor 1981). The original method Uunderhill and Kirkland 1971) sililated the intact glucosinolate without purification. Thies (1974, 1976, 1977, 1978, 1979, 1980) and Heaney and Fenwick (1980) improved the method by introducing ion exchange chromatography to remove impurities with on-column desulfation with aryl sulfatase enzymes commercially extracted from edible snails to improve derivatization, and temperature programmed gas liquid chromatographic to facilitate separation and quantitation.

To meet the canola standard oil-extracted seed meal must contain less than 30 μmoles/g of the four main aliphatic glucosinolates, 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl and 2-hydroxy-4-pentenyl glucosinolate. The meal must also not contain significant amounts of other aliphatic glucosinolates e.g. 2-propenyl (allyl) or 4-hydroxybenzyl. The TMS method is capable of measuring all six glucosinolates normally found in seed meals rapeseed including, in addition to the above aliphatic glucosinolates, 3-indolylmethyl and 4-hydroxy-3-indolylmethyl glucosinolate.

Allylglucosinolate, the main glucosinolate in seed meals of brown or Oriental mustard or stinkweed (*Thpaspi arvense* L.), and 4-hydroxybenzyl glucosinolate, the main glucosinolate of white and wild mustard (*B. kaber* (D.C.) L.C. Wheeler) can also be detected and quantitated. Stinkweed and wild mustard are common Cruciferous weed seeds on the Canadian prairies.

4. ADVANTAGES/DISADVANTAGES

In addition to determining glucosinolate content, gas liquid chromatography of trimethylsilyl derivatives of glucosinolates provides information on glucosinolate composition.

The method has been specifically designed to faclitate analysis of large numbers of samples by allowing for batch processing of several samples at one time, and automated gas liquid chromatography. The use of methanol to inactivate endogenous myrosinase allows the glucosinolates to be extracted at room temperature. Derivatization is

also carried out at room temperature allowing for use of less expensive silylating reagents and improving recovery of the indole glucosinolates. The use of acetone and dimethylformamide as the solvents for derivatization substantially reduces problems with plugging of the autosampler.

Compared to other methods of analysis which provide information on glucosinolate content, sample preparation is long and tedious and some reagents are relatively expensive or difficult to obtain.

5. EQUIPMENT

Standard laboratory apparatus and in particular:

5.1 Disposable plastic syringes, 1 mL:

Fisher Scientific Ltd.
Cat. No. 14-823-226

5.2 Porous polyethylene sheeting, (Bel-Art) 1.6 mm 35 um pore size:

Mandel Scientific Co. Ltd
Cat. No. F13638-5116

5.3 Cork Bore, #1.

5.4 Oxford pipette tips, 1 mL:

Fisher Scientific Ltd.
Cat. No. 21-240-20

5.5 Disposable syringe needles, 20 guage, 38 mm long:

Fisher Scientific Ltd.
Cat. No. 14-826-5C

5.6 Centrifuge, bench top.

5.7 Vials, teflon-lined screw-cap, 4 mL:

Canlab Ltd.
Cat. No. V3020-1 (vial)
Chromatographic Specialties Ltd.
Cat. No. C669112 (teflon liner)

5.8 Balance, 0.1 mg accuracy.

5.9 Eppendorf Repeater Pipette with 50,5 and 2.5 mL tips:

Fisher Scientific Ltd.
Cat. No. 21-380-8
Cat. No. 21-380-8B (2.5 mL)
Cat. No. 21-381-101 (5 mL)
Cat. No. 21-381-102 (50 mL)

5.10 Eberbach shaker:

Fisher Scientific Ltd.
Cat. No. 14-255
Cat. No. 14-266 (carrier)

5.11 Dispensettes (3), 2 mL:

Fisher Scientific Ltd.
Cat. No. 13-688-100

5.12 Holder for ion-exchange columns and corresponding holder for 4 mL vials.

5.13 Auto sampler vials, teflon-lined caps, 1 mL.

5.14 Dry block heater, to accommodate 4 mL screw-cap vials, Reacti-Therm:

Chromatographic Specialties Ltd.
Cat. No. P18800

5.15 Compressed air and air maifold, Reacti-Vap Evaporator:

Chromatographic Specialties Ltd.
Cat. No. P18782

5.16 Gas chromatograph with flame ionization detector and autosampler:

Equipped with a 0.32 mm ID by 12.5 m fused silica capillary column coated with OV-1 0.5 um thick or Glass column 2 mm ID by 1 m packed with 2% OV-7 coated on Supelcoport.

5.17 Hamilton syringes, 10 mL:

Chromatographic Specialties Ltd.
Cat. No. H80366 (10 μL) (fixed needle)

6. REAGENTS

Standard laboratory reagents and in particular:

6.1 Acetone:

Fisher Scientific Ltd.
Cat. No. A18-500

6.2 Barium acetate:

Fisher Scientific Ltd.
Cat No. B24-500

6.3 Benzyl glucosinolate, tetramethyl ammonium salt:

Biocatalysts
or
Canola Council of Canada

6.4 Dimethylformamide (DMF):

Fisher Scientific Ltd.
Cat. NO. D119-500

6.5 Ethanol, absolute:

Fisher Scientific Ltd.
Cat. No. A407-4

6.6 Glacial acetic acid:

Fisher Scientific Ltd.
Cat. No. A38-500

6.7 Hydrochloric acid:

Fisher Scientific Ltd.
Cat. No. A144-500

6.8 Lead acetate:

Fisher Scientific Ltd.
Cat. No. L33-500

6.9 Methanol:

Fisher Scientific Ltd.
Cat. No. A412-500

6.10 1-Methyl imidazole:

Sigma Chemical Co.
Cat. No. M 8878

6.11 N, O-bis (Timethylsilyl) (tetrafluoroacetamide(BSTFA)
or
N,O-bis (Trimethylsilyl) acetamide (BSA):

Sigma Chemical Co.
Cat. No. T 1506 (BSTFA)
Cat. No. T 4377 (BSA)

6.12 Pyridine:

Fisher Scientific Ltd.
Cat. No. P368-500

6.13 Sephadex DEAE A25, Anion exchanger 40-120u bead size:

Sigma Chemical Co.
Cat. No. A-25-120

6.14 Sinigrin (allyl glucosinolate), potassium salt:

Sigma Chemical Co.
Cat No. S 7508

6.15 Sodium acetate:

Fisher Scientific Ltd.
Cat. No. S209-500

6.16 Sodium hydroxide:

Fisher Scientific Ltd.
Cat. No. S318-100

6.17 Sulfatase, type H-1:

Sigma Chemical Co.
Cat. No. S-9626

6.18 Trimethylsilylchlorosilane(TMCS):

Sigma Chemcial Co.
Cat. No. T 4252

7. SUPPLIERS

7.1 Sigma Chemical Co.

P.O. Box 14508
St. Lois, MO
USA 63178
Telephone (800) 325-8070
(314) 771-5750
Telefax (800) 325-5052
(314) 771-5757

7.2 Biocatalysts

Main Avenue
Treforest Industrial Estate
Pontypridd, Wales
CF37 5YT
Telephone 044385 3712
Telex 497126 BIOCAT G

7.3 Canola Council of Canada

Room 301, 433 Main Street
Winnipeg, MB
CANADA R3B 1B3
Telephone (204) 944-9494

8. PREPARATION

8.1 Benzyl glucosinolate, 1 mM

- Weigh 24.1 mg of benzyl glucosinolate, tetreamethyl ammonium salt, into a 50 mL volumetric flask and make to volume with water.

8.2 Barium-lead acetate, 0.6 M

- Weigh 7.66 g of barium acetate and 11.38 g lead acetate into a 100 mL

volumetric flask.
- Add 0.29 mL glacial acetic acid.
- Make to volume with water.

8.3 DEAE Sephadex column, 0.2 mL

- Cut a 1 mL plastic syringe at the 0.2 mL mark.
- Insert a disk of porous polyethylene cut from a sheet with a #1 cork bore into the shorter bottom piece of the syringe.
- Fill this part of the syringe with DEAE Sephadex A-25, preswollen in water overnight.
- Cut a 1 mL Oxford pipette tip at the collar and insert the larger piece over the syringe tip filled with DEAE Sephadex.
- Place a 38 mm, 20 guage needle on the Luer lock tip of the syringe.

8.4 Methanol, 67%:

- Transfer 67 mL of methanol to a 100 mL volumetric flask and make to volume with water.

8.5 Pyridine acetate, 0.02 M:

- Transfer 2 mL pyridive and 1.5 mL glacial acetic acid to a 1000 mL volumetric flask and make to volume with water.

8.6 Purification of sulfatase:

- Weigh 70 mg of sulfatase into a centrifuge tube.
- Add 3 mL of water and swirl to dissolve sufatase,minimizing formation of foam as much as possible.
- Add 3 mL absolute ethanol and mix.
- Centrifuge at 2000 g for 20 minutes.
- Decant the supernatant into a second centrifuge tube and discard the precipitate.
- Add 9 mL absolute ethanol to the supernatant, mix and centrifuge at 2000 g for 15 minutes.
- Discard the supernatant and dissolve the precipitate in 2.5 mL water.
- Store frozed and thaw just before use.

8.7 Methanol, 60%:

- Transfer 60 mL of methanol to a 100 mL volumetric flask and make to volume with water.

8.8 Sodium acetate, 1M:

- Weigh 8.2 g of sodium acetate into a 100 mL volumetric and make to volume with water.

8.9 Sodium hydroxide, 0.5 M:

- Weigh 2 g of sodium hydroxide into a 100 mL volumetric flask.
- Dissolve and make to volume with water.

8.10 Hydrochloric acid, 0.5 M:

- Place 4.3 mL of concentrated hydrochloric acid in a 100 mL volumetric flask containing water and make to volume with water.

9. PROCEDURE

9.1 Glucosinolate Extraction:

- Weigh 100 ±10 mg of oil-extracted meal into a 4 mL vial an record weight to the nearest 0.1 mg.

  (**NOTE:** This is a critical measurement. Be accurate.)

- Add 2 mL of methanol, 1 mL 1 mM benzyl glucosinolate standard solution and 0.1 mL of 0.6 M barium-lead acetate, cap vial and mix.

  (**NOTE:** The order of addition of these reagents is critical. Water must not contact the meal in the absence of methanol. The addition of benzyl glucosinolate is a critical measurement. Be accurate.)

- Shake vials in an Eberbach shaker (200 strokes per minutes) for 1 hr.
- Centrifuge at 2000 g for 10 min.

9.2 Sulfatase Hydrolysis:

- Transfer approximately 1.5 mL of the supernatant to a 0.2 mL DEAE Sephadex A25 column.
- Wash column with 1.8 mL 67% methanol, 1.8 mL water and 1 mL 0.02 M pyridine-acetate.
- Add 0.05 mL of purified sulfatase to the column, cap and let stand overnight at room temperature.
- Elute column twice with 0.45 mL 60% methanol into a 1 mL auto-sampler vial or 4 mL screw-cap vial.
- Regenerate the column after use by washing with 1 mL 1M sodium acetate, 1 mL 0.5 M sodium hydroxide and 1 mL 0.5 M

hydrochloric acid followed by two washings of 1.8 mL water.

9.3 Derivatization:

- Place the vial in a dry block heater at 60 °C and take to dryness by passing over the sample a stream of air, removing the vials when they are dry.
- Immediately before addition premix acetone, DMF, BSA (or BSTFA), TMCS and 1-methyl-imidazole (40:20:20:2:1).
- Add 40 µL of the derivatization mixture to the vial, cap immediately with a teflon lined cap and let stand for 15 minutes at room temperature.

9.4 Chromatography:

9.4.1 Manual

- Inject approximately 2-3 µL of the derivatized sample onto the capillary column.

9.4.2 Autoinjection

- Adjust the flush time of the autosampler to approximately 20 µL of sample before injection.
- Set the autosampler to inject approximately 2-3 µL.

(**NOTE**: If the autosampler is to be turned off for an extended period flush with one vial of DMF and then one vial of ethanol after the last sample, and when restarting the autosampler flush with one vial of DMF to remove the ethanol and ensure that the autosampler is working properly.)

9.4.3 Chromatographic conditions:

9.4.3.1 Packed column

Carrier gas (Helium) (mL.minute) ....optimized
Air(mL/minute)............................optimized
Hydrogen (mL/minute).......................optimized
Detector range.................................100
Detector attenuation...........................64
Injector temperature (°C).......................300
Detector temperature (°C).......................300
Initial column temperature (°C).................200
Initial time (minute)............................ 0
Program rate (°C/minute).........................10
Final temperature (°C)..........................280
Final time (minute)...............................4

Approximate retenion times (minute)
Allyl (mustard or stinkweed)....................4.0
Butenyl...4.3
Pentenyl..4.7
Hydroxy-butenyl.................................5.1
Hydroxy-pentenyl................................5.4
Benzyl(internal standard).......................6.0
Hydroly-benzyl (mustard or wild mustard).........6.8
Indolyl...9.4
4-hydroxy-indolyl...............................9.9

9.4.3.2 Capillary column

Carrier gas (Helium) (mL/minute)...........optimized
Air(mL/minute)............................optimized
Hydrogen(mL/minute).......................optimized
Detector range....................................1
Detector attenuation.............................64
Injector temperature (°C)........................300
Detector temperature (°C)........................300
Initial column temperature (°C)..................245
Initial time (minute).............................1
Program rate (°C/minute).........................13
Final temperature (°C)...........................281
Final time (minute)...............................2

Approximate retenion times (minute)
Allyl (mustard or stinkweed)....................1.4
Butenyl...1.6
Pentenyl..1.8
Hydroxy-butenyl.................................2.0
Hydroxy-pentenyl................................2.2
Benzyl (internal standard)......................2.6
Hydroly-benzyl (mustard or wild mustard).........3.8
Indolyl...5.0
4-hydroxy-indolyl...............................5.5

10. CALCULATION AND REPORTING OF RESULTS

Area glucosinolate/area benzyl glucosinolate standard
***response factor * 0.86 * 1000/100 =µmoles/g oil-extracted meal**

where:
- The **response factor** is the ratio of the carbon number of trimethylsilyl derivative of the glucosinolate to the carbon number of the trimethyl- silyl derivative of the internal standard (benzyl glucosinolate) 0.2 is the µmoles myo-inositol added to the sample.
- The factor **0.86** adjusts for the response of benzyl glucosinolate relative to allyl glucosinolate.
- The factor **1000/100** converts to a gram basis (1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

(**NOTE**: The relative response of benzyl glucosinolate to allyl glucosinolate

may vary from day to day. The relative response factor (i.e. 0.86) may be checked by injecting a calibration mixture of equimolar amounts of allyl and benzyl glucosinolate.)

Glucosinolate	TMS carbon number	Response factors
Allyl	25	1.16
3-butenyl	26	1.12
4-pentenyl	27	1.07
2-hydroxy-3-butenyl	29	1.00
2-hydroxy-4-pentenyl	30	0.97
Benzyl	29	1.00
Hydroybenzyl	32	0.91
3-indolylmethyl	31	0.94
4-hydroxy-indolylmethyl	34	0.85

11. REFERENCES

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_______. 1980. Analysis of glucosinolates via "on column" desulfation. Analytical Chemistry of Rapeseed and Its Products. Ed. by J. Daun, D.I. McGregor, and E.E. McGregor. The Canola Council of Canada, Winnipeg.

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DETERMINATION OF GLUCOSINOLATE CONTENT BY GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF GLUCOSE

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1. APPLICATION

The method is intended for determining the glucosinolate composition and content (1 to 200 µmoles per gram oil-extracted meal) of seed or meal samples of rapeseed/canola (*Brassica campestris* L. and *B. napus* L.). It may also be applied to the analysis of seed or meal samples of brown or Oriental (yellow seeded) mustard, *B. juncea* (L.). Coss., and white mustard, *Sinapsis alba* L.

2. PRINCIPLE

Endogenous enzymes, including myrosinase, are inactivated and glucosinolates extracted using boiling water. The glucosinolates are purified on an ion-exchange column then hydrolyzed to release glucose with the addition of myrosinase to the column. The glucose released is derivatized to form volatile trimethylsilyl (TMS) ethers which are separated and quantified, using myo-inositol as internal standard, by gas liquid chromatography.

3. BACKGROUND

Gas chromatography of trimethylsilyl derivatives of desulfated glucosinolates is one of the most useful approaches to date for determining the glucosinolate content of rapeseed (McGregor et al. 1983). In addition to determining glucosinolate content it also provides information on glucosinolate composition as almost all of the glucosinolates known to be present in rapeseed are well resolved. However, as individual glucosinolates are measured separately it suffers from possible accumulative errors resulting from the summing of the individual peaks.

Gas chromatography of trimethylsilyl derivatives of myrosinase released glucose avoids this problem. The method was originally proposed by Olsson and Theander (Olsson and Theander 1976) and was thought to be particularly suited to the determination of the glucosinolate content of low glucosinolate rapeseed, rapeseed protein concentrates, and derived foods and feeds. The method has high sensitivity as only two peaks, α-D-glucose and ß-D-glucose are summed to obtain total gluco-sinolate content. Quantification is facilitated by the use of myo-inositol as internal standard. Relative to trimethylsilyl derivatives of de-sulfated glucosinolates, peaks for trimethylsilyl derivatives of glucose and myo-inositol have short retention times, but are well resolved. Background levels of glucose, from contamination of the myrosinase, may be avoided by dialyzing the myrosinase before use or corrected with appropriate controls.

4. ADVANTAGES/DISADVANTAGES

Gas chromatography of trimethylsilyl derivatives of glucose is a particularly sensitive method for determining glucosinolate content in low glucosinolate samples. Glucosinolate extraction and ion-exchange column purification steps lend themselves to batch type processing of samples and, along with short retention times of the chromatographic peaks, increase throughout (number of samples per day).

5. EQUIPMENT

Standard laboratory apparatus and in particular:

5.1 Culture tubes, 125 x 15 mm with PTFE-lined screw-cap:

Fisher Scientific Ltd.
Cat. No. 14-930-10E

5.2 Water bath, 100 °C (boiling).

5.3 Vortex mixer.

5.4 Centrifuge, bench top.

5.5 Disposable plastic syringes, 1 mL:

Fisher Scientific Ltd.
Cat. No. 14-823-226

5.6 Porous polyethylene sheeting, (Bel-Art) 1.6 mm 35 um pore size:

Mandel Scientific Co. Ltd.
Cat. No. F13638-5116

5.7 Cork bore, #1.

5.8 Oxford pipette tips, 1 mL:

Fisher Scientific Ltd.
Cat. No. 21-240-20

5.9 Disposable syringe needles, 20 guage, 38 mm long:

Fisher Scientific Ltd.
Cat. No. 14-826-5C

5.10 Holder for ion-exchange columns and corresponding holder for 4 mL vials.

5.11 Vials, teflon-lined screw-cap, 4 mL:

Canlab Ltd.
Cat. No. V3020-1 (vial)
Chromatographic Specialties Ltd.
Cat. No. C669112 (teflon liner)

5.12 Dry block heater, to accommodate 4 mL screw-cap vials, Reacti-Therm:

Chromatographic Specialties Ltd.
Cat. No. P18800

5.13 Compressed air and air maifold, Reacti-Vap Evaporator:

Chromatographic Specialties Ltd.
Cat. No. P18782

5.14 Hamilton syringes, 10 μL, 50 μL, 250 μL:

Chromatographic Specialties Ltd.
Cat. No. H80366 (10 μL) (fixed needle)
Cat. No. H80900 (50 μL) (fixed needle)
Cat. No. H81130 (250 μL) (Leur Lock removable needle)

5.15 Forced-air oven.

5.16 Gas chromatograph with flame ionozation detector:

Equipped with a 0.2 mm ID by 25 m fused silica capillary column coated cross-linked methyl-silicone 0.5 um thick.
Hewlett-Packard
Cat. No. 19091A 002
556-2-10A

6. REAGENTS

6.1 Acetic acid, glacial:

Fisher Scientific Ltd.
Cat. No. A38-500

6.2 Myrosinase:

Prepared from yellow mustard *Sinapsis alb*a L.) seed.
or
Biocatalysts Ltd.
or
Boehringer Mannheim Ltd.
Cat. No. 1088 769

6.3 N-Methyl-N-trimethyl-silylheptafluor (o) butyramide (MHSFBA):

Macherey-Nagel GmbH and Co.
Cat. No. 70126

6.4 Myo-inositol:

Sigma Chemical Co.
Cat. No. I-5125

6.5 Parafilm:

Fisher Scientific Ltd.
Cat. No. 13-374-5

6.6 Pyridine (silylation grade):

Chromatographic Specialties Ltd.
Cat. No. 27530

6.7 Sephadex DEAE A25:

Anion exchanger
40-120u bead size

Sigma Chemical Co.
Cat. No. A-25-120

6.8 Sodium hydroxide:

Fisher Scientific Ltd.
Cat. No. S318-100

6.9 Trimethylchlorosilane (TMCS):

Chromatographic Specialties Ltd.
Cat. No. 88530

7. SUPPLIERS

7.1 Biocatalysts Ltd.:

Main Avenue, Treforest Industrial Estate, Pontypridd, Wales CF37 5YT
Telephone: 044385 3712
Telex: 497126 BIOCAT G

7.2 Boehringer Mannheim Ltd.

11450 Cote de Liesse, Dorval, PQ
CANADA H9P 1A9
Telephone: (514) 636-6760
Telex: 05-8222677

7.3 Canlab Ltd.

11620 181 St., Edmonton, AB
CANADA T5S 1M6
Telephone: (403) 453-3921

7.4 Chromatographic Specialties Ltd.

P.O. Bag 1150, 300 Laurier Blvd.
Brockville, ON, CANADA K6V 5W1
Telephone: (613) 342-4678

7.5 Fisher Scientific Ltd.

P.O. Box 3840 Station D
Edmonton, AB, CANADA T5L 4K2
Telephone: (403) 483-2123

7.6 Macherey-Nagel GmbH and Co.

Postfach 307, 5160 Duren
WEST GERMANY

7.7 Mandel Scientific Co. Ltd.

9840-47th Ave., Unit #2
Edmonton, AB, CANADA T6E 5P3
Telephone: (403) 436-0665

7.8 Sigma Chemical Co.

P.O. Box 14508, St. Lois, MO
USA 63178
Telephone: (800) 325-8070
(314) 771-5750
Telefax: (800) 325-5052
(314) 771-5757

8. PREPARATION

8.1 DEAE Sephadex A-25:

- Weigh 10 g DEAE Sephadex A-25 into a beaker.
- Add 150 mL water and allow the Sephadex to swell overnight.
- Slurry onto a 20 x 400 mm column.
- Pass 500 mL 0.5 N sodium hydroxide (10 g dissolved in water and made up to 500 mL) through the column.
- Wash the column with 250 mL water to remove excess sodium hydroxide checking to ensure the pH has dropped to neutrality.
- Pass 400 mL 0.5 M pyridine-acetate (19.8 mL pyridine and 15 mL glacial acetic acid made up to 500 mL with water) through the column.
- Wash with 250 mL water.
- Slurry into a flask for storage.

8.2 DEAE Sephadex column, 0.2 mL:

- Cut a 1 mL plastic syringe at the 0.2 mL mark.
- Insert a disk of porous polyethylene cut from a sheet with a #1 cork bore into the shorter bottom piece of the syringe.
- Fill this part of the syringe ith DEAE Sephadex A-25, preswollen in water overnight.
- Cut a 1 mL Oxford pipette tip at the collar and insert the larger piece over the syringe tip filled with DEAE Sephadex.
- Place a 38 mm, 20 guage needle on the Luer lock tip of the syringe.

8.3 Myo-inositol, 1 mM:

- Weigh 45.1 mg into a 250 mL volumetric flask and make to volume with water.

9. PROCEDURE

9.1 Glucosinolate Extraction

- Weigh 100 mg of oil-extracted meal into a 125 x 15 mm culture tube.
- Place the tube in a boiling water bath for 1 minute.
- Add 2 mL of hot (<90 °C) water to the tube and, without allowing the contents to cool, mix to ensure that the meal is thoroughly wetted and continue heating for 3 min.
- Centrifuge at 2000 g for 10 min. and transfer the supernatant to a 5 mL volumetic flask.
- Wash the pellet twice with 1.5 mL of water, pool the supernatants, and make to volume with water.

9.2 Myrosinase Hydrolysis:

- Add 1 mL of the glucosinolate extract to a 0.2 mL DEAE Sephadex A25 column.
- Wash the column twice with 1 mL water discarding the washes.
- Transfer the column to a 4 mL vial covered with Parafilm to prevent evaporation.
- Add 0.1 mL of freshly prepared solution of myrosinase (3 mg/mL in water) collecting the column eleuate.
- Cap the column and let stand overnight at room temperature.
- Elute the column with 1 mL water pooling the eluate with the eluate collected upon addition of the myrosinase.

NOTE: It may be necessary to determine and correct for background glucose in the myrosinase preparation by setting up at least one additional column to which myrosinase is added, allowed to stand overnight and eluted as above.)

- Add 0.2 mL myo-inositol standard to the sample eluate and 0.05 mL myo-inositol standard and 0.15 mL water to the myrosinase background eluate.

9.3 Derivatization:

- Place the vial in a dry block heater at 60 °C and take to dryness by passing over the sample a stream of air, removing the vials when they are dry.
- Add in order, 100 μL pyridine, 100μL MSHFBA and 10 μL TMCS and immediately cap the vial.
- Heat at 120 °C for 20 minutes.

NOTE: It is essential that all reagents be dry. It is advisable to add the reagents to one sample at a time to minimize exposure to moisture in the atmosphere.

9.4 Chromatography:

- Inject approximately 1 μL of the derivatized sample onto the capillary column.

Carriergas(Helium)(mL/minute)..optimized
Air (mL/minute)................optimized
Hydrogen (mL/minute)...........optimized
Detector range.........................1
Detector attenuation..................16
Injector temperature(°C)..............220
Detector temperature(°C)..............300
Initial column temperature (°C).......180
Initial time (minute)..................4
Program rate(°C/minute)...............10
Final temperature(°C).................280
Final time (minute)....................4

Approximate retenion times (minute)
α-D-glucose..........................3.9
ß-D-glucose..........................5.1
Myo-inositol.........................6.5

10. CALCULATION AND REPORTING OF RESULTS

Area α-D-glucose + area ß-D-glucose/ area myo-inositol
*** 1.14285 * 0.2 * 5.0/1.0 * 1000/100**
= μmoles/g oil-extracted meal

where:

-**1.14285** is the ratio of the carbon number of the trimethylsilyl derivative of myo-inositol (24) to the carbon number of the trimethylsilyl derivative of glucose (21).
-**0.2** is the μmoles myo-inositol added to the sample.
-The factor **5.0/1.0** adjusts for the amount (1.0 mL) of the glucosinolate extract (5.0mL) placed on the ion-exchange column.
-The factor **1000/100** converts to a gram basis(1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

11. REFERENCES

MCGREGOR, D.I., W.J. MULLIN AND G.R. FENWICK. 1983. Review of analysis of glucosinolates. Analytical methodology for determining glucosinolate composition and content. J.Assoc. Off. Anal. Chem. 66: 825-849.

OLSSON, K., O. THEANDER AND P. AMAN. 1980. Determination of total glucosinolate content in rapeseed and turnip rapeseed meals by gas chromatography. Sewdish J. Agric. Res. 6:225-229.

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DETERMINATION OF TOTAL GLUCOSINOLATE AND TOTAL INDOLE GLUCOSINOLATE CONTENT OF RAPESEED/CANOLA USING GLUCOSE OXIDASE TO MEASURE GLUCOSE AND FERRIC NITRATE TO MEASURE FREE THIOCYANATE ION

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1. APPLICATION

The method is intended for determining the glucosinolate content of seed or meal samples of canola or near canola quality (1 to 70 µmoles per gram oil-extracted meal) rapeseed (*Brassica campestris* L. and *B. napus L.*), although it may be applied to the entire range (1 to 200 µmoles per gram oil-extracted meal) of rapeseed glucosinolate content. It may also be applied to the analysis of seed or meal samples of brown or Oriental (yellow seeded) mustard, (*B. juncea* L.) Coss., and white mustard, (*Sinapsis alba* L.).

2. PRINCIPLE

Glucosinolates are extracted from oil-extracted meal using boiling water and purified by binding to a DEAE Sephadex A25 column. Myrosinase is added to the column to hydrolyze the glucosinolates releasing isothiocyanates and sulfate which remain bound to the column, and glucose which is eluted with water.

Total glucosinolate content is determined by converting glucose to gluconic acid and hydrogen peroxide in the presence of the glucose-specific enzyme glucose oxidase. The hydrogen peroxide is reacted with phenol and 4-aminoantipyrene (4-aminophenazone) in the presence of peroxidase to produce a red color complex which is measured. A glucose standard curve is used to quantify the results.

Total indole glucosinolate content is determined by adding sodium hydroxide to the column to convert the unstable indole isothiocyanates to inorganic thiocyanate ion. The inorganic thiocyanate ion, eluted with the sodium hydroxide and a nitric acid wash, is reacted with ferric chloride to form a red color complex which is measured. An ammonium thiocyanate standard curve is used to quantify the results.

Total non-indole (aliphatic) glucosinolate content may be obtained by subtracting the total indole glucosinolate content from the total glucosinolate content.

Analysis for total glucosinolate and total indole glucosinolate content is facilitated by measuring absorbances with a microtiter plate reader, capturing the data with a computer, and transferring it directly to a spreadsheet program for calculation of micromolar amounts.

3. BACKGROUND

Accuracy, precision, speed, simplicity and low cost are desirable attributes of any analytical method particularly when there is a need for large numbers of analyses, such as in breeding programs or in quality control associated with production, transportation and marketing. Over the last four decades many methods have been proposed or used to determine glucosinolate content. In part this proliferation has been due to deficiencies in one or other of the above attributes. Generally methods of analysis can be divided into two classes: those which are the state of the art with respect to accuracy and precision and those which sacrifice some accuracy or precision for increased speed and simplicity. The latter methods are frequently referred to as "screening" methods since they are often used in combination with the former to reduce the number of samples which must be analyzed for accurate and

precision measurements by more tedious and time consuming means.

To date one of the most popular analytical approaches to screen for glucosinolate content has involved myrosinase hydrolysis to release glucose and colorimetric measurement of the glucose utilizing glucose oxidase, peroxidase and a chromogen (Lein and Schon 1969, Lein 1970, McGregor et al. 1983, Daun and McGregor in press). In plant breeding programs the so-called "hammer test", in which a few seeds are crushed with a hammer, water added to allow endogenous myrosinase to hydrolyze the glucosinolates and the glucose measured with a test paper containing the glucose oxidase, peroxidase and a chromogen (Comer 1956), was exten-sively used in the development of canola cultivars. In the market place the so-called "mortar test", in which a small mortar was used to analyze a larger sample of seed and powdered carbon added to adsorb inhibitors thus enhancing the color development (McGregore and Downey 1975), was extensively used to identify and segregate canola seed during the conversion in Canada to commercial production of canola cultivars. More recently efforts have been made to improve on the glucose oxidase approach to glucosinolate determination. Noteable attempts include the use of automation to increase throughput (Smith et al. 1985), the use of a reflectometer to estimate the color development and thereby improve precision (Thies 1985, Robbelen 1987), and the varying reagent to improve both accuracy and precision (Saini and Wratten 1987, Smith and Dacombe 1987).

Recently evidence has been published which indicates that rapeseed contains enzymes other than myrosinase which release glucose from non-glucosinolate sources if samples are autolysed (Smith and Donald 1988). Studies at Agriculture Canada, Saskatoon have shown that this non-glucosinolate glucose can amount to as much as 6 to 8 micromoles per gram oil-extracted air-dried meal. By inactivating endogenous enzymes including myrosinase before glucosinolate extraction, substantial over estimate of glucosinolate content, particularly with low glucosinolate samples, is avoided.

Rape and mustard seed or meal contain inhibitors of peroxidase which if present can reduce the sensitivity and precision of analyses performed using glucose oxidase and peroxidase. Binding the glucosinolates extracted from seed or meal to an ion exchange column removes these inhibitors.

Sound rapeseed also contains about 6 to 10 micromoles of free glucose while poor quality rapeseed may contain even greater amounts of free glucose. By binding the glucosinolates to an ion exchange column free glucose is removed. Overnight hydrolysis with exogenous myrosinase also allows sufficient time for the ß-D-glucose released from the glucosinolates to mutarotate to equilibrium between the α- and ß-D-glucose forms.

Although most of these isothiocyantes are stable and when treated with base will from thioureas or cyclize to form oxazolidinethiones, noteable exceptions are the indole gluco-sinolates and 4-hydroxybenzyl gluco-sinolate which hydrolyze further to from free thiocyanate ion. Analysis of free thiocyante ion has been used to determine both indole glucosinolates (Johnston and Jones 1966) and 4-hydroxybenzyl glucosinolate (Josefsson 1968). Isothiocyanates released by myrosinase hydrolysis bind to DEAE Sephadex A25. By binding the isothiocyantes to the DEAE Sephadex A25 column, then adding base to release the thiocyante ion indole glucosinolates (or 4-hydroxybenzyl glucosinolate) can be determined separately. When combined with the measurement of glucose to determine total glucosinolate content, non-indole glucosinolates to be determined by difference.

4. ADVANTAGES/DISADVANTAGES

Separation on an ion exchange column of the glucose released by myrosinase hydrolysis from the inorganic thiocyante ion released by subsequent hydrolysis with base of indole isothiocyanates allows for simultaneous determination of total glucosinolate, total indole glucosinolate and, by difference, total non-indole glucosinolate content. Purification and myrosinase hydrolysis of glucosinolate extracts on ion exchange columns followed by colorimetric analysis of glucose using glucose oxidase and peroxidase and of thiocyante ion using ferric nitrate lends itself to batch type operation and therefore high throughput (numbers of samples per day). The method is highly accurate since calibration is easily performed with glucose and inorganic thiocyanate. The method also has high precision

since only one measurement is made, glucose or thiocyanate ion, to determine total content. Both glucose oxidase/ peroxidase and ferric nitrate are sensitive colorimetric analyses.

5. EQUIPMENT

Standard laboratory apparatus and in particular:

5.1 Chromatography column, 20x400 mm.

5.2 Culture tubes, 125 x 15 mm with PTFE-lined screw-cap:

Fisher Scientific Ltd.
Cat. No. 14-930-10E

5.3 Water bath, boiling.

5.4 Vortex mixer.

5.5 Centrifuge, bench top.

5.6 Disposable plastic syringes, 1 mL:

Fisher Scientific Ltd.
Cat. No. 14-823-226

5.7 Porous polyethylene sheeting, (Bel-Art) 1.6 mm 35 um pore size:

Mandel Scientific Co. Ltd.
Cat. No. F13638-5116

5.8 Cork bore, #1.

5.9 Oxford pipette tips, 1 mL:

Fisher Scientific Ltd.
Cat. No. 21-240-20

5.10 Disposable syringe needles, 20 guage, 38 mm long:

Fisher Scientific Ltd.
Cat. No. 14-826-5C

5.11 Vials, 4 mL:

Canlab Ltd.
Cat. No. V3020-1

5.12 Oxford pipette, 50, 100 and 200 μL with 1-200 μL tips:

Fisher Scientific Ltd.
Cat. No. 21-218
Cat. No. 21-240-10 (1-200 μL)

5.13 Eppendorf repeater pipette with 2.5 and 50 mL tips:

Fisher Scientific Ltd.
Cat. No. 21-380-8
Cat. No. 21-380-8B (2.5 mL)
CAT. No. 21-381-102 (50 mL)

5.14 Microtiter plates, flat-bottom:

Fisher Scientific Ltd.
Cat. No. 08-772-9

5.15 Microtiter plates, (EIA) reader, Model 2550:

Bio-Rad Laboratories (Canada) Ltd.

5.16 Apple Macintosh computer, Macintoch Plus or SE.

5.17 Macintoch/Model 2550 EIA reader interface cable:

Bio-Rad Laboratories (Canada) Ltd.
Cat. No. 170-6597

5.18 Microplate manager software:

Bio-Rad Laboratories (Canada) Ltd.
Cat. No. 170-6607

5.19 Microsoft Excel spreadsheet software:

Microsoft (Canada) Ltd.

6. REAGENTS

Standard laboratory reagents and in particular:

6.1 Acetic acid, glacial:

Fisher Scientific Ltd.
Cat. No. A38-500

6.2 4-Aminoantipyrene (4-amino-phenazone):

Sigma Chemcial Co.
Cat. No. A-4382

6.3 Ammonium thiocyanate standard solution (N/10):

BDH Chemicals Ltd.
Cat. No. R01007

6.4 Barium acetate:

Fisher Scientific Ltd.
Cat. No. B24-500

6.5 Ferric nitrate:

Fisher Scientific Ltd.
Cat. No. I110-500

6.6 ß-D-Glucose:

Sigma Chemical Co.
Cat. No. G-5250

6.7 Glucose oxidase:
Sigma Chemical Co.
Cat. No. G-7141

6.8 Imidazole:

Fisher Scientific Ltd.
Cat. No. 03196-100

6.9 Lead acetate:

Fisher Scientific Ltd.
Cat. No. L33-500

6.10 Mercuric chloride:

Fisher Scientific Ltd.
Cat. No. M155I-100

6.11 Myrosinase:

Prepared from yellow mustard (*Sinapsis alba* L.) seed.
or Biocatalysts Ltd.
or Boehringer Mannheim Ltd.
Cat. No. 1088 769

6.12 Nitric acid, 61.4%, 13.3 moles/L:

Fisher Scientific Ltd.
Cat. No. A202-500

6.13 Parafilm:

Fisher Scientific Ltd.
Cat. No. 13-374-5

6.14 Peroxidase:

Sigma Chemcial Co.
Cat. No. P-8250

6.15 Phenol:

Fisher Scientific Ltd.
Cat. No. A92-500

6.16 Pyridine:

Fisher Scientific Ltd.
Cat. No. P368-500

6.17 Sephadex, DEAE A-25:

Sigma Chemical Co.
Cat. No. A-25-120

6.18 Sodium azide:

Fisher Scientific Ltd.
Cat. No. S227I-100

6.19 Sodium hydroxide:

Fisher Scientific Ltd.
Cat. No. S318-100

7. SUPPLIERS

7.1 BDH Chemicals Ltd.

501-45th Street West Saskatoon, SK
CANADA S7L 5Z9
Telephone: (306) 424-3414

7.2 Biocatalysts Ltd.

Main Avenue, Treforest Industrial Estate, Pontypridd, Wales CF37 5YT
Telephone: 044385 3712
Telex: 497126 BIOCAT G

7.3 Bio-Rad Laboratories (Canada) Ltd.

3140 Universal Drive, Mississauga, ON, CANADA L4X 2C8
Telephone: (416) 624-0713
Telex: 06-961 233

7.4 Boehringer Mannheim Ltd.

11450 Cote de Liesse, Dorval, PQ
CANADA H9P 1A9
Telephone: (514) 636-6760
Telex: 05-8222677

7.5 Canlab Ltd.

11620 181 St., Edmonton, AB
CANADA T5S 1M6
Telephone: (403) 453-3921

7.6 Fisher Scientific Ltd.

P.O. Box 3840 Station D, Edmonton, AB, CANADA T5L 4K2
Telephone: (403) 483-2123

7.7 Mandel Scientific Co. Ltd.

9840-47th Ave., Unit #2, Edmoton, AB
CANADA T6E 5P3
Telephone: (403) 436-0665

7.8 Microsoft (Canada) Ltd.

6300 Northwest Drive, Mississauga, ON, CANADA L4V 1J7

7.9 Sigma Chemical Co.

P.O. Box 14508, St. Lois, MO
USA 63178
Telephone: (314) 771-5750
Telefax: (314) 771-5757

8. PREPARATION

8.1 Barium acetate 0.6 M/lead acetate 0.6 M (1:1 v/v):

- Dissolve 3.07 g barium acetate ($Ba(C_2H_3O_2)_2$ in water and make to 100 mL with water.
- Dissolve 4.55 g lead acetate Pb $C_2H_3O_2).3H_2O$ in water and make to 100 mL with water.
- Combine equal volumes of the barium and lead acetate solutions.

8.2 DEAE Sephadex A-25:

- Weigh 10 g DEAE Sephadex A-25 into a beaker.
- Add 150 mL water and allow the Sephadex to swell overnight.
- Slurry onto a 20 x 400 mm column.
- Pass 500 mL 0.5 N sodium hydroxide (10 g dissolved in water and made up to 500 mL) through the column.
- Wash the column with 250 mL water to remove excess sodium hydroxide checking to ensure the pH has dropped to utrality.
- Pass 400 mL 0.5 M pyridine-acetate (19.8 mL pyridine and 15 mL glacial acetic acid made up to 500 mL with water) through the column.
- Wash with 250 mL water.
- Slurry into a flask for storage.

8.3 DEAE Sephadex column, 0.2 mL:

- Cut a 1 mL plastic syringe at the 0.2 mL mark.
- Insert a disk of porous polyethylene cut from a sheet with a #1 cork bore into the shorter bottom piece of the syringe.
- Fill this part of the syringe with DEAE Sephadex A-25, preswollen in water overnight.
- Cut a 1 mL Oxford pipette tip at the collar and insert the large piece over the syringe tip filled with DEAE Sephadex.
- Place a 38 mm, 20 guage needle on the Luer lock tip of the syringe.

8.4 Acetic acid, 4 M:

- Dilute 23 mL glacial acetic acid to 100 mL with water.

8.5 Sodium hydroxide, 0.5 N:

- Dissolved g sodium hydroxide in water and make up to 500 mL.

8.6 Imidazole buffer:

- Into a 1 L volumetric flask weigh:
  Imidazole, 9.82 g,
  Sodium azide, 0.29 g,
  Glacial acetic acid, 4.45 g (4.24 mL).
- Make to volume with water.

(Colour reagent 1 stable up to 14 days when stored at 4°C).

- Into a 25 mL volumetric flask weigh:
  Glucose oxidase, 14.4 mg,
  4-Aminoantipyrene, 28.8 mg.
- Make to volume with buffer.

(Colour reagent 2 stable up to 14 days when stored at 4°C)

- Into a 100 mL volumetric flask weigh:
  Peroxidase 2.2 mg
  Phenol, 144.3 mg.
- Make to volume with buffer.
  Myrosinase-glucose oxidae-peroxidae reagent
- Mix together:
  Colour reagent 1, 5 mL
  Colour reagent 2, 5 mL

8.7 Nitric acid, 0.5 N:

- Transfer 9.25 mL of concentrated (61.4%, 13.3 moles/L) nitric acid to a 250 mL volumetric flask and make to volume with water.

8.8 Nitric acid, 1.5 N:

- Transfer 28.2 mL of concentrated (61.4%, 13.3 moles/L) nitric acid to a 250 mL volumetric flask and make to volume with water.

8.9 Ferric nitrate reagent (prepared fresh daily):

- Weigh 6 g ferric nitrate into a small beaker.
- Add 5 mL 1.5 N nitric acid to dissolve.
- Transfer to a 25 mL volumetric flask and make to volume with 1.5 N nitric acid.

8.10 Mercuric chloride 6%:

- Weigh 1.5 g into a 25 mL volumetric flask and make to volume with 1.5 N nitric acid.

8.11 Ferric nitrate with mercuric chloride:

- Add 30 μL of 6% mercuric chloride in nitric acid to 2 mL ferric nitrate reagent.

9. PROCEDURE

9.1 Glucosinolate Extraction:

- Weigh 100 mg of oil-extracted meal into a 125 x 15 mm culture tube.
- Place the tube in a boiling water bath for 1 minutes.
- Add 1.5 mL of hot (<90 °C) water to the tube and, without allowing the contents to cool, mix to ensure that the meal is thoroughly wetted and continue heating for 3 minutes.
- Add 0.5 mL of a 0.12 M barium acetate lead acetate solution and mix.
- Centrifuge at 2000 g for 10 minutes and transfer the supernatant to a 5 mL volumetric flask.
- Wash the pellet twice with 1.5 mL of water, pool the supernatants, and make to volume with water.

9.2 Myrosinase Hydrolysis:

- Add 2.5 mL of the 5 mL extract to a 0.2 mL DEAE Sephadex column.
- Wash twice with 0.5 mL 4 M acetic acid and twice with 0.1 mL water.
- Wash twice with 1.0 mL water.

To correct for free glucose in the myrosinase preparation, if any, also set up at least one blank column:
- Wash twice with 0.5 mL 4 M acetic acid and twice with 0.1 mL water.
- Wash twice with 1.0 mL water.

To hydrolyse with myrosinase and collect the glucose released:
- Transfer the columns to a clean 4 mL vial covered with Parafilm to prevent evaporation.
- Add 0.1 mL of a freshly prepared myrosinase solution (3 mg/mL).
- Cover top of column to prevent drying out and let stand overnight.
- Elute with 1.0 mL of water.
- For samples with 30 to 200 μmoles glucosinolate/g oil-extracted meal make a 10 fold dilution of the eluate.

To release the inorganic thiocyanate ion:
- Transfer the column to a 4 mL clean vial covered with Parafilm to prevent evaporation.
- Add 1.0 mL 0.5 N sodium hydroxide to the column and let stand 15 minutes.
- Add 1.0 mL 1 N nitric acid and pool eluate.

9.3 Measurement of glucose and thiocyanate Ion:

9.3.1 <u>Spectrophotometer</u>:

9.3.1.1 <u>Measurement of Glucose</u>:

Unknown samples
- Add 0.1 mL of the eluate from the myrosinase hydrolysis to a 4 mL vial.
- Add 0.3 mL water.
- Add 0.2 mL glucose oxidase-peroxidase reagent.
- Mix and let stand 1 hour.
- Transfer to a 1 mL cuvette and record the absorbance at 505 nm.

Background samples
- Add 0.1 mL of the blank column eluate to a 4 mL vial
- Add 0.3 mL water
- Add 0.2 mL glucose-oxidase-peroxidase reagent
- Mix and let stand for 1 hour

Standards
- To each of 5 vials add 0.1 mL of a 1.5 μmole/mL solution of glucose, and half serial dilutions (0.5, 0.25, 0.125 and 0.0625 μmole/mL, respectively).
- Add 0.3 mL water.
- Add 0.2 mL glucose oxidase-peroxidase reagent.
- Mix and let stand 1 hour.
- Transfer to a 1 mL cuvette and record the absorbance at 505 nm.

9.3.1.2 <u>Measurement of thiocyanate ion</u>:

Unknown samples
- Add 0.1 mL of the sodium hydroxide eluate to a 4 mL vial.
- Add 0.3 mL water.
- Add 0.2 mL ferric nitrate reagent.
- Mix and let stand in the dark at room temperature for 15 minutes (time carefully).
- Transfer to a 1 mL cuvette and record the absorbance at 470 nm.
- Add 10 μL of 6% mercuric chloride, mix and record absorbance at 470 nm.

Standards
- To each of 5 vials add 0.1 mL of a 1 μmole/mL solution of 1 μmole/mL solution of ammonium thiocyanate, and half serial dilutions (0.5, 0.25, 0.125 and 0.0625 μmole/mL, respectively).
  . Add 0.3 mL water.
- Add 0.2 mL ferric nitrate reagent.
- Mix and let stand in the dark at room temperature for 15 minutes (time carefully).
- Transfer to a 1 mL cuvette and ecord the absorbance at 470 nm.
- Add 10 μL of 6% mercuric

chloride, mix and record absorbance at 470 nm.

9.3.2 Multiscan:

9.3.2.1 Measurement of Glucose:

Unknown samples

- Place unknown samples in plate wells A1 to H9.
- Add 0.1 mL of the eluate from the myrosinase hydrolysis to a microtiter plate well.
- Add 0.1 mL water to the plate well.
- Add 0.1 mL glucose oxidase-peroxidase reagent to the plate well.
- Let stand 1 hour.
- Read plate on the microtiter plate reader using the 492 nm filter.

Background samples

- Add 0.1 mL of the blank column eluate to plate wells A11, A12, H11 and H12.
- Add 0.1 mL water to the plate well.
- Add 0.1 mL glucose oxidase-peroxidase reagent to the plate well.
- Let stand 1 hour.
- Read plate on the microtiter plate reader using the 492 nm filter.

Standards

- Add 0.1 mL of a 1 μmole/mL solution of glucose to plate wells B11 and B12.
- Add 0.1 mL of a 1/2 serial dilution (0.5 μmole/mL glucose) to plate wells C11 and C12.
- Add 0.1 mL of a 1/2 serial dilution (0.25 μmole/mL glucose) to plate wells D11 and D12.
- Add 0.1 mL of a 1/2 serial dilution (0.125 μmole/mL glucose) to plate wells E11 and E12.
- Add 0.1 mL of a 1/2 serial dilution (0.0625 μmole/mL glucose) to plate wells F11 and F12.
- Add 0.1 mL of the background eluate from the blank column to the plate wells.
- Add 0.1 mL glucose oxidase peroxidase reagent to the plate well.
- Let stand 1 hour.
- Read plate on the microtiter plate reader using the 492 nm filter.

9.3.2.2 Measurement of Thiocyanate Ion:

Unknown samples

- Add 0.2 mL of the sodium hydroxide eluate to a microtiter plate well.
- Place unknown samples in plate wells A1 to D9.

For determination of background using mercuric chloride:

- Add duplicate 0.2 mL of the sodium hydroxide eluate to a microtiter plate well.
- Place duplicate samples in plate wells E1 to H9.

Background sample

- Add 0.2 mL background eluate from the blank column to plate wells A11, A12, H11 and H12.

Standards

- Add 0.2 mL of a freshly prepared 1 μmole/mL solution of ammonium thiocyanate to plate wells B11 and B12.
- Add 0.2 mL of a 1/2 serial dilution (0.5 μmole/mL ammonium thiocyanate) to plate wells C11 and C12.
- Add 0.2 mL of a 1/2 serial dilution (0.25 μmole/mL ammonium thiocyanate) to plate wells D11 and D12.
- Add 0.2 mL of a ½ serial dilution (0.124 μmole/mL ammonium thiocyanate) to plate wells E11 and E12.
- Add 0.2 mL of 1/2 serial dilution (0.0625 μmole/mL ammonium thiocyanate) to plate wells F11 and F12.
- Add 0.1 mL ferric nitrate reagent to plate wells A1 to D9, and A11 to F12.
- Add 0.1 mL ferric nitrate reagent containing mercuric chloride to plate wells E1 to H9, H11 and H12.
- Let stand in the dark at room temperature for 15 minutes.
- Read plate on the microtiter plate reader using 492 nm filter.

10. CALCULATION AND REPORTING OF RESULTS

10.1 Spectrophotometric:

10.1.1 Total Glucosinolate

For samples with 0 to 30 μmoles glucosinolate/g oil-extracted meal:

ΔA * 1/m * 1.1 * 5.0/2.5 * 1000/100 = μmoles total glucosinolate/g oil-extracted meal

where:
-**ΔA** is the absorbance of the unknown sample minus the absorbance of the background blank.
-**m** is the slope of a plot of the glucose standards (μmoles/mL) (x) versus their absorbance (y).
-The factor **1.1** adjusts for the dilution (1.1 mL) of the gucosinolate extract (5.0 mL) placed on the ion-exchange column.
-The factor **5.0/2.5** adjusts for the amount (2.5 mL) of the glucosinolate extract (5.0 mL) placed on the ion-exchange column.
-The factor **1000/100** converts to a gram basis (1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

For samples with 30 to 200 μmoles glucosinolate/g oil- extracted meal:

ΔA * 1/m * 1.1 * 5.0/2.5 * 1000/100 * 10 = μmoles total glucosinolate/g oil- extracted meal

where:
-The factor of **10** adjusts for the 10 fold dilution of the ion-exchange eluate.

10.1.2 <u>Total Indol Glucosinolate</u>:

For samples with 0 to 200 μmoles glucosinolate/g oil-extracted meal:

ΔA * 1/m * 2.0 * 5.0/2.5 * 1000/100 =μmoles total glucosinolate/g oil-extracted meal

where:
-**ΔA** is the absorbance of the unknown sample minus the absorbance of the background blank.
-**m** is the slope of a plot of the ammonium thiocyanate standards (μmoles/mL) (x) versus their absorbance (y).
-The factor **2.0** adjusts for the dilution (1.1 mL) of the eluate from the ion-exchange column.
-The factor **5.0/2.5** adjusts for the amount (2.5 mL) of the glucosinolate extract (5.0 mL) placed on the ion-exchange column.
-The factor **1000/100** converts to a gram basis (1000 mg) the weight of oil- extracted meal (100 mg) from which the glucosinolates were extracted.

10.1.3 <u>Total Non-Indol Glucosinolate</u>:

Total non-indole glucosinolate content may be estimated by subtracting the value for total indole glucosinolate content from the value for total glucosinolate content.

10.2 Multiscan:

10.2.1 <u>Total Glucosinolate</u>

Absorbance for the background blank is subtracted from the absorbance of the unknown sample and the μmole/mL equivalent determined from the glucose standards using the Microplate manager software to obtain an adjusted absorbance. Subsequent calculations, outlined below may be performed using Microsoft Excel software.

For samples with 0 to 30 μmoles glucosinolate/g oil-extracted meal:

Adjusted absorbance * 1.1/0.1 * 5.0/2.5 * 1000/100 = μmoles total glucosinolate/g oil-extracted meal

where:
-The **adjusted absorbance** is the absorbance of the unknown sample minus absorbance for the background blank adjusted according to the absorbances of the glucose standards using the Microplate manager software.
-The factor **1.1/0.1** adjusts for the portion (0.1 mL) of the total column eluate (1.1 mL) placed in the plate well.
-The factor **5.0/2.5** adjusts for the amount (2.5 mL) of the glucosinolate extract (5.0 mL) placed on the ion-exchange column.
-The factor **1000/100** converts to a gram basis (1000 mg) the weight of oil-extracted meal (100 mg) from which the gluco-sinolates were extracted.

For samples with 30 to 200 μmoles glucosinolate/g oil-extracted meal:

ΔA* 1/m * 11 * 5.0/2.5 * 1000/100 * 10 = μmoles total glucosinolate/g oil-extracted meal

where:
-The factor of **10** adjusts for the 10 fold dilution of the ion-exchange

column eluate.

10.2.2 Total Indole Glucosinolate

Absorbance for the sample with ferric nitrate/mercuric chloride reagent added is subtracted from the absorbance of the sample with just ferric nitrate reagent added and the µmole/mL equivalent determined from the thiocyanate standards using the Microplate manager software to obtain an adjusted absorbance. Subsequent calculations, outlined below may be performed using Microsoft Excel software.

For samples with 0 to 200 µmoles glucosinolate/g oil-extracted meal:

Adjusted absorbance * 2.0/0.2 * 5.0/2.5 * 1000/100 = µmoles total glucosinolate/g oil-extracted meal

where:
-The **adjusted absorbance** is the absorbance of the unknown sample minus absorbance for the background blank adjusted according to the absorbances of the glucose standards using the Microplate manager software.
-The factor **2.0/0.2** adjusts for the portion (0.2 mL) of the total column eluate (2.0 mL) placed in the plate well.
-The factor **5.0/2.5** adjusts for the amount (2.5 mL) of the glucosinolate extract (5.0 mL) placed on the ion-exchange column.
-The factor **1000/100** converts to gram basis (1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

10.2.3 Total Non-Indole Glucosinolate

Total non-indole glucosinolate content may be estimated by subtracting the value for total indole glucosinolate content from the value for total glucosinolate content.

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DETERMINATION OF TOTAL GLUCOSINOLATE CONTENT OF RAPESEED/CANOLA USING IMMOBILIZED MYROSINASE AND GLUCOSE OXIDASE

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1. APPLICATION

The method is intended for determining the glucosinolate content of seed or meal samples of canola or near canola quality (1 to 70 µmoles per gram oil-extracted meal) rapeseed (*Brassica campestris* L. and *B. napus* L.), although it may be applied to the entire range (1 to 200 µmoles per gram oil-extracted meal) of rapeseed glucosinolate content. It may also be applied to the analysis of seed or meal samples of Brown or Oriental (yellow seeded) mustard, *B. juncea* L. Coss., and white mustard, *Sinapsis alba* L.

2. PRINCIPLE

Endogenous enzymes including myrosinase are inactivated glucosinolates extracted from oil-extracted meal using hot (boiling) water. The extracted glucosinolates are hydrolyzed in a bioreactor containing immobilized myrosinase. The glucose released is converted to gluconic acid and hydrogen peroxide and the amount of hydrogen peroxide measured in a biosensor consisting of a membrane containing immobilized glucose oxidase (GOD) attached to the end of a hydrogen peroxide sensitive electrode.

3. BACKGROUND

Accuracy, precision, speed, simplicity and low cost are desirable attributes of any analytical method particularly when there is a need for large numbers of analyses such as in breeding programs or in quality control associated with production, transportation and marketing. Over the last four decades many methods have been proposed or used to determine glucosinolate content. In part this proliferation has been due to deficiencies in one or other of the above attributes. Breeders, nutritionists and industry have required a simple, low cost method which is sufficiently rapid to allow identification of low glucosinolate seed and meal in only a few minutes time. Such a method would not only be useful to identify and segregate canola seed and meal in commerce, but would be particularly useful to pay premiums or penalties based on glucosinolate content in countries where low glucosinolate winter type cultivars are currently being introduced into commercial production. Also required is a method which, in addition to being rapid, is sufficiently sensitive to be suitable for analyzing seed and meal with very low glucosinolate content (<10 micromoles glucosinolate expressed per gram oil-extracted air-dried meal). Other methods with sufficient sensitivity are either too slow or too expensive to facilitate screening of very large numbers of samples. The availability of a rapid, sensitive low cost method for determining glucosinolate content could facilitate conversion of commercial production to glucosinolate free cultivars expected in the near future. Identification and segregation of glucosinolate free seed and meal throughout the production, transpor-tation and marketing system could give Canada the competitive edge enjoyed for the last decade with canola.

To date the most popular analytical approach to screen for glucosinolate content has involved myrosinase hydrolysis to release glucose and colorimetric measurement of the glucose utilizing glucose oxidase, peroxidase and a chromogen (Lein and Schon 1969, Lein 1970, McGregor et al. 1973, Daun and McGregor in press). In plant breeding programs the so-called "hammer test", in which a few seeds are crushed with a hammer, water added to allow endogenous myrosinase to hydrolyze the glucosinolates and the glucose measured with a test paper containing the glucose oxidase, peroxidase and a chromogen (Comer 1956), was extensively

used in the development of canola cultivars. In the marketplace the so-called "mortar test", in which a small mortar was used to analyze a larger sample of seed and powdered carbon added to adsorb inhibitors thus enhancing the color development (McGregor and Downey 1975), was extensively used to identify and segregate canola seed during the conversion in Canada to commercial production of canola cultivars. More recently, efforts have been made to improve the glucose oxidase approach to glucosinolate determination. Noteable attempts include the use of automation to increase throughput (Smith et al. 1985), the use of a reflectometer to estimate the color development and thereby improve precision (Thies 1985, Robbelen 1987), and the varying reagent to improve both accuracy and precision (Saini and Wratten 1987, Smith and Dacombe 1987). Nevertheless, an assay which is sufficiently simple, rapid and inexpensive which can be used by relatively unskilled analysts to obtain answers in a matter of minutes with little cost, which is sufficiently sensitive to be used to identify low glucosinolate, canola and very low glucosinolate seed and meal, and which is sufficiently accurate and precise to be used to pay premiums and penalties based on glucosinolate content, remains elusive.

A solution is the use of enzyme immobilization. Studies have been reported recently on the immobilization of myrosinase on solid supports (Iori and Palmieri 1988, Wang et al. 1989a, Wang et al. 1989b) and on the coupling of immobilized myrosinase with immobilized glucose oxidase to measure glucose released from glucosinolate extracts (Koshy et al. 1989, Wang et al. 1989). The later study showed that myrosinase can be immobilized using readily available commercial supports and bonding agents. Sufficient activity can be bound to as little as 0.2 g of support to allow hydrolysis the glucosinolates from seed and meal extracts in as little as 5 minutes. When incorporated into a bioreactor at least 500 analyses can be performed without the need for replenishment of the myrosinase. When the bioreactor is coupled to a biosensor, in which glucose oxidase is immobilized by entrapment on the end of a hydrogen peroxide sensitive electrode, the complete analysis, including the initial extraction of the glucosinolates from seed or oil-extracted meal, can be completed in less than an hour.

4. ADVANTAGES/DISADVANTAGES

Immobilization of myrosinase in a bioreactor in combination with immobilization of glucose oxidase to form an enzyme electrode offer some particular advantages over other methods for determining of glucosinolate content. Since both the myrosinase and the glucose oxidase are reusable the analysis is essentially "reagentless". This substantially reduces the cost of analysis. Using a hydrogen peroxide sensitive electrode eliminates the need for peroxidase.

Rapeseed and rapeseed meal contain inhibitors of peroxidase which must be removed, either with powdered carbon or ion-exchange chromatography, in methods that rely on glucose oxidase and peroxidase to colorimetrically determine myrosinase released glucose. Measuring the hydrogen peroxide formed by the action of glucose oxidase on myrosinase released glucose eliminates the need to isolate the glucosinolates or glucose from these inhibitors and thus speeds up the analysis substantially.

Recently it has been shown that rapeseed contains enzymes other than myrosinase which release glucose when endogenous myrosinase is used to hydrolyze the glucosinolates (Smith and Donald 1988). Studies (McGregor, unpublished) have shown that this non-glucosinolate glucose can amount to as much as 6 to 8 micromoles expressed per gram of oil-extracted air-dried meal and thus provide a substantial background interference in very low glucosinolate material. By in activating endogenous enzymes during extraction and relying on exogenous myrosinase in the bioreactor, the enzyme immobilization approach avoids the problem of non-glucosinolate glucose.

Sound rapeseed contains about 6 to 10 micromoles of free glucose while poor quality rapeseed may contain even greater amounts of free glucose. By bypassing the bioreactor and analyzing a sample of the glucosinolate extract directly with the enzyme electrode background glucose can be determined and a correction applied. This does not add substantially to the time required for analysis as analysis of glucose with the enzyme electrode requires only 90 seconds. Correction for non-glucosinolate background glucose in combination with high sensitivity allows for accurate and precise analyses of samples with less than 10 micromoles of glucosinolate per gram

oil-extracted air-dried meal.

Analysis of myrosinase released glucose estimates true glucosinolate content. Indole glucosinolates are estimated in addition to aliphatic glucosinolates. This rapid and simple method which estimates the true glucosinolate content of seed or meal with high accuracy, precision and throughput is ideally suited as a standard method of analysis. The improved ease of analysis and standardization can facilitate trade of canola and of glucosinolate-free seed and meal.

Unlike gas chromatography, or high performance liquid chromatography, this method does not provide detailed information on glucosinolate profiles. Unlike the glucose oxidase/ferric nitrate method, it does not provide information on the amount of the various (aliphatic and indole) glucosinolate classes.

Compared to the hammer method, mortar method, or microtiter plate method of glucosinolate analysis accuracy and precision is considerably higher, but throughput is lower.

5. EQUIPMENT

Standard laboratory apparatus and in particular:

5.1 **Water bath, 70 °C.**

5.2 **Scintered glass funnel, 60 mL:**

Fisher Scientific Ltd.
Cat. No. 1- 358H

5.3 **Forced-air oven.**

5.4 **Vibrax shaker, Model VXR 7:**

Terochem Scientific Ltd.

5.5 **Eppendorf repeater pipete tips, 5 mL:**

Fisher Scientific Ltd.
Cat. No. 21-381-101

5.6 **Disposable plastic syringes, 5 mL Disposable syringe needles, 20guage, 38 mm long:**

Fisher Scientific Ltd.
Cat. No. 14-823-85
Cat. No. 14-826-5C

or, preferably: **Eppendorf repeater pipette tips, 5 mL**

Fisher Scientific Ltd.
Cat. No. 21-381-101

5.7 **Porous polyethylene sheeting, (Bel-Art) 1.6 mm 35 um pore size:**

Mandel Scientific Co. Ltd.
Cat. No. F13638-5116

5.8 **Cork bore, #6.**

5.9 **Disposable micropets, 50:**

Fisher Scientific Ltd.
Cat. No. 21-164-2G

5.10 **Vials, 10 mL with polyethylene cap:**

Fisher Scientific Ltd.
Cat. No. 03-339-10C

5.11 **Cuvette, 0.1 cm pathlength:**

Sargent-Welch Scientific Ltd.
Cat. No. S75735-11-A

5.12 **Culture tubes, 125 x 15 mm with PTFE-lined screw-cap:**

Fisher Scientific Ltd.
Cat. No. 14-930-10E

5.13 **Vial, 8 mL:**

Fisher Scientific Ltd.
Cat. No. 06-408C

5.14 **Water bath, boiling.**

5.15 **Centrifuge, bench top.**

5.16 **Water bath, 70 °C.**

5.17 **Glucose analyzer, Model 27 with 25μL syringepet:**

Yellow Springs Instrument Co.

6. REAGENTS

Standard laboratory reagents and in particular:

6.1 **-Aminopropyltriethoxysilane (APTS):**

Pierce Chemical Co.
Cat. No. 80370

6.2 **Citric acid, monohydrate.**

6.3 **Controlled Pore Glass, CPG/Uncoated:**

500Å pore diameter
80/120 mesh
125-177um particle size

Pierce Chemical Co.
Cat. No. 23808

6.4 Controlled pore glass, CPG/ Aminopropyl 80/120 mesh 125-177 um particle size:

Pierce Chemical Co.
Cat. No. 23909

6.5 ß-D-Glucose:

Sigma Chemical Co.
Cat. No. G-5250

6.6 Glutaraldehyde, 25% in water:

Eastman Kodak Ltd.
Cat. No. P 8648

Available from North American Scientific Chemical Ltd.

6.7 Myrosinase:

Prepared from yellow mustard (*Sinapsis alba* L.) seed.
or
Biocatalysts Ltd.
or
Boehringer Mannheim Ltd.
Cat. No. 1088 769

6.8 Parafilm:

Fisher Scientific Ltd.
Cat. No. 13-374-5

6.9 Sodium borate.

6.10 Sodium chloride.

6.11 Sinigrin, potassium salt, monohydrate:

Sigma Chemical Co.
Cat. No. S7508

6.12 Sodium phosphate, dibasic anhydrous.

6.13 2,4,6-Trinitrobenzenesulfonic acid, hydrate (TNBSA):

Pierce Chemical Co.
Cat. No. 28999

7. SUPPLIERS

7.1 Biocatalysts Ltd.

Main Avenue, Treforest Industrial Estate Pontypridd, Wales CF37 5YT
Telephone: 044385 3712
Telex: 497126 BIOCAT G

7.2 Boehringer Mannheim Ltd.

11450 Cote de Liesse, Dorval, PQ
CANADA H9P 1A9
Telephone: (514) 636-6760
Telex: 05-8222677

7.3 Chromatographic Specialties Ltd.

P.O. Bag 1150, 300 Laurier Blvd.
Brockville, ON, CANADA K6V 5W1
Telephone: (613) 342-4678

7.4 Fisher Scientific Ltd.

P.O. Box 3840 Station D, Edmonton, AB, CANADA T5L 4K2

7.5 Mandel Scientific Co. Ltd.

9840-47th Ave., Unit #2, Edmonton, AB, CANADA T6E 5P3
Telephone: (403) 436-0665

7.6 North American Scientific Chemicals Ltd.

7058 "F" Farrell Road S.E.
P.O. Box 5961 Station "A"
Calgary, AB, CANADA T2H 1Y4
Telephone: (403) 253-0456
Telex: 03-825749

7.7 Pierce Chemical Co.

P.O. Box 117, Rockford, IL
USA 61105
Telephone: (910) 631-3419
Telefax: (815) 968-7316

7.8 Sargent-Welch Scientific Ltd.

285 Garyray Drive, Weston, ON
CANADA M9L 1P3
Telephone: (416) 741-5210

7.9 Sigma Chemical Co.

P.O. Box 14508, St. Lois, MO
USA 63178
Telephone: (314) 771-5750
Telefax: (314) 771-5757

7.10 Terochem Scientific Ltd.

5729 92 Street, Edmonton, AB
CANADA T6E 3A4
Telephone: (403) 438-2222
Telex: 0373022
Telefax: (403) 434-3362

7.11 Yellow Springs Instrument Co.

Yellow Springs OH, USA 45387
Telephone: (513) 767-7241
Telex: 205437
Telefax: (513) 767-9353

8. PREPARATION

8.1 Silanization of CPG Glass

. Weigh 1 g of CPG\uncoated glass

into a 100 mL round bottom flask.

- Add 20 mL of 10% APTS (18 mL water and 2 mL APTS).
- Reflux in a water bath with the flask fitted with a cold water-jacketed condenser at 70 °C for 4 hours.
- Transfer the glass to a medium porosity scintered-glass filter funnel and wash with water under suction to remove the APTS.
- Dry in a forced-air oven at 120 °C for 5 hours.
- To a small amount of silanized CPG glass (approximately 10 mg) add approximately 2 mL of saturated sodium borate.
- Add 5 drops of a 3% aqueous solution of TNBSA and mix. If the glass has been sufficiently silanized it should turn orange within a couple of minutes.

8.2 Construction of the Bioreactor:

(**NOTE:** The bioreactor may be constructed with a 5 mL plastic syringe. However, if available, a 5 mL Eppendorf Repeater Pipette tip is preferred. Its one piece construction and flat base, which allows for snug fitting of the porous polyethylene frit, minimizes retention of eluate. Also, the plastic plunger has less tendency to stick with repeated use.)

- Insert a disk of porous polyethylene cut from a sheet with a #6 cork bore into the shorter bottom piece of the Eppendorf Repeater Pipette tip.

8.3 Activation of the Silanized Glass:

- Weigh 0.2 g of alkylamine glass (as prepared above, or preferably Controlled Pore Glass, CPG/Aminopropyl), into the 5 mL bioreactor.
- Add 2 mL 6.6% glutaraldehyde in citrate-phosphate buffer (2.5 mL 25% glutaraldehyde and 7.5 mL 0.2 M citrate-phosphate buffer pH 7) 14.1 g sodium phosphate, dibasic anhydrous, dissolved in 500 mL water and 2.1 g citric acid, monohydrate, dissolved in 100 mL water, citric acid solution added to 450 mL sodium phosphate to obtain pH 7).
- Seal the tube with its plunger and a small piece of Parafilm and shake on the Vibrax shaker for 2 hours.
- Wash with water until all of the glutaraldehyde has disappeared.

8.4 Covalent Attachment of Myrosinase:

- Add 2 mL of freshly prepared myrosinase (7 mg/mL) to the 5 mL bioreactor.
- Seal the tube with its plunger and a small piece of Parafilm and shake on the Vibrax shaker overnight at 4 °C.
- Wash with 0.5 N NaCl (2.9 g dissolved in 100 mL of water), water, then 0.05 M citrate-phosphate buffer pH 7 (0.2 M citrate-phosphate buffer pH 7 diluted 4 fold.)
- Store with sufficient 0.05 M citrate-phosphate buffer to cover the glass at 4 °C.

8.5 Analysis of Immobilized Myrosinase Activity:

- Transfer a known weight (approximately 5 mg) of CPG glass containing the immobilized myrosinase to a second 5 mL bioreactor.

(**NOTE:** Transfer of a known weight of CPG glass may be facilitated by constructing a transfer device consisting of a 5 mL plastic syringe or Eppendorf Repeater Pipette tip to which is attached a short piece of tygon tubing containing a porous polyethylene frit disk and a short length of glass tubing (30 mm of a 50 disposable Micropet) into which can be drawn and expelled a known weight of glass. The porous frit permits removal of the buffer.)

- Rinse the bioreactor twice with 0.5 mL 2 mM sinigrin (41.5 mg/100 mL) and remove With aspiration.
- Without delay add 1 mL 2 mM sinigrin after last washing.
- After 3,6,9,12 or 15 minutes transfer the filtrate to a 4 mL vial, cap and heat at 70 °C for 5 minutes.
- Transfer the filtrate from the vial to a 1 mm pathlength cuvette and read the absorbance at 227 nm.
- Rinse the CPG glass by addition of water and with removal by aspiration, and repeat the above steps for additional times.
- Upon completion replace the CPG glass with immobilized myrosinase in the original bioreactor.

To express activity in μmoles per minute per 10 mg CPG glass: (ΔT) after 3 minutes, i.e. the initial change in absorbance per minute excluding the initial 3 minutes.

- **$(\Delta A/\Delta T)/\epsilon$ * 1/(pathlength) * 10/(mg of glass assayed) =μmoles min^{-1} per 10 mg CPG glass**

where:
-**$\Delta A/\Delta T$** is the change in absorbance per minute.
-**ϵ(6.784)** is the extinction coefficient for sinigrin.
-**1/(pathlength)** is the correction for the sort (less that 1 cm) pathlength of the cuvette.
-**10/(mg of glass assayed)** is the correction to express the result per 10 mg CPG glass

Thus if:
-The change in absorbance per minute is 0.0475,
-The pathlength of the cuvette is 0.1 cm,
-The weight of glass assayed in 4.16 mg,
then:

0.0475/6.784 * 1/0.1 * 10/4.16 = 0.144 μmoles min^{-1} per 10 mg CPG glass.

An activity of 0.1 μmoles min^{-1} per 10 mg CPG glass is sufficien or a 0.2 g bioreactor to hydrolyse 0.5 mL of an extract of 30 μmole glucosinolate per g oil-extracted meal in 5 minutes, or 0.5 mL of an extract of 180 μmole glucosinolate /g oil-extracted meal in 15 min.

The performance of the bioreactor should be checked periodically with either the above procedure or by applying a meal extract and hydrolyzing for varying periods of time.

8.6 Glucose standard:

- Weigh 180.2 mg of b-D-glucose in to an 100 mL volumetric flask, dissolve and make to volume with water.
- Let stand overnight for mutarotation to equilibrium between α- and ß-D-glucose forms, 37% and 63%, respectively.

9. PROCEDURE

9.1 Glucosinolate Extraction:

- Weigh 250 mg of oil-extracted meal into a 125 x 15 mm culture tube.
- Place the tube in a boiling water bath for 1 minute.
- Add 3 mL of hot (<90 °C) water to the tube and, without allowing the contents to cool, mix to ensure that the meal is thoroughly wetted and continue heating for 5 min.
- Centrifuge at 2000 g for 10 min. and transfer the supernatant to a 5 mL volumetric flask.
- Wash the pellet twice with 1.5 mL of water, pool the super-natants, and make to volume with water.

9.2 Myrosinase Hydrolysis:

- Ensure that the bioreactor has been rinsed well with water and excess water removed by aspiration.

(**NOTE:** It is important to rinse the bioreactor with water prior to addition of the sample. Rinsing the bioreactor with sample results in apparent binding of gluco-sinolate to the active sights of the immobilized myrosinase. This leads to over estimation of the glucosinolate content of the sample.)

- Transfer 0.5 mL of sample into a test tube and draw up into the bioreactor.
- Shake the bioreactor and sample on the Vibrax shaker for 10 minutes.
- Transfer the sample to a 8 mL screw-cap vial.
- Cap and heat in water bath (70 °C) for 1 minute.

(**NOTE:** It is important to ensure that the glucose released from the glucosinolates is completely mutarotated to equilibration between the α-glucose and ß-glucose forms. Glucose released from the gluco-sinolates is the ß-glucose form. As glucose oxidase reacts only with ß-glucose and the glucose oxidase reacts only with ß-glucose and the glucose analyzer is calibrated with an equilibrated α-glucose and ß-glucose solution, it is important to heat the sample to ensure that the ß-glucose released from the gluco-sinolate is completely mutarotated to equilibration between the α-glucose and ß-glucose forms.

Failure to equilibrate the sample can result in an artificially high measurement.

9.3 Glucose Measurement

- Stabalize glucose analyzer and perform analyses using 0.05 M citrate-phosphate buffer pH 7 (0.2 M citrate-phosphate buffer pH 7 diluted 4 fold).

- Inject 25 μL of a 30 mg/dL or 180

mg/dL glucose standard and calibrate the glucose analyzer if necessary.

(**NOTE**: For best results calibrate before analyzing each sample. Use a standard concentration of glucose close to that of the glucosinolate content of the sample.)

- Inject 25 μL of the sample and record the glucose value.

To correct for dilution of the sample in the bioreactor:

- Ensure that the bioreactor has been rinsed well with water and excess water removed by aspiration.
- Transfer 0.5 mL of a 30 mg/dL or 180 mg/dL glucose solution into a test tube.
- Draw up into the bioreactor.
- Shake the bioreactor and glucose standard on the Vibrax shaker for 1 minute.
- Transfer the filtrate to an 8 mL vial.
- Inject 25 μL of the sample and record the glucose value.

10. CALCULATION AND REPORTING OF RESULTS

10.1 μMoles per Gram Meal:

- Divide the concentration of the glucose standard (30 mg/dL or 180 mg/dL) by the glucose analyzer value obtained with addition of the glucose standard to the bioreactor to determine the dilution factor, i.e. 30 (mg/dL)/value (mg/dL) obtained for 30 mg/dL glucose standard in bioractor = dilution factor.

- Correct the glucose analyzer reading for the hydrolyzed sample for dilution by multiplying by the dilution factor to obtain a dilution corrected glucose analyzer reading.
- Subtract the corrected glucose analyzer reading for the hydrolyzed sample from the non-hydrolyzed (non-corrected) glucose analyzer reading.

Glucosinolate content is determined as:

▲glucose analyzer reads (mg/dL)/20 * 1000/250 * 1/0.1802 = μmoles/g oil-extracted meal

where:
-The factor **20** converts from 1 dL to the extract volume (5 mL).
-The factor **1000/250** converts to a gram basis the weight of oil-extracted meal (250 mg) from which the glucosinolates were extracted.
-The factor **1/0.1802** converts to μmoles from miligrams.

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SECTION 2
MANUAL OF ADDITIONAL TRAINING LECTURES AND PAPERS

TOTAL GLUCOSINOLATE CONTENT IN RAPESEED USING REFLECTANCE

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Several methods are available for the quantitative determination of glucosinolates in rapeseed. The High Pressure Liquid Chromatography (HPLC) method for separating desulfoluco-inolates can provide a measure of the content of individual glucosinolates including indole glucosinolates(3). This method, however, is not the method of choice for most routine plant breeding purposes where a total glucosinolate figure is all that is required.

A number of methods are available for the estimation of total glucosinolate content in rapeseed for example, thymol(1), chlorophalladate(4) and techniques based on glucose release(2). Some of the advantages and disadvantages of these methods are discussed at the end of this paper. The aim of this project was to develop a method for measuring total glucosinolate content which was rapid, simple, lacked any chromatographic steps and which did not require the use of expensive equipment.

EXPERIMENTAL DETERMINATION OF GLUCOSINOLATE CONTENT IN RAPESEED

1. Weigh 200 mg air-dried rapeseed into a 10 mL centrifuge tube.
2. Add 3.0 mL of 50 mM Glycine-NaOH buffer (pH 9.0) and homogenise thoroughly (15 seconds). Rinse the ultra turrax shaft with 2x1.0 mL aliquots of buffer solution dispensed through the top hole in the shaft.
3. Mix the tube gently and leave for 10 minutes.
4. Add 1 mL of chloroform, seal the tube, mix thoroughly by shaking.
5. Add 50 µl 10% chlorohexidine diacetate in methanol and mix.
6. Add 1.0 mL of 100 mM Citric Acid/Sodium Citrate buffer pH 5.0.
7. A scoop of activated charcoal (0.25 g) is added and the tube is sealed and vortexed.
8. Centrifuge the sample at 4000 g for 2 minutes. A clear colourless supernatant must be obtained.
9. Dip a Clinistix strip into the supernatant (4 seconds). Shake off excess liquid. Leave for 2 minutes for colour to develop.
10. Calibrate the meter setting the high end at 90 using a strip dipped in 1.0 mM glucose and the low end at 0 using water.
11. Determine the reflectance of the sample and read the corresponding figure glucose concentration from the standard curve. Convert to µmole glucosinolate/g seed by multiplying by the factor 30 (i.e. Glucose mNx30=Glucosinolate µmole/g seed). The appropriate correction for the moisture content of the seed sample can be applied.

Standard Curve Construction:

Preparation of curve A, Fig.1, involves aliquoting 27,45,90,135 and 180 µl of the 5.56 mM standard glucose into separate vials containing 166 µl of 0.1M Citric Acid/Sodium Citrate buffer pH 5.0. The total volume in each is adjusted to 1.0 mL giving 0.10,0.25,0.50,0.75 and 1.0 mM glucose.

Preparation of curve B, Fig.1, is as follows:

1. Weigh 200 mg rapeseed into a 10 mL centrifuge tube.
2. Immerse in a boiling water bath and add 3.0 mL of boiling water, leave for at least 10 minutes.
3. Homogenise in an ultra turrax for 15 seconds. Wash shaft with 2x1.0 mL aliquots of water.
4. The extract is then treated according to steps 4-8 of sample preparation.
5. Prepare standards using 5.56 mM glucose as described previously and adjust total volume to 1.0 mL using the extract.

Thymol Method:

The thymol method for glucosinolate determination was essentially that of *Brzezinski* and *Mendelewski*.

Suppliers of Equipment and Chemicals:

Ultra turrax TP 18/10 with 8 n shaft Janke and Kunkel. Reflectance meter Digital 4000, Australian Biotransducers Pty.Ltd. Glucose test strips-Clinistix, cat no. 2847, Ames, Miles Laboratory Australia. 10 mL Polypropylene centrifuge tubes, Bio-rad. Activated Charcoal (powder) cat no. 33032; Tri-sodium citrate, BDH. Standard Glucose solution cat.no. 635-100; Chlorohexidine diacetate, Sigma. Glycine; Citric Acid, Ajax Chemical Co.

RESULTS

Clinistix strips were selected from a range of commercially available glucose test strips, since they were found to provide the greatest sensitivity and reproducibility. Although Clinistix strips are marketed for visual glucose estimation in urine, they have been found, when used in conjunction with reflectance measurement, to provide a quantitative measure of glucose content. This is illustrated in Fig. 1.

Selective hydrolysis of glucosinolates by endogenous myrosinase is achieved at pH 9.0; the reaction being completed within 5 mins. At lower pH values seed enzymes other than myrosinase may be acting to liberate free glucose from other sources, Fig. 2.

Inhibitors of the clinistex enzymes are removed using activated charcoal. Tests showed that 50 mg is sufficient and up to 500 mg may be used without any significant adsorption of glucose being observed. The addition of 250 mg of activated charcoal also plays a role in lowering the pH of the final extract which should be approximately 5 to ensure a clear colorless supernatant.

Clinistix strips were found to reach optimum colour intensity 2 minutes after dipping, Fig.3. The stability of the colour of clinistix strips as a function of time of exposure to light is shown in Fig. 4. Thus optimum sensitivity and reproducibility is achieved by allowing 2 minutes for colour development, after which time, fading of the dye is observed especially when the strip is exposed continually to the reflectance meter light source. Exposure of the strip to sunlight during the 2 minutes period, however, does not significantly affect colour development. The clinistix pads were found to take up 9 µL of solution and this volume did not appear to alter significantly with different batches of clinistix strips.

The values for glucosinolate content of four rapeseed varieties were determined by reflectance and checked against those determined by the thymol method(2). The results are shown in Table 1. The close correlation between the glucosinolate contents obtained by the two methods suggests that the reflectance method can give accurate glucosinolate values over the range normally found in rapeseed samples (5-30 µmole/g seed). If seed samples contain high levels of glucosinolates, 100 mg seed can be taken for analysis.

Table 1. Comparison of glucosinolate contents obtained by the reflectance method and the Thymol method

	Thymol			Reflectance		
Rapeseed Variety*	Mean umol/g seed	S.D.	C.V. %	Mean umol/g seed	S.D.	C.V. %
CS503	6	0.87	14	7	0.71	10
CRX9	13	1.12	9	12	0.83	7
Marnoo	21	1.12	5	22	1.12	5
Bunyip	31	1.41	4	31	0.83	3

* Each rapeseed variety was analysed four times by both methods.

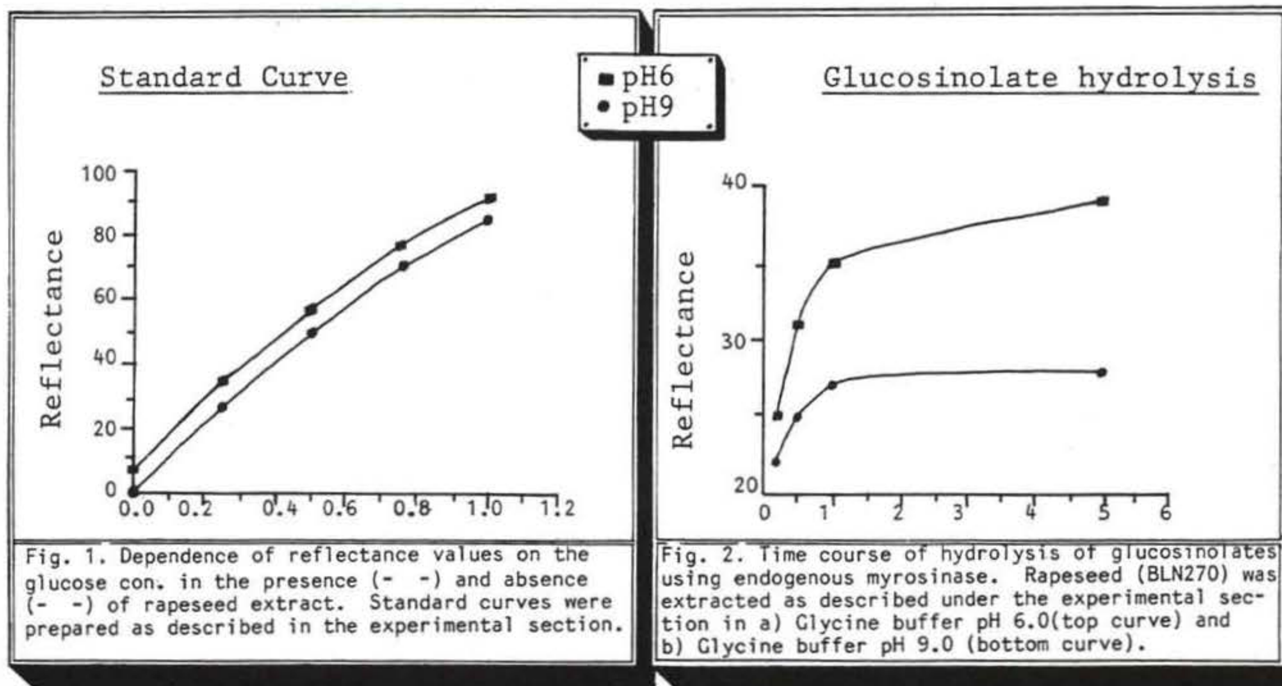

Fig. 1. Dependence of reflectance values on the glucose con. in the presence (- -) and absence (- -) of rapeseed extract. Standard curves were prepared as described in the experimental section.

Fig. 2. Time course of hydrolysis of glucosinolates using endogenous myrosinase. Rapeseed (BLN270) was extracted as described under the experimental section in a) Glycine buffer pH 6.0(top curve) and b) Glycine buffer pH 9.0 (bottom curve).

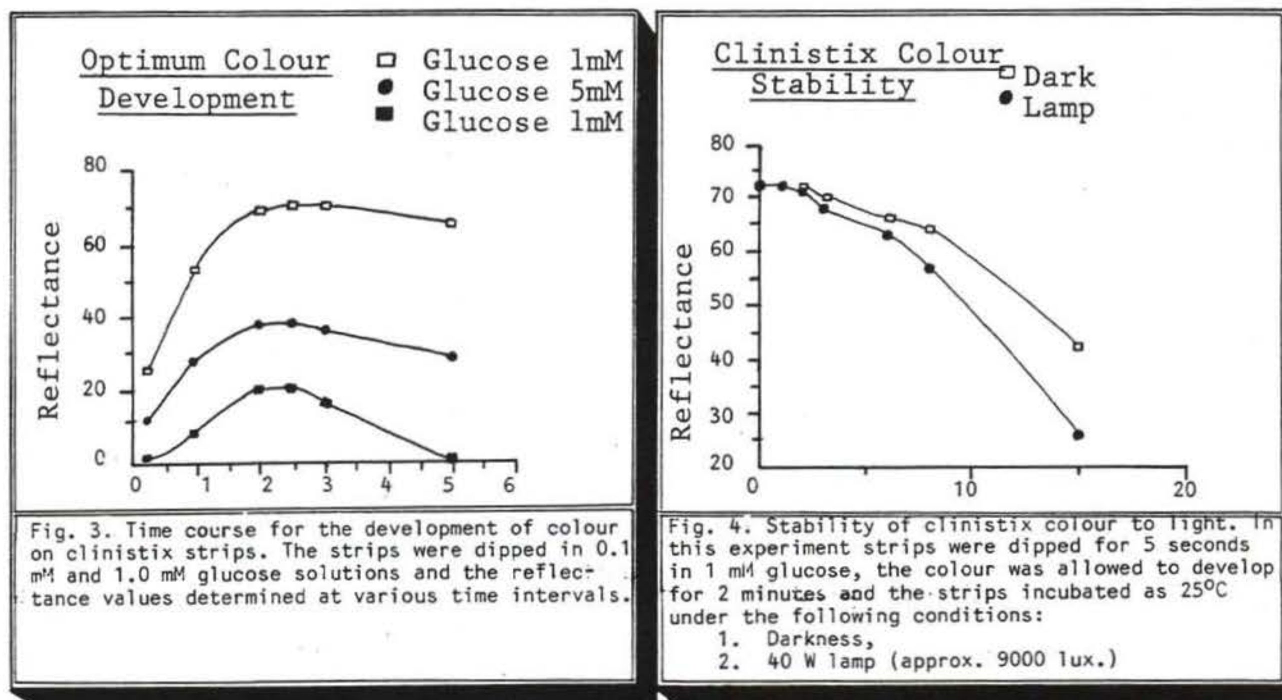

Fig. 3. Time course for the development of colour on clinistix strips. The strips were dipped in 0.1 mM and 1.0 mM glucose solutions and the reflectance values determined at various time intervals.

Fig. 4. Stability of clinistix colour to light. In this experiment strips were dipped for 5 seconds in 1 mM glucose, the colour was allowed to develop for 2 minutes and the strips incubated as 25°C under the following conditions:

1. Darkness,
2. 40 W lamp (approx. 9000 lux.)

DISCUSSION

The current methods available for total glucosinolate determination include thymol(1), chloropalladate(4), and cholorimetric glucose methods(2). The aim of this project was to develop a method which could readily be adopted by a non-specialist chemical laboratory. To this end we aimed to develop a rapid yet simple assay capable of being used by plant breeders or at collection points of silos. A similar procedure based on reflectance measurement of Glucotest paper strips cut by hand from a paper roll has been reported by Thies(5). The thymol method(1) can provide accurate total glucosinolate measurement, however, this method suffers several drawbacks, such as the need for a chromatographic step, marked sensitivity to interferences such as dust, fibre etc. and it involves the use of concentrated sulfuric acid solutions. The chloropaladate method is also subject to considerable interference from endogenous seed metabolites(4), but can provide an estimation of the total glucosinolate content.

Reflectometry coupled with a sensitive enzyme linked dye assay (clinistix), gives excellent sensitivity and due to the specificity of the enzymes involved, the result seems less influenced by the presence of other metabolites.

THe reflectance method relies on free glucose in seed being consistent between varieties. Ten rapeseed varieties were examined and free glucose was found to be in the range of 1.5-3.0 μmole/g seed. For a given variety, this can be readily checked by firstly boiling the seed for 15 minutes to inactivate the endogenous enzymes prior to extraction.

Experimentally, it was found that the strips needed to be immersed in the glucose solution for a period of at least 4 seconds. The diping time of the strips should not be excessive as this increases the likelihood of dye being leached from the pad into the sample solution. Care must be exercised during dipping to avoid any suspended fine particles of activated charcoal from adhering to the clinistix pad, as these will alter the reflectance significantly. If this is a problem an aliquot of the supernatant can be removed using a pasteur pipette and dropped onto the clinistix strip. Alternatively a fluted filter paper can be immersed in the solution and the filtrate used for the clinistix assay. This removes the need for a centrifugation step.

As reported by others(6) colour development was found to be inhibited in the absence of activated charcoal, however excessive amounts may lead to adsorption of glucose. With our protocol, glucose adsorption is not a problem, due possibility to the high sensitivity of the clinistix strips. The activated charcoal plays an additional role in lowering the pH of the extract, which is finally buffered at approximately pH 5.5. This is important for obtaining a clear and colourless extract.

At present, work is underway to produce a reflectance meter capable of giving a direct readout of gluco-sinolate content.

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A SIMPLE METHOD FOR IDENTIFYING LOW-ERUCIC ACID AND LOW-GLUCOSINOLATE RAPESEED-TURBIDITY TITRATION-COLORIMETRY

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A simple analytical method for rapeseed breeding, producing, purchasing and processing is reported. It is based on turbidity titration(1). Determination of erucic acid and glucosinolate for 5 samples can be carried out within a quarter. Absolute error for erucic acid estimation less than 0.5% when the content of erucic acid below 6%, and for glucosinolate less than 2 μmole/g when glucosinalate content below 30 μmole/g. Very similar results have been required by different operators.

The main studies presented in the paper are the condition of extracting polar glucosinalate and nonpolar fat from rapeseed at one time in room temperature, the curve about turbidity titration and the effects of various factors in glucosinolate colormetry.

EXPERIMENTAL CONDITION

I. Instrument and reagents:

1. Extract: dissolve 5 mL Triton X-100 in 500 mL absolute ethand.
2. Titration solution: 75-80% aqueous ethanol.
3. Oxide silicon dust (SiO_2)
4. $PdCl_2$ solution: put 177 mg dCl_2 in the mixture of 2 mL 2M HCl and 10 mL H_2O, than heat and solutize and make up to 1 litre with H_2O.
5. $CHCl_3$
6. Little spoon (0.5g) made by self.
7. Glass mortar
8. Test tube with plug (10m)
9. Graduated pupet (2mL, 10mL)
10. Sucking globe
11. Centrifuge (or hand set centrifuge)
12. Standard rapeseed (erucic acid: 2% and 5%, glucosinolate: 15 and 25 μmole/g)

II. Extract fat and glucosinolate from rapeseed at one time:

1. Choice of extract:
Effort was made to search for an extract which can extract polar glucosinolate and nonpolar fat at one time in room temperature so as to determine erucic acid and glucosinolate with same extracting solution. It proved that 1% Toriton X-100 (neutral surface active agent) absolute ethanol solution is efficient to extract erucic acid and glucosinolate from rapeseed at one time in room temperature. Data presented in Tables 1 and 2 were obtained from the determination of glucosinalate with colormetry and of fatty acid methyl ester by GC.

Table 1. Relation between glucosinolate content and absorbance.

Glucosinolate (μmole/g)	12.500	20.000	24.600	31.200	40.000
Absorbance (E)	0.030	0.048	0.060	0.192	0.200

Table 2. Percentage of fatty acid in extracting solution (%).

	Fatty Acid			
	C_{16}	C_{18}	C_{20}	C_{22}
avg. of 5 analyses	4.62	88.25	4.04	2.93
standard value	4.26	88.01	4.68	3.06

2. Other factors:
Fat can't disolve in water. The moisture content in extract had great effect on extracting of fat, especially with the fat containing erucic acid. So the extract-Triton X-100 solution must be prepared with absolute ethanol.

Disposing the extract in the air for 21 hours (no test for much long), different temperature and humidity have no effects on the result.

III. Effective factors in the determination of glucosinolate with colormetry:

1. Tentative plan and test for the concentration of color-developing agent: This method is used in identifying low-glucosinolate rapeseed. Distinguishing vaults are suggested that .30 μmole/g rape cake (about 15 μmole/g rapeseed) for original seed and 50 μmole/g rape cake (about 25 μmole/g rapesed) for commercial rapeseed(2). So we expected that the sudden change of colour is clear for visual photometry when glucosinolate content above 50 μmole/g rape cake, so as to identifying commercial rapeseed. And expected that there was Linear relate between the content and colour when glucosinolate content below 50 μmol/g rapecake, so as to know the valid content for screening original rapesed. Optimum content of color-developing agent and sample amount used were chosen and get ideal effect expected, Table 1.

2. Removal of the effects of fat and pigment: Glucosinolate and fat were extracted together. Adding color-developing agent into the extracting solution made fat colloidal flocculent precipitate. Pigment also interfered with visual photometry. An effect and simple method was adopted. That was adding $CHCl_3$ into the extracting solution before adding color-developing agent. By $CHCl_3$ extracting fat and pigment, the extracting solution was transparent, decoloured and to the benefit of visual photometry.

3. Effect of colour developing time:

   Colour developing by glucosinolate and $PdCl_2$ need a long time-more than 10 hours. Color thickness is about 70% of the maximum in 5 minutes. Standard rapeseed and sample rapeseed parallel analysis can remove effect of color develop-ing time.

IV. Turbidity Titration Curve:

Water content in titration solution had great effect. In order to identify the rapeseed in which erucic acid content below 5%, 75-80% aqueous ethanol was adopted to have great difference in solution volume. Titration curve is shown in Fig. 1.

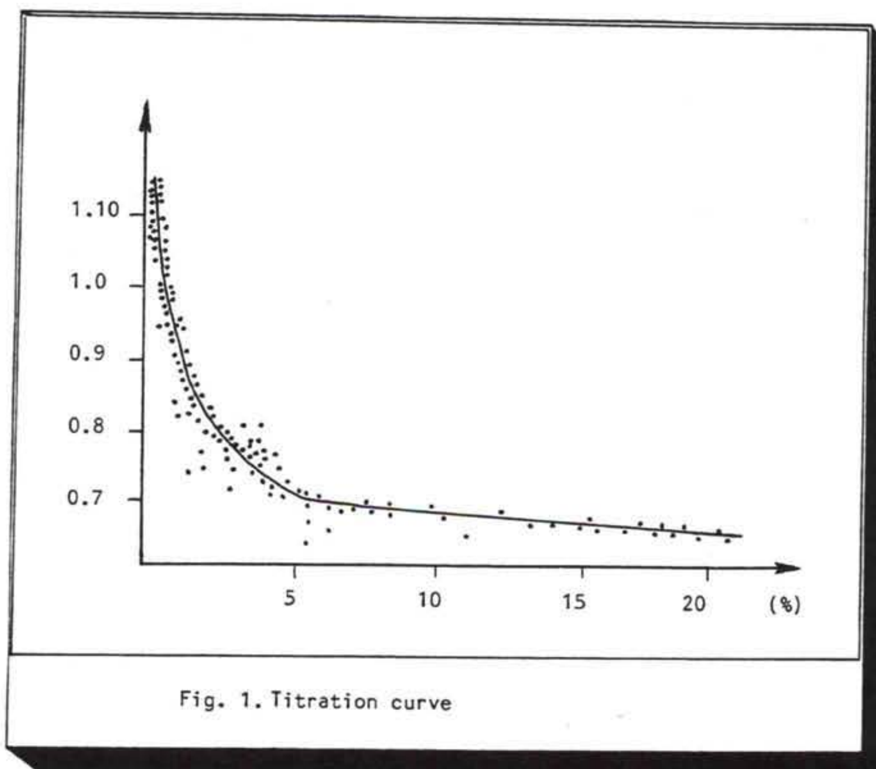

Fig. 1. Titration curve

SAMPLE ANALYSIS

Turbidity titration-colormetry procedure:

1. Representative rapeseed of different position is about 20g.
2. Put the sample put into a little clean bottle and mix.
3. Then abandon half of the rapeseed, shake and abandon another half.
4. Repent the operation till about 2.5 g rapeseed remains.
5. Take 0.5g sample with little spoon and put into glass mortar.
6. Add 0.5 g drug oxide silicon dust.
7. Grind.
8. Put into a test tube (10mL, with plug).
9. Add 8 mL extract solution.
10. Accurately extract 3 mins.
11. Centrifug.
12. Take 1 mLx2 of the supernatant liquid into test tube 1 and 2.

Determination of erucic acid:

1. Add 2mL of extract into tube 1.
2. Mixing with 75-80% aqueous ethanol titrate till turbidity.
3. Recording volume of titrating solution.
4. Erucic acid content in sample was estimated by comparing with titration volume of standard rapeseed (2% of 5% erucic acid) of same operating procedure.

Determination of glucosinolate:

1. Add 1 mL $CHCl_3$ into tube 2.
2. Mix.
3. Add 1 mL $PdCl_2$ solution.
4. Mix.
5. Draw out $CHCl_3$ below.
6. 5 mins.
7. Compare the color thickness between sample and standard rapeseed (15 or 25 umol/g rapeseed) and estimate the glucosinolate content of sample.

RESULT

Turbidity titration-colormetry have been tested and verified for three years. Table 3 is comparing the result with GC.

Table 3. Comparison between the method and GC.

erucic acid	>5%	5-2 %	<2 %
coincidence*	99%	98.7%	99.2%

* For glucosinolate, coincidence was 100%.

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AN OUTLINE OF RESEARCH ON RAPESEED QUALITY ANALYSIS

Wu Xingyong

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Rape is a very important oil crop in China. The planting area is close to 80,000,000 mu (5.3 million hectar annually. In the late of 70s', our institute began to study improving rapeseed quality, and quality analysis. The Department of Quality Analysis was established equipped with GLC. HPLC UV-vice, High Speed Centrifuge, and NMR (for oil content determination), with 25 staff.

The research on rapeseed quality analysis includes two parts: the first is analysis method and the second is studying the chemical composition of rapeseed germplasm resources. In the past ten years, the state has paid great attention to support this project, and the project ranked among the national key research projects in the sixth-five and the seventh-five years plan. At the same time, this project has been helped and supported by Canada and Australia through the Sino-Canadian and Sino-Australia Cooperating Projects. Great appreciation is extended to International Development Research Center (IDRC) and Australia Center for International Agriculture (ACIAR).

The major progress of the project is summerized below:

A. Introduction in analysis method of rapeseed quality and further study:

1. Establishing a national standard method of testing crude oil content in oil seed: This method was set up by Oil Crops Institute of CAAS, in cooperation with Shanghai Academy of Agricultural Sciences (SAAS), Jiangsu Academy of Agricultural Sciences (JAAS), Huazhong University of Agriculture and etc. The method was admitted by the government, which was utilized by the institutions all over China.
2. Establishing the national standard method for determining erucic acid content in rapeseed: This work was carried out by the four units mentioned above. The method also was admitted by the state, which is expanding its use in China.
3. Paper chromatograph method for erucic acid test: This method was introduced from abroad, and was widely used in the institutions of agriculture for chemical selection.
4. NMR for oil content determination: It is schemed by Oil Crop Institute of CAAS, Wuhan, Physics Institute of Academia of Sinica, Wuhan Measuring Equipment Factory and it is in a small lot production.
5. Palladium method of rapeseed glucosinolate quantitation: Set up by Huazhong University of Agriculture and Oil Crop Institute of CAAS, also has been widely used.
6. ITC-OZT method: After we established this method, the procedure has been applied to detect the glucosinolate content in food stuff.
7. TMS method for single glucosinolate tasting: It was established with the help of Canadian chemists.
8. Reflectometry: A quantitative method of total glucosinolate established with the help of Australian chemists.
9. Thymol method: Was established with the help of Australian chemists.
10. HPLC method: Was established with the help of Australian chemists.
11. Testape method: We are thankful to IDRC and ACIAR providing with testapes, we have used the quick test for several years.
12. Thiourea method: A quantitative

method of total glucosinolate in rapeseed plant organ. It was set up in our lab.

B. Study on the chemical composition of rapeseed germplasm resources:

There are rich germplasm resources of rapeseed (*B. campestris*, and *B. juncea*) varieties and a number of *B. napus* varieties. In the recent years, cooperating with relevant institutions of China, our institute have investigated the oil content, fatty acid composition, total and individual glucosinolate content of germplasm resources and wild rapeseed. Also we have primarily studied the bionomic and geographical factors affecting the chemical constituents of rapeseed, and provided the evidence for making breeding materials to different areas.

C. The problems existed:

1. The research condition ought to be improved: We need more study fee and improvement of the equipments in labratory and import of the special chemicals and reagents e.g. standard glucosinolates.

2. The fast methods of erucic acid and glucosinolate need to be further studied: As we know, before the through changeover of double high by double low rapeseed varieties, the double low, single low and double high varieties may be planted in adjacent area. So we request fast methods and equipments to distinguish the different seed quality, which suit our commercial and processing departments.

3. The standard methods of total and single glucosinolate contents: Although we have made a lot of progress in this aspect, we cannot meet the need of breeding. Because the results from different methods are not the same, we must evaluate and improve the methods we have now, so as to set up the standard methods.

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NEW METHODS OF MYROSINASE BIOREACTOR AND GLUCOSE SENSOR FOR RAPID AND ACCURATE ASSAY OF GLUCOSINOLATES IN RAPESEEDS

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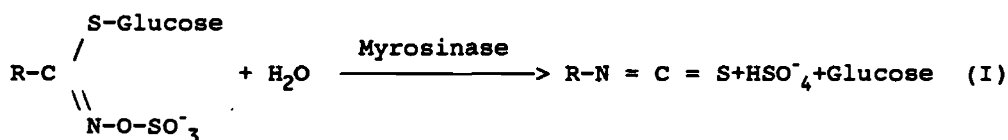
As the demand for protein is on the increase, the selection and development of rapeseed species with low glucosinolates is a challenge to the developing countries. A fast, simple, accurate, and precise method is essential for such a program.

Three years ago, we succeeded in constructing a glucose sensor, Fig. 1. An immobilized glucose oxidase membrane was attached on the top of Pt-Ag/AgCl electrode. The glucose sensor was also utilized for clinical diagnosis of blood sugar and monitoring of glucose change in the fermentation broth. The good linearity of glucose between 10^{-3} to $2x10^{-4}$mol/L with r=0.999 and CV<3% made it possible to use 20 μl sample for each assay. Response time is 40 seconds. The glucose oxidase membrane possesses high operational stability that may be used for over 3000 assays, and also stable for storage at 4°C for 2.5 years or at 37°C for 4 months.

In the view of principle of enzyme catalysis, we believe that the combination of glucose sensor with myrosinase-hydrolysis is certainly an approach of promise for measurement of glucosinolate. This is illustrated as follows:



METHODOLOGY DEVELOPMENT

To determine the total glucosinolates in rapeseeds, we have been developing three types of combined techniques of bioreactor and biosensor:

1. Combination of free myrosinase and GOD electrode
   ↓
2. Combination of immobilized myrosinase and GOD electrode
   ↓
3. Co-immobilized myrosinase-glucose oxidase membrane/O_2 electrode

Fig. 2 indicates that pure sinigrin can be hydrolyzed by free myrosinase and the hydrolysates can be assayed on the glucose sensor to obtain a linear response between 1-5 μmoles/mL.

In the case of rapeseeds or de-fatted meal, glucosinolates were extracted from the crushed seeds or meal powder with boiling water, and then hydrolyzed by exogenous myrosinase for 5 minutes.

Monitor

O_2 electrode

"O" ring

Inlet

Reaction chamber

Glucose oxidase membrane

Peristaltic pump

Cleaning solution

Magnetic stirrer

Waste

Fig. 1. Schematic diagram of the glucose sensor.

```
/|- OH       O           O                              /|-OH          O
/|           |           |         Etherification       /|             |
/|- OH + OH-S-O-CH2CH2-S<    >NH2   ----------->        /|-O-CH2-CH2-S-<    >NH2
/|           |           |         Alkaline pH          /|             |
/|- OH       O           O                              /|-OH          O

Agarose  p, (Sulphato-                                   ABSE-agarose
         ethylsulphonyl)aniline

                                  O                                        O
 Diazotization   /|-OH            |            Coupling    /|-OH           |
 ----------->    /|-O-CH2-CH2-S-<   > N≡NCl   -------->    /|-O-CH2CH2-S-<  >N=N  [Myrosinate]
 NO2, 0-4°C      /|-OH            |            Myrosinase  /|-OH           |
                                  O                                        O

   Diazonium salts of ABSE-                                Immobilization Myrosinase
      agarose

          O               CHO          O
     /|   |                |      /|   |
 CPG /|-O-S1(CH2)3NH2+(CH2)3      /|-O-S1(CH2)3 N=CH(CH2)3CHO
     /|   |                |      /|   |
          O               CHO          O
                                              | Myrosinase
                                  /|   O
                                  /|   |
                                  /|-O-S1(CH2)3-N=CH(CH2)3CH=N  [Myrosinase]
                                  /|   |
                                  /|   O
```

Fig. 2. Immobilization of Myrosinase

Finally, withdrew aliquots of hydrolysate and injected into glucose sensor. The response is obtained in one minute. As shown in Fig. 3, glucosinolate contents between 3 to 130 μmoles/g were linearly related to the response data. The precision and accuracy of the procedure are good as data listed in Tables 1 and 2.

Table 1. Analytical recovery rate of sinigrin in measurement of glucosinolates using the enzyme-enzyme electrode procedure.

Concntration of gluco-sinolate in rape-seeds (μmoles)	Added amount of Sinigrin (μmoles)	Detected amount of total gluco-sinolates (μmoles)	Recovery rate of Sinigrin (%)
	16.0	38.5	103.1
22.0	74.5	92.5	94.6
("86.66")	131.5	153.0	99.6
	16.0	109.5	96.9
94.0	74.5	176.0	110.1
("909.2")	131.5	236.5	108.4

Table 2. Precision of measurement of glucosinolates in rapeseeds using the combined procedure of enzyme and glucose sensor.

Sample	Times of measurement	Mean content of gluco-sinolates (μmol/g)	SD (%)	CV (%)
"24144-3"	10	7.5	0.27	3.6
"902-2"	9	105.7	1.51	1.4

Immobilization of enzymes is a well known technique to stabilized enzyme. We separated and purified myrosinase from seeds of white mustard (*Sinapis alba*) by ethanol frac-tionation, ion exchange chromatography and affinity chromatography on Con A-Agarose. Based on the developed methods and supports of enzyme-immobilization in our laboratory, the high activity of myrosinase was covalently bound on ABSE-Agarose (CL) and porous glass beads. This is illustrated as follows:

In the second procedure of determination of glucosinolates, the extract mentioned above was added in a bioreactor (a column containing 1 g immobilized myrosinase) and shaked for 5 minutes. An aliquot of effluent was injected into the glucose sensor. The relation between sinigrin degradated by immobilized enzyme and response on glucose sensor is shown in Fig. 4. Immobilized myrosinase could be repeatedly used for 500 times of hydrolysis. In the case of determination of total glucosinolates in rapeseed, a satisfactory correlation between immobilized myrosinase-glucose sensor method and the routine TMS-gas chromatography was obtained Fig. 5. We are pleased to know that this procedure was confirmed at Jiang Su Academy of Agriculture sciences.

In comparison with the conventional methods of glucosinolate assay, these combined procedures of bioreactor and biosensor have many advantages, such as accuracy, precision, simplicity, time saving, and cost saving, Table 3. Encouraged by those results, we are now turning our attention to prepare a Co-immobilized bi-enzyme system on the same membrane. In the preliminary experiments, we found that only extraction of glucosinolates is needed before assay on glucose sensor.

Table 3. A comparison of different methods for glucosinolates detection.

Method	Time
Enzyme-Enzyme electrode	15 min.
Gravimetric	6 hr.
Thiourea-UV	6 hr.
Palladium Chloride	3 hr.
GOD Kit	3 hr.
GOD paper	20 min.
TMS-GC	36 min.

ACKNOWLEDGEMENT

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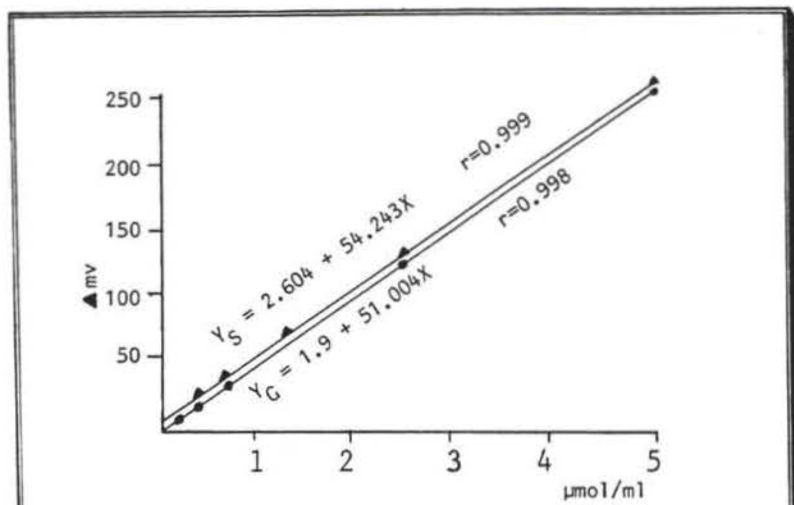

Fig. 3 Response-concentration relationship of glucose and sinigrin on GLUCOSE SENSOR. A sample containing various amounts of glucose or myrosinase-hydrolyzed sinigrin was injected into the GLUCOSE SENSOR.

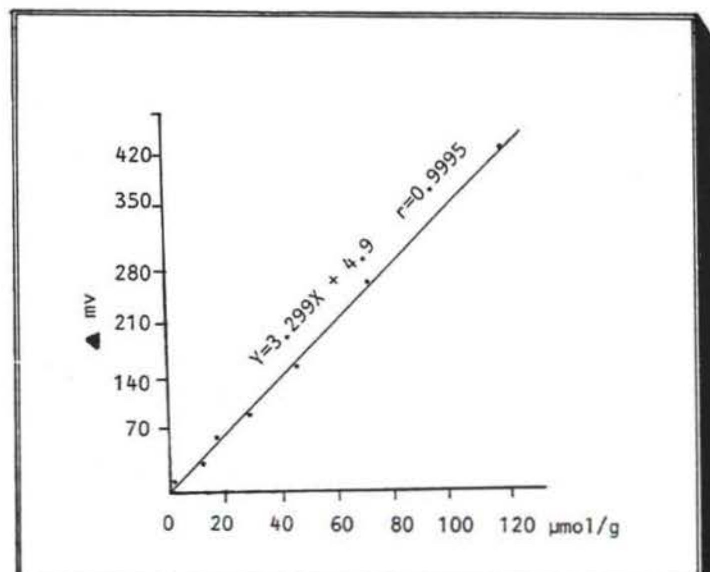

Fig. 4 Glucosinolate content in myrosinase hydrolysate and response on glucose sensor.

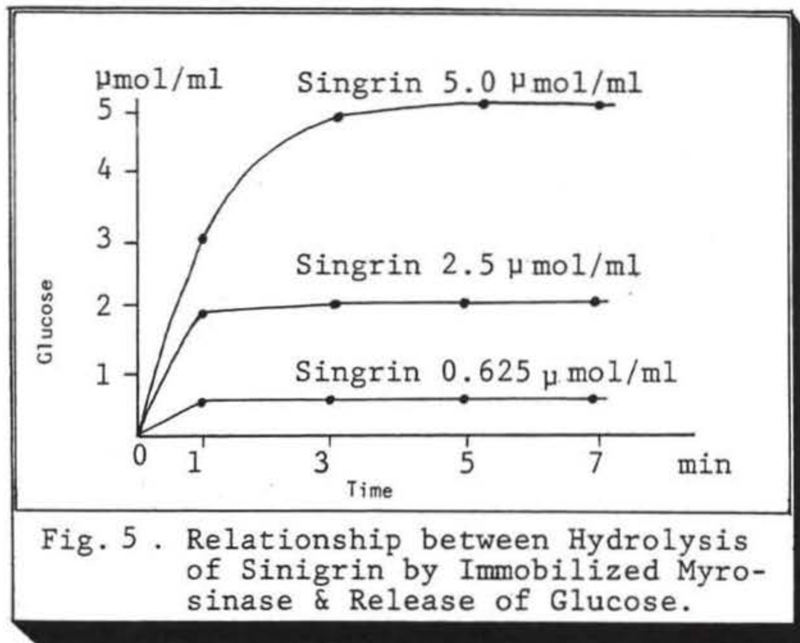

Fig. 5 . Relationship between Hydrolysis of Sinigrin by Immobilized Myrosinase & Release of Glucose.

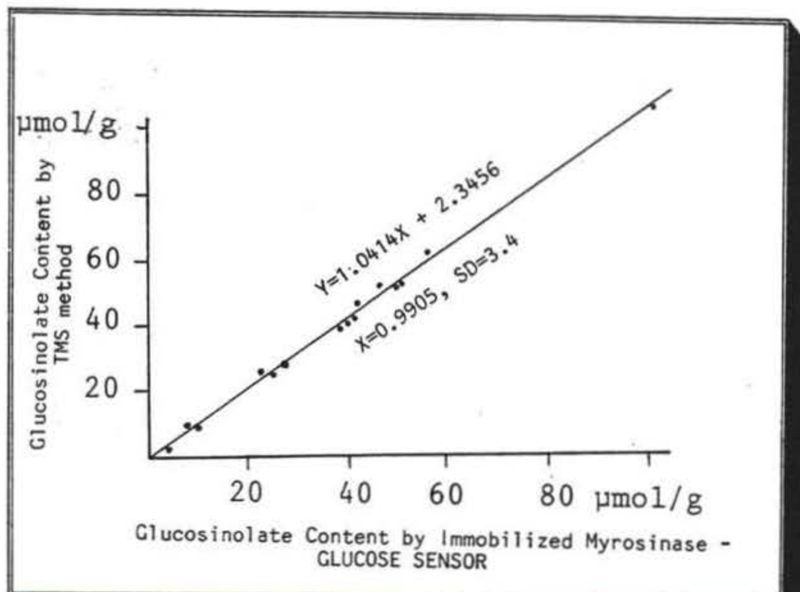

Fig. 6 . Correlation between Immobilized Myrosinase-Glucose Sensor Method and A Routine TMS Method.

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PART II

A FINAL SUMMARY REPORT OF SINO-CANADIAN RAPESEED BREEDING PROJECT

(OCTOBER, 1983 TO APRIL, 1990)

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With

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Acknowledgement

Since 1983, the Sino-Canadian Rapeseed Breeding Project was placed, as a key research project of the Ministry of Agriculture, in four Academies of Agricultural Sciences in China. The project has been supported by International Development Research Center (IDRC), Canada, and the Ministry of Agriculture, China. We would like to appreciate IDRC support and also to greatly express our sincere appreciation of Chinese and Canadian scientists, institutions, universities and the Oilcrops Network who have made great efforts for scheme and enforcement of the project. This report will present the scientific achievements. Training and other aspects will be presented with the common report of 1991.

1. SHANGHAI ACADEMY OF AGRICULTURAL SCIENCES (SAAS), SHANGHAI, CHINA

Yan Zhang and Guanghua Fang

The good quality rapeseed breeding progremme in SAAS was begun in 1981. Since 1983, 1,100 domestic and foreign good quality rapeseed germplasms were collected, preserved and evaluated. Crosses for good quality were made. Low erucic acid (single low) and low erucic acid and glucosinolate (double low) strains were selected and demonstrated in a certain area. The breeding techniques, analysis methods for quality and feeding broilers with low glucosinolate rapeseed meal were studied. Many Canadian scientists visited SAAS and held training courses in the last six years. Four postgraduates and two visiting scholars went to Canadian Universities and research stations to get their master's degrees and to engage in advanced studies, respectively. The equipments of the chemistry laboratory have been supported by IDRC and is expected to be supported further. All of these laid solid foundations for selection, demonstration and popularization of rapeseed varieties with good quality.

GENERAL OBJECTIVES OF THE PROJECT

A. Screening and evaluating rapeseed varieties (*Brassica napus* L.).
B. Improving rapeseed breeding techniques.
C. Studying the methods of analyzing erucic acid and glucosinolate of rapeseed oil.
D. Expanding production area of good quality rapeseed variety and satisfying the needs of people and animals.

PROGRESS OF THE PROJECT

A. <u>Screening and evaluating rapeseed varieties (*B. napus* L.)</u>

a. In 1984, the first year of the project, the yield comparison test of four lines with single low was conducted. The result indicated that the yield of the tested lines decreased, compared with check "Huyou 9". The decrease of yield ranged from 14.0% to 23.0%. However, the yield comparison test of 21 lines with single and double low was carried out, among which the yields of six tested lines were higher than that of check "Huyou 50" in 1989. The yield increase varied from 6.6% to 20.7%.

b. "84 - 24016" is a double low line selected in 1984. It was derived from the cross between the local early maturing and high yielding variety "No. 23" and Canadian canola cultivar "Regent". Its maturity suits to the ecological conditions of Shanghai. The experimental results showed that this line was of good resistance, of high quality oil and meal, but of comparatively low seed yield. Siliqua numbers/plant and seed numbers/siliqua of "84 - 24016" was from 79 to 139 and from 3 to 4 less than that of check "Huyou 9", respectively. The oil content of "84 - 24016" was 2 - 3% less and the seed yield was 10% less than that of the check "Huyou 9". "No. 23" was used as a recurrent parent, backcrossed to "84 - 24016" for improving the agronomic characters. The result indicated that the siliqua numbers/plant have been increased, the seed numbers/siliqua has reached 18, but the oil content has not been increased yet. Its erucic acid in oil is still low, but its glucosinolate content in meal is more than 30 µmole/g. This line now is still being improved.

c. "8701" is a single low strain derived from the cross between the local early maturity and high yield strain "8201" and the Australian cultivar "Wesbell" in 1983. After several years of field and chemical selections, mass selection was made in F_4. Since 1987, it entered into the seed yield comparison test, such as the cooperative tests in 1987 and 1988, Shanghai Rapeseed Variety

Performance Pre-test in 1988, Shanghai Rapeseed Variety Performance Test and the National Variety Performance Test in the lower reaches of the Yangtze River in 1989. Eight good quality strains and two check varieties, "Ningyou 7" and local check, were put into the National Variety Performance Test in the lower reaches of the Yangtze River. Among them, the seed yield of "8701" ranked third, and the oil yield ranked second in the test. Seed yield of "8701" was the same compared with check "Ningyou 7". There was no significant difference in comparison of the mean, Table 1. However, seed yield of "8701" was significantly different from the local commercial varieties in Jiangsu, Zhejiang, Anhui provinces and Shanghai, Table 2. The yield of 8701 was stable in seven locations, Table 1. According to the determination in 1989, linoleic and erucic acid contents were 22% and 0.25%, respectively, in oil of "8701". Oil content was 41% in dry seed. The growth phase was 235 days. It is 1-2 days earlier than that of the check "Huyou 50". Thus, "8701" has reached the objective of the project. This line has been checked and accepted by the experts of the Industrial Crops Group of Shanghai Crops Examination Committee.

B . **Improving rapeseed (*B. napus*)**

a. Anther and pollen culture: The laboratory of rapeseed biotechnology was built under the support from Canadian IDRC and the Ministry of Agriculture, China. Since 1985, several factors influenced the embryoid induction frequency, and plant regeneration rate were investigated. These factors are genotypes of materials inoculated, anther/pollen inoculation time, sucrose and auxin concentrations in the medium, changing temperature treatment and embryoid types. Fifty eight lines from haploid are planted in the field for selection in 1990.

Table 1. The Comparison of the Seed Yield and Stability among Eight Good Quality Rapeseed Varieties (B. napus L.), 1989.

Strains	Seed Yield* (kg/ha)	Difference** 5%	Difference** 1%	Interactive Variance	C.V.(%)
Ningyou 7(CK)	1859.9	a	A	0.273	14.05
Jian 7	1824.8	ab	A	0.090	8.22
1026	1824.8	ab	A	0.218	12.79
8701	1799.9	ab	A	0.039	5.49
4039	1749.8	b	AB	0.021	4.12
75-01-1	1649.9	c	B	0.090	9.10
D89	1464.9	d	D	0.131	12.34
126	1154.9	e	D	0.137	16.02
135	1124.9	e	D	0.105	14.40

* The average of the seed yield of 7 testing locations.
** Varieties with the same letter in the column are not significantly different.

Table 2. The Comparison of the Seed Yield between 8701 and Local variety, 1989; showing no significant differences.

Place	Strains	Seed Yield (kg/ha)
Shanghai	8701	1669.5
	Huyou 50 (CK)	1540.5
Jiangsu	8701	2079.9
	Shenyou 1 (CK)	1922.6
Zhenjiang	8701	1657.5
	92-13-58 (CK)	1622.5
Anhui	8701	1721.3
	Dangyouzao 1(CK)	1725.0

b. Artificial vernalization: Artificial vernalization for winter *B. napus* was studied for speeding up breeding. The result indicated that the germinating seeds of early/medium materials can develop, bloom, and set siliquae normally after artificial vernalization. The rate of setting siliquae can be increased using artificial supplementary pollination in growth room. Thus, the breeding materials on hand can add one growth cycle in summer of Shanghai.

C. **Studying the methods of analyzing erucic acid and glucosinolate**

The chemistry analysis laboratory for rapeseed quality was set up in 1984. Assay methods of UV thiourea, TMS, thymol and palladium were introduced. Three technical evaluations namely the modified UV thiourea assay method, infrared spectrum method and modified TMS method have been established. Standard analysis method for erucic acid has also completed the technical evaluation under cooperating with the Institute of Oil Crops of Chinese Academy of Agricultural Sciences, Huazhong University of Agriculture and Jiangsu Academy of Agricultural Sciences. Cooperated with Shanghai Institute of Biochemistry, Academia Sinica, a rapid determination method of total glucosinolate in rapeseed was studied using enzyme electrode principle. GUL - 01 glucosinolate meter has been developed by Shanghai Institute of Biochemistry, Academia Sinica, based on this principle.

D. **Expanding production area of good quality rapeseed variety and satisfying the needs of people and animals**

a. In 1986, a natural isolation condition was found in a small island of Qingpu county. There, a base of reproduction foundation seed has been built in order to insure the seed purity.

b. The double low strain "84 - 24016" was put into demonstrational experiment in Songjiang and Nanhui counties in an area of 34 ha from 1986 to 1988. The single low strain "8701" was demonstrated in Songjiang, Qingpu counties and Baosheng district covering the area of 16 ha.

c. The feeding broilers test with low glucosinolate rapeseed meal has been studied according to schedule.

d. Salad oil, salad sauce and margarine have been processed using good quality rapeseed oil.

RESEARCH METHODS

1. This project was conducted in the experimental farm of SAAS mainly. There were 250 germplasm materials each year in a complete systemized design (CSD). About 700 hybrid materials were planted in the nursery in CSD each year. About 250 lines were grown in the line plot in CSD. Ten to 20 strains were tested per year in a completely randomized design (CRD) with three replications. The regional experiment tested 8 - 10 strains in CRD with three replications.

2. The main breeding method was that the Chinese high seed yield and early maturing cultivar was used as maternal plant and the Chinese/ foreign single/double low cultivar was used as parent plant and intervarietal cross was made. The hybrid progeny was selected in field and chemistry laboratory. Backcross is a common method for improving some agronomic characters in hybrid progeny.

3. The production demonstration experiment was carried out in Shanghai suburbs. We discussed the cultivation techniques with local technicians and agrono-mists. The local agriculture company is held responsible for this experiment.

4. The experiences and lessons of the research method:

a. The research area of the rapeseed breeding with good quality is extensive. We organized scientists of breeding, agronomy, chemistry, plant protection and biotechnology specialties to discuss the research proposal. Also, we have made cooperation with other institutions.

b. Artificial vernalization test was studied with success. Two growth cycles can be finished within one year in field and growth room in Shanghai. The rapeseed breeding programme will be speeded up.

c. At the beginning of the project, the selection of the quality characters received a great attention due to transferring good quality characters into local cultivars. With the development of rapeseed quality breeding, the most important thing is to select the high yield traits. Backcrossing was used for improving agronomic traits of the breeding materials.

d. Virosis is the main disease in Shanghai. Resistant source has not been found in *B. napus* yet. A few materials possess tolerance. Resistant source will be searched in

other species of *Brassica*.

e. Due to the lack of parent research, a certain blindness in making hybrids is existent.

f. The F_2 population is too small. The selection efficiency is affected.

g. A set of reproduction systems of fine variety which is suited to the *Cruciferae* vegetable crops intercropping area in Shanghai will be studied in the future.

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2. INSTITUTE OF OILCROPS CHINESE ACADEMY OF AGRICULTURAL SCIENCES, WUHAN, CHINA

Chengqing Liu

GENERAL OBJECTIVES

1. **Selecting and identifying rapeseed varieties:**

   To select and breed low erucic acid varieties and low erucic acid-low glucosinolate varieties which are adaptable to the upper and middle reaches of Yangtze River in China.

2. **Improving breeding techniques:**

   To investigate methods of shortening breeding time and identifying *Sclerotinia sclerotiorum* resistance or tolerance in rapeseed varieties.

3. **Investigating suitable methods for the analysis of erucic acid and glucosinolates.**

ACHIEVEMENTS AND RESULTS

1. <u>Selection of low erucic acid and double-low varieties</u>

During the first and second periods of the project, three single-low varieties and three double-low varieties (*B. napus*) were bred up. Among these, 5 have been licensed by the provincial government. Their names and origins are described as follows:

Zhongdi* No. 1 (81002), by systematic selection from Ganyou 5.
Zhongdi* No. 2 (81007), from the cross of Ganyou 5 x Expander.
Zhongdi* No. 3 (81008), from the cross Oro x Shanghai 2413.
Zhongshuang** No. 1 (84001), from (84008 x Ganyou 5) x PB52.
Zhongshuang** No. 2 (84004), from Start x Ganyou 5.
Zhongshuang** No. 3 (84039), from Ru3 x Ganyou 5.

<u>Note</u>: *Zhongdi means single low (erucic acid), and ** Zhongshuang means double-low (erucic acid and glucosinolates).

A. <u>Quality</u>:

The qualities of the six varieties are in accordance with the requirements of quality determinants. Results of several years' testings showed that contents of erucic acid and glucosinolates of those six varieties were less than 1% and 25 µmole/g respectively, oil contents varied from 41% to 43%, all of which were higher than the oil contents of the double high varieties cultivated widely in our country, Table 1.

B. <u>Yields</u>:

Yields of the improved varieties were equal to or higher than those of the check local commercial cultivated varieties.

<u>Single-low varieties</u>: Zhongdi No. 2 and Zhongdi No. 3 were approved with high yields in regional tests. The yield of Zhongdi No. 2 was almost equal to that of the check Ganyou 5 while the yield of Zhongdi No. 3 was even higher than that of check, Table 2.

The three varieties have been popularized and put into production in upper and middle reaches of Yangtze River as well as in Henan and Anhui provinces. According to statistics, accumulative cultivated areas of Zhongdi No. 1, Zhongdi No. 2 and Zhongdi No. 3 have achieved 600,000 mu (40,000 ha), 900,000 mu (60,000 ha) and 1,846,800 mu (123,100 ha) individually. It was proven by productional activities both in Hubei and Yunnan that Zhongdi No. 2 and Zhongdi No. 3 possessed good production potentials, exceeding

Table 1. Quality characters of 5 improved varieties

Varieties	Components of fatty acid (%)							glocos. (umol/g)	Oil Content (%)
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1		
Ganyou 5(CK)	3.57		15.15	13.95	9.49	10.78	47.06		39.43
8 2 1 (CK)	3.51		12.25	13.74	9.87	40.96	47.52		41.00
Zhongdi No.1	3.42	1.75	63.72	22.14	9.81	1.02	0.21		42.00
Znongdi No.2	3.58	1.19	63.24	22.12	9.09	0.79	0.00		42.60
Zhongdi No.3	3.25	1.51	64.94	20.58	8.66	1.08	0.00		41.00
Zhongshuang 1	3.26	1.19	64.01	22.13	9.01	0.61	0.23	14-23.79	42.00
Zhongshuang 2	3.27	1.21	64.18	22.05	8.97	0.68	0.05	13-22.10	43.00
Zhongshuang 3	3.29	0.31	63.76	21.98	9.10	0.72	0.16	14-25.13	42.00

Table 2. Yields of 3 single-low varieties in regional tests during 1982.

Place	Improved variety			Check variety	
	Name	Yield (kg/mu)*	+/-over Check (5)	Name	Yield (kg/mu)
<u>National Trials</u>					
Middle Reaches of Yangtze River	Zhongdi No. 1	92.9	-13.0	Ganyo 5	106.8
Middle Reaches of Yangtze River	Zhongdi No. 2	97.6	-8.6	Ganyo 5	106.8
Upper Reaches of Yangtze River	Zhongdi No. 3	130.0	+4.4	Xinan 302	124.2
<u>Provinancial Trials</u>					
Hubei Province	Zhongdi No. 1	111.2	-15.4	Ganyo 5	131.5
Hubei Province	Zhongdi No. 2	121.2	-7.8	Ganyo 5	131.5
Sichvan Province	Zhongdi No. 3	134.0	+19.1		112.5
Yunnan Province	Zhongdi No. 3	185.8	+4.6	Yungyou 31	177.7
Guizhou Province	Zhongdi No. 3	133.4	+2.2	Qianyou 9	130.5

* 1 ha = 15 mu

the yields of the check varieties by 12-15% with a total area of 671,800 mu in Yunnan.

b. <u>Double-low varieties</u>: Results of regional tests conducted in the upper region along Yangtze River and in Yunnan province during 1986 to 1988 indicated that Zhongshuang No. 1 (84001) yielded as much as, or more than the check varieties. However, yields of Zhongshuang No. 2 (84004) and Zhongshuang No. 3 (84039) were significantly lower than that of the check Zhongyou 821, according to results of regional tests in the middle region along Yangtze River and Hubei during 1986 to 1989. Nevertheless, there were still 3 - 4 test spots where those two double low varieties yielded significantly more than the check, Table 3.

Productional experiment is an essential and important process in our country in complement to regional adaptation test. Its sample area is usually o.5 - 1 mu multiplied 2 - 3 times. Results of productional experi-ments in various provinces showed that those three double-low varieties yielded much more than checks did in all cases, Table 4.

When those three double-low varieties were put into regional and productional tests, they were also cultivated widely at suitable places for demonstration. Although influenced by extremely low temperature and heavy infection of insects and diseases, those three varieties still increased in yield compared to check varieties, Table 5.

The cultivated area of Zhongshuang No. 1 reached 62,000 mu (4,133 ha) in three provinces of Southwest China, that of Zhongshuang No. 2

Table 3. Yields of 3 double-low varieties in regional trials, 1986-88.

Regions	Varieties (lines)	Sample yield (kg)	Yield kg/mu)	+/- over Check (%)	Comparison (5%)
Upper reaches of Yangtze	Zhongshuang 1	4.5936	153.21	+2.19	not signifi.
	CK(Xinan302)	4.4976	149.92		
Middle reaches of Yangtze	Zhongshuang 2	2.8130	93.77	-10.71	significant
	Zhongshuang 3	2.8951	96.50	-8.51	not signif.
	CK(Ganyou 5)	3.1501	105.01		

Table 4. Yields of 3 double-low varieties in productional experiments.

Place	Year	Varieties (lines)	Yield (kg/mu)	+/- over Check (%)	Comparison
Yunnan province	1986-89	Zhongshuang 1	230.00	+16.0	Significant
		CK(Yunyou 31	193.20		
Sichuan province	1988-89	Zhongshuang 2	153.70	+30.9	High signif.
		CK(Xinan 302)	117.50		
Guchen of Hubei province	1986-87	Zhongshuang 2	192.75	+25.44	Significant
		CK (821)	143.72		
Dayong of Henan province		Zhongshuang 2	157.10	+18.52	High signif.
		CK(Xiangyou 11)	128.01		
Runan of Henan province	1987-88	Zhongshuang 2	244.54	+33.03	Significant
		CK (Yuyou 1)	183.83		
Qicun of Hubei province	1987-88	Zhongshuang 3	125.60	+15.60	Significant
		CK (Ganyou 5)	106.01		
Jian of Jiangxi province	1987-88	Zhongshuang 3	101.20	+14.80	Significant
		CK (Xinan 302)	87.22		

Table 5. Yield of 3 double-low varieties in large scale cultivations at various places, 1987-90.

Place	Varieties	Year	Area (mu)	Yield (kg/mu)	+/- over Check (%)	Area (mu)	Yield (kg/mu)
Yunnan province	Zhongshuang 2	1987	34.18	231.62		0.33	325.75
	Zhongshuang 2	1989	11000.00	190.20	+23.80	1.27	442.23
	Zhongshuang 2	1990	44930.00				
Gucheng of Hubei province	Zhongshuang 2	1987	378.10	153.20	+24.00	935.00	
	Zhongshuang 2	1988	9000.00	90.00	+15.70	2.10	154.00
	Zhongshuang 2	1989	28000.00	108.00			243.80
	Zhongshuang 2	1990	38000.00				
Qichun of Hubei province	Zhongshuang 3	1987	36.20	113.50		1.20	
	Zhongshuang 3	1988	375.00	113.80		3.20	136.10
	Zhongshuang 3	1989	869.50	127.40			156.30
	Zhongshuang 3	1990	5500.00				
Jian of Jiangxi	Zhongshuang 3	1989	5000.00	66.00	3.2-14.2		
Wanzai ofJiangxi	Zhongshuang 3	1989	200.00	125.20	15.27		
	Zhongshuang 3	1990	4000.00				

accumulated about 400,000 mu (26,000 ha) in four provinces of Central China, that of Zhongshuang No. 3 got more than 25,000 mu (1,666 ha) in Hubei and Henan. It is expected for them to get a great development in production this year.

C. Adaptability:

All six varietie possessed. Relatively high adaptability. Among them Zhongdi No. 3, Zhongshuang no.1 and Zhongshuang No 3 matured early and were best adapted to upper and middle reaches of Yangtze River where 3 seasons of crops were harvested, Table 6. Zhongdi No. 1, Zhongdi No. 2 and Zhongshuang No. 2 best adapted to middle and lower reaches where only 2 seasons of crops were harvested.

D. Resistance:

a. Cold hardiness: After several years' tests, especially after the happening of the serious cold in 1987 and long rains, low temperature, and rains, low temperature, and rare light during the spring of that year, it could be seen that, excluding Zhongdi No. 1 and Zhongshuang No. 3, the cold hardness of the other forur varieties were significantly stronger than that of the check varieties. For example, Zhongshuang No. 1 was successfully cultivated for the first time at Beisa village of Lijian county, Yunnan province, where rapessed had not been suitableto grow originally because of high altitude (1,800 - 2,400) m and low temperature. Owing to its strong cold hardiness, Zhongshuang No. 1 yielded the highest in history at that village with the yield of 200 kg/mu. During the Winter of 1987, the temperature at Hubei province decreased rapidly from 22°C to about - 7°C, and continued over a few days. In the Spring of 1988 there was a great snow fall when rape was flowering in Hubei. Tests of cold hardiness in these two seasons indicated that Zhongshuang No. 2 was much stronger than the check in Hubei, Table 7.

b. Resistance or tolerance to diseases: Diseases identified by natural inoculation in large area tests and by artificially inoculating in the laboratory had been made for years. Among the six single- and double-low varieties, the tolerance to *S. sclerotiorum* of "Zhongshuang No. 2" and "Zhongdi No. 2" can reach or surpass double-high "Zhongyou 821" which is considered as the best tolerant variety in China, and "Zhongshuang No. 2" also resists virus disease, Tables 8 & 9. The two varieties have the best resistance to *S. sclerotiorum* among the same kind of varieties in China.

2. Research on improving breeding techniques

A. Simple technique of vernalization to double generations in Wuhan region:

In Wuhan region rapeseed is harvested in Summer. The seeds are taken to sprout. The sprouts are put under 2°C - 5°C condition for 25 - 30 days to be vernalized. Then they are transferred to grenhouses. Next January, the second generation can be harvested. Having been artificially vernalyzed again, the third generation can mature late June. This method can keep the full and half winter hardiness of varieties and avoid that these varieties can not flower normally by the traditional method where the seeds are planted in high mountain areas and are vernalized by natural low temperature. It is also cheaper.

B. Breeding for *Sclerotinia* resistance:

It has been confirmed that differences of tolerance to *Sclerotinia* between varieties really exist. Heritability of tolerance is high. High to low tolerance is partially dominant. The crossing effects is high x high > high x low > low x low. There are no significant difference between the directions of reciprocal crossing. Back - crossing effects are obvious, Tables 9,10 &11.

3. Research on assay methods of erucic acid and glucosinolates

Under the support of Canadian experts, by cooperation of scientists from Shanghai, Jiangsu Agricultural Sciences Academy and Huazhong Agriculture University, the achievements were made

as follows:

a) The standardization of determination of erucic acid contents of rapeseed has been finalized. Now it has been adopted in breeding.
b) The standardization of determination of glucosinolates is being established.
c) There are a number of rapid methods for determination of erucic acid and glucosinolates, which are further being compared.

4. The cooperation experiment of identifying tolerance to *S. sclerotiorum* between Manitoba University and China

The cooperative experiment was decided in October, 1989. Becuase the seeds were not received on time, sowing was late. In flowering time, identification was made by inoculating on ex-leaves. The results showed that 2 - 3 varieties are with higher tolerance.

Table 6. Growth duration and adaptable regions of the six varieties.

Variety	Growth duration	Maturing date	Maturing date compared to CK	Regions best suited
Zhongdi 1	220-225	17-18 May	3-4 days later	Middle & lower reaches of Yangtze River, Henanand Anhui provinces
Zhongdi 2	220-225	18-20 May	3-4 days later	Same as Zhongdi 1
Zhongdi 3	205-210	4-6 May	0	Upper reaches of Yangtze River
Zhongshuang 1	206-210	4-6 May	0	Same as Zhongdi 3
Zhongshuang 2	220-227	18-20 May	3-4 days later	Same as Zhongdi 1
Zhongshuang 3	210-215	4-6 May	2-3 days earlier	Same as Zhongdi 1

Table 7. Resistance to freezing of Zhongshuang 2 in regional trials and large area test.

	Regional trials		Large area tests		
	1*	2*	3*	4*	5*
CK (821)	72.90	40.10	57.70	36.30	57.30
Zhongshuang 2	68.80	36.80	16.00	10.00	3.20

* 1. Frigid damage plant propor, 2. Index of frigid damage, 3. Frigid damage plants propor. in seedling time, 4. Broken plants propor. in flowering time, 5. Broken branches propor. in flowering time.

Table 8. Naturally identifying results of resistance to Sclerotinia of Zhongdi 2 and Zhongshuang 2.

Regional trials	Varieties	Years	Sclerotinia (%)		Virus disease	
			Disease rates	Disease index	Disease rates	Disease index
Hubei provinance	Zhongdi 2	1982-85	29.59	15.17		
	CK (Ganyou 5)		40.37	19.58		
	Zhongshuang 2	1987-89	13.10	4.90	14.33	5.32
	CK (821)		19.10	6.50	22.22	8.26
The middle reaches of Yangtze River	Zhongdi 2	1982-85	12.86	6.87	1.12	1.16
	CK (Ganyou 5)		20.67	11.44	1.82	1.79
	Zhongshuang 2	1986-88	13.57	6.87	11.80	4.53
	CK (Ganyou 5)		26.85	15.24	14.30	5.32
The lower reaches of Yangtze River	Zhongdi 5	1982-85	21.79	11.24	10.57	7.43
	CK (Ningyou 7)		30.71	19.47	8.78	6.16
The reaches of Huang and Huai Rivers		1988-89	5.70	3.56	4.17	2.45
	Zhongshuang 3		41.01	37.15	9.13	7.51
	CK (Qinyou 3)					
Large area test in gucheng of Hubei		1987-88	8.83	6.20		
	Zhongshuang 2		28.40	8.16		
	CK (Ganyou 5)					

Table 9. Artificially inoculating in lab, results of resistance to to sclerotinia*

Varieties**	Size of disease spots (cm²)	Comparison
039	17.86	a
381	15.79	ab
Ganyou 5	14.02	ab
Zhongdi 2	13.12	bc
081	13.00	bc
821	9.05	cd
Zhongshuang 2	8.43	d

* Ex-leaves inoculating given by Builey D.T. was adopted
** 039 is double-low line. 381 and 081 are double-high lines

Table 10. Hertibility of tolerance to Sclerotinia.

Inoculation time	Materials	Average lengths of disease spots	Variance	Broad-sense hertibility
Seedling period	84039	4.00	0.25	
	821x84039	3.40	0.50	56.0
	84039x821	3.26	0.56	60.9
	821	2.94	0.18	
	Rul	3.85	0.54	
	8400xRul	2.74	1.20	47.0
	Rulx84004	2.94	1.32	52.0
	84004	2.70	0.73	
Flowering period	84039	5.45	1.15	
	821x84039	3.68	2.13	64.8
	84039x821	2.73	2.13	59.5
	821		0.35	

Table 11. Back-cross effects on the tolerance from back-cross parents.

	(821x8439)F2	[(821x8439)x8439]B2	[(821x8439)F1x821]B1
Average lengths of disease spots (cm)	3.67	4.30	3.36
Ranges of Varieties	1.6-5.4	3.1-5.6	1-5.0

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3. QINGHAI ACADEMY OF AGRICULTURE AND FORESTRY

Zeng Ke Tian

Sino-Canadian rapeseed breeding project was conducted under direct leadership of Agriculture Department, Qinghai Academy of Agriculture and Forestry, mainly undertaking the tasks of spring *B. campestris* breeding and research. During the two phases, Qinghai Academy of Agriculture and Forestry and IDRC supported the project by cash, instruments, reagents and training. This stimulated improvement of scientific research level of persons taking part in the project, thus assuring the project to be carried out smoothly. Varieties bred have been released in the spring rapeseed regions of our country, and farmers have obtained great economic benefits.

OBJECTIVES OF PROJECT

A. First phase:

a) To select and breed two good single-low breeding lines from each of *B. napus*, *B. campestris*, and a good double-low line of *B. campestris* or *B. napus*.

b) To determine the technique of seed production for elite lines and prevent the good characters from being lost during propagation.

B. Second phase:

a) To breed *B. campestris* lines with early maturity (five days earlier than Tobin), cold tolerance and double-low adapted to regions of high sea level and high latitude in west China.

b) To breed double-low lines of *B. napus* adopted to irrigable regions of Qinghai. Maturity of these lines should be similar to that of Oro, but have better yield than Oro.

c) To breed *B. napus* lines with early maturity and double-low suitable for planting in drought field of spring sowing of north China. These lines should be similar or superior to Tower in yield and early maturity.

d) To screen germplasm of *B. campestris* with superior early maturity, frost tolerance and root rot resistance under the environment of China and Canada.

RESEARCH ACHIEVMENTS AND PRESENT SITUATION

We introduced the low erucic acid variety Oro in 1974 and double-low variety, Tower, in 1976. In the same time of experimentally planting them, we utilized them as single and double low parents to make cross combinations. At that time, because of lack of indispensable analytical equipment and accurate breeding methods, the advancement of the quality breeding was slow. After taking part in the project in 1983, we built the first lab of rapeseed quality analysis in the northwest region and gradually equiped it with the analytical instrument under the support of Qinghai provincial government and aid of IDRC. Specially, through investigating the rapeseed quality breeding of Canada and four countries of western Europe and exchanging the technology with them, our breeding methods were perfected and we made a great progress in the work of quality breeding. According to the research plan, we think that we have achieved the breeding objectives in the two phases of the project.

A. First phase (October 11, 1983 - March 31, 1987)

We bred two *B. campestris* varieties with low erucic acid and extended two introduced varieties of *B. napus*. We respectively put forward the propagating regulations for these two different rapeseed species through elite production technology. The regulation effectively prevented the verieties from losing the good characters during the propagating

process. In addition, a production base of single-low and double-low rapeseed has been formed fast in the nothern spring rapeseed regions of our country.

These varieties and varietal studies are:

a) QingYou No. 11 (the former code name 8201): it is an early maturing low erucic acid line produced in 1982 from the line of Men-24-1 with medium erucic acid using half-seed screening method. Duration of growth period is 91-120 days, seeds black and brown, 1000-seed weight 2.84 g, oil content 42.61%, content of erucic acid in oil 0.24%, and average yield 1686.75 kg/ha. The yield increased 9.44% than control variety. Its expression is adapted to cultivation in early maturing region with an elevation of 2,700 - 2,900 meters and average annual temperature of 1 - 2°C in Qinghai. It was registered by Qinghai province in 1989. Its growing areas approached 2560 ha in 1989. It showed increasing production over the spring rapeseed regions, such as Qinghai, Guanshu, Zinjiang, Tibet, and Helongjiang.

b) QingYou No. 13 (the former name-82011-4): It is a low erucic acid line produced in 1982 from a selected line, 82-286-1, of a combination of multiple cross by half-seed screening. Duration of growth is 94-124 days, seeds are mostly black, but a few are yellow, 1000-seed weight 2.85 g, oil content 41.5%, content of erucic acid in oil 0.25% and average yield 1609.5 kg/ha. The yield increased 4.43% than control variety. Its expression is adapted to cultivation for early and medium maturing region with an elevation of 2,600 - 2,800 meters and average temperature of 2°C. It was evaluated and registered by Qinghai province in January, 1989. The growing areas were 1,660 ha in the spring rapeseed regions, as Qinghai, Xinjiang, Gianshu, Tibet, Helongjiang and so on in 1989. Its yield was similar to that of local variety with high erucic acid.

c) Oro: It is the first spring rapeseed variety of low erucic acid bred by Canada, which was introduced into Qinghai in 1974. The result of test growing of multiple locations indicated that its adaptability is strong and the increasing production is significant. In 1983-1984, it was recommended by Qinghai Academy of Agriculture and Forestry to participate in regional trials of the spring rapeseed varieties of north China, the per hectare yield was 967.6-4300 kg. It showed increasing production all over Qinghai, Xinjiang, Ganshu, Tibet, Heilongjiang, Inner Mongolia and so on. It was formally defined as the released variety in the northern spring rapeseed regions in the national meeting of rapeseed regional trials in 1984. The planting areas reached 85,000 ha in 1989, and it is the low erucic acid variety with the largest growing area in the spring rapeseed regions and is also one of the earliest fine varieties with low erucic acid to be put into production in China.

d) Tower: It is the first double-low variety bred by Canada, which was introduced into China in 1976. Its drought resistance and early maturity are better than Oro and double-low character is stable through many years of appraisal. In 1983-1984, it was recommended to participate in the regional test of the spring rapeseed variety of north China. Per ha yield was 1,216.2 - 1,985.25 kg. The yield has identically shown increase on fertile land and stable on dry land in Qinghai, Xinjiang, Guanshu, Inner Mongonlia, Tibet, Heilongjinag. It was identified as a good popularized variety in spring rapeseed regions of north china by Regional Test summing-up Meeting of National Single-low and Double-low Rapeseed Varieties in 1984. The cultivated area was 46,000 ha. It is the earliest double-low variety to be put into production in China.

e) Technical study for multiplication of *B. campestris*: The out-crossing rate of *B. campestris* is over 90%. According to the biological characters of multiplication, we adopted some methods, such as bagging single plant, self-crossing by opening the bud, covering the plant lines with net and shaking the plant to

assist pollination, making the strains and varieties in the isolation plot free pollinating and simultaneously carrying out mass pollination. Using these methods, we have basically prevented the good quality trait from being lost during the propagating process and simultaneously avoided the problem of inbreeding depression. We stipulated the technical regulation for elite production. After the Department of Seed Production adopted it, the good effect was shown. The regulation passed the natonal technical appraisal and was defined as the technical standard of elite production of *B. campestris* in August 1984.

f) Technical study for multiplication of *B. napus*: Oro and Tower are the earliest single-low and double-low varieties popularized in the spring rapeseed region of China. After cultivating from the begining we encountered the coexistent situation of single-low and double-low varieties with double-high variety applied simultaneously in production. According to this situation, we arranged the following tests: test of distance of high erucic acid variety to low erucic acid variety, test of annual variation of erucic acid under natural crossing planting conditions, tests of effect for the reserved seeds in diffeent methods of purity preservation, such as covering bags for main inflorescences, covering screens for single plants, covering nets for line and variety test plots, etc. The methods have proved that covering bags, screens and nets are all able reliably to keep the characters and qualities not to change basically, and have no significant effect on pollination and bearing fruit of *B. napus*. After crossing, planting of varieties with high and low erucic acid, ranges of erucic are increased to 2.2 - 5.6%. In the conditions without insect pollination, the safely isolation distance of wind pollination is over 750 m. According to these test results, under the conditions of natural shield to ensure acquisition and artificial blockade for the pollen source, we have respectively built up a special multiplication farm from the elite of both varieties, Oro and Tower. Content of erucic acid of reproduced seeds for three years in succession varied between 0 and 1.6%, the average was 0.72%. The assumption of erucic acid of seed for the use of production below 1% and marketable rapeseed below 3% has been realized basically, and a plan of technical regulations for production of the elite of *B. napus* has been put forward. That ensured the commodity production of rapeseed with low erucic acid.

B. Second phase (April 1, 1987 - March 31, 1990)

We bred two double-low *B. campestris* strains and one double-low *B. napus* strain, and introduced and spread one double-low *B. napus* variety. These made the rapeseed production of northern spring rapeseed regions to grow from low erucic acid variety to double-low variety.

a) 86049: It is a double-low *B. campestris* strain bred from introduced strain S-13 from Sweden through selection of single plant and Chemical test. Its growth period is 106-128 days, seeds brown, 1000-seed weight 2.48 g, oil content 39.83%, content of erucic acid 0, and glucosinolate content 28 μmole/g. Yield/hectar took an average of 1540 kg, which is equal to that of double-high variety. Through the northern regional trials for two years in 1988-1989, it began to be spread in the Hailan state of Qinghai and Lasha and Hequ of Tibet.

b) 88-533: It is the early maturing double-low *B. campestris* strain bred from combination "Menyuan Rapeseed x (Tobin x Menyuan Rapeseed)". It showed the highest yield in all strain contrast tests of two years. Its growth duration is 88-106 days, seeds brown, 1000-seed weight 2.58 g, oil content 35.31%, erucic acid content 1.76% and glucosinolate content 49 μmole/g. Per hectare yield took an average of 1950 kg, increasing production 8.4% than low erucic acid controlled variety. It is ten days earlier in maturity than Tobin. It has been recommended to participate in the spring rapeseed regional trials of north China and

is a promising early maturing double-low *B. campestris* strain.

c) Dan Di: It is a double-low *B. napus* strain produced from introduced winter breeding material from Denmark. The result of characters appraisal and yield contrast for many years indicated that it has good agronomical characters and tolerance to *Sclerotinia* stem rot. It was recommended to take part in the spring regional trials of north China by our Academy in 1988-1989. It showed increasing production and the yield was the highest at 18 locations in two successive years. The average yield was 2407.4 kg/ha, 15.6% increase over Oro. Growth duration is 128 days with one day earlier than Oro, seeds brown and black, 1000-seed weight 3.3 g, oil content 44.7%, erucic acid content 0.64%, and glucosinolate content 45 μmole/g. It is adapted to irrigable areas at 2000-2600 m in Qinghai. Through the appraisal of specialists in the Regional Trials Summing-up Meeting of North China, it was regarded as good reserve line replacing low erucic variety Oro. There were about 730 ha grown in Qinghai, Guanshu, and Tibet, in 1989. It will be estimated and named this year.

d) Topas: It is a double-low variety of *B. napus* bred by Sweden and introduced into Qinghai in 1985. The result of characters appraisal and yield contrast indicated that it is adapted to mountain areas in Qinghai, and its growth duration is analogous to that of Tower. Oil content is 44.19%, erucic acid 0 and glucosinolate content 27 μmole/g. Its drought resistance and many economic characters are superior to those of Tower. It was recommeded to participate in the northern spring rapeseed regional trials by our Academy in 1988-89, yield was 827.3-4014.0 kg/ha increasing 5.2-18.9% than control variety,and it was next to the best variety. It is suitable for planting in mountain areas at 2400-2700 meters in Qinghai. Through the appraisal of specialists, it was regarded as the variety replacing Tower in mountain areas. It is initially being expanded in Qinghai, Inner Mongonlia, Tibet and Guanshu. There were 926 ha grown in 1989.

e) Screening of *B. campestris* germplasm with early maturity and cold tolerance: We have planted 87 germplasm lines of *B. campestris* in recent years. There are 28 varieties with single-low and double-low mainly from Canada and Sweden. Their growth duration are above 10 days longer than control variety with early maturity of our province. They can't mature normally at 3000 m and have weak tollerance to frost. We have planted 23 germplasm lines from Nepal, Japan and South China. Their growth duration are shorter and only 3-6 days longer than control variety, but their tolerance to frost and cold is weak. They can't also be adapted to mountain areas of our province. We have planted 30 lines from mountain areas of our province, most of them are varieties with early maturity and cold tolerance, and some may germinate at low temperature of 3°C also, but the erucic acid and glucosinolate content are all above middle level. Some good varieties (like Menyan rapeseed, Babao rapeseed, B43, Haoyou No. 5, Linjiao No. 5 and Qingyou No. 9) with both early maturity and cold tolerance, have been used as parents of crosses in breeding.

RESEARCH METHODS

A. Methods adopted in breeding

a) Breeding procedure in breeding of *B. campestris*: We mainly adopted the method of crossing and oriental selection. On making cross combination, we generally used native traditional variety as basic parent and introduced single-low and dobule-low variety as compensable parent. According to the need, sometimes we also carried out a few back-crosses in order to combine the dobule-low character with cold tolerance and breed variety with high yield. According to the difference of genetic behavior between agronomic characters and quality traits, we put emphasis on the ecological adaptability and yield traits. After ecological adaptability and yield traits became stable, we began to screen quality trait in colony containing single- and double-low genes.

b) Selection of early maturity: Because early opening of the first flower in main raceme is the mark of early maturity, it may be used as base for selection of early maturity. Because outcrossing rate of *B. campestris* is especially high, to make the colony oriental development, we must pick 3-5 siliquaes with the earliest maturity at low part of main raceme from the earliest flowering plant in every generation. Because all generations of *B. campestris* are inbreds, hybrids are formed by free pollination under isolation. In this kind of colony, if we select early maturing pods of early flowering plant, its maternal plant is certaily early maturing and the pollen is also from early flowering plant. This combination of early with early easily produce early maturing progeny exceeding parents. If this continues for several generations, the early maturity comes up at once.

c) Selection of cold resistance: Low temperature and frost are the main natural disasters of planting regions of *B. campestris* in Qinghai. Low ground temperature is often encountered during growth process of rapeseed. The temperature is -11°C in emergence period, -7°C in stem elongation period and -2.5°C in flowering period. The variety bred will not be released if it is not able to tolerate the temperature. In the breeding process, we imitated this temperature to identify some important materials in refrigerator. In addition, we mainly grew materials in production regions in order to conduct identification and selection under natural situation. We set up two locations for ecological test at 3,900 - 3,100 m. The materials selected after F_2 were grown in these locations in order that we could screen freeze-tolerance materials with the aid of adverse natural climate there. This method is economic and reliable.

d) Adoption of isolating equipment: Because outcrossing rate of rapeseed is high, we must control this process in rapeseed for oriental selection. Especially for *B. campestris*, we must not only prevent contamination of alien pollen, but also avoid depression brought by selfing and inbreeding. So, every generation must be grown in the isolating plot in the breeding process. Generally, the early generations were all isolated by net; pollination of the colony in the net was free. The colony in the net might attain certain purity through oriental selection. To elimnate the effect that the net obstructed light, we generally shorten the time of net covering at flowering period. In multiplication period of massive seeds, varieties were all planted in areas with natural shields or safety distance.

B. Breeding materials and their origin

Variety	Origin
B. campestris	
Span	Canada
Torch	"
Candle	"
Tobin	"
Emma	Sweden
SONJA	"
KOVA	"
9166	Nepal
9128	"
Men Yan	China
Ba Bao	"
Haoyou No. 5	"
Xiaoleiqi	"
B. napus	
Oro	Canada
Tower	"
Altax	"
Westar	"
Regent	"
Middas	"
Topas	Sweden
Hanna	"
Ww1258	"
Ww1256	"
Dan Di	Denmark
Marnoo	Australia
Qinyou No.4	China
Luzhou No.5	"

C. Arrangement of field experiments and regional trials

Field experiments were mainly arranged in three locations, Xiling, Fuzhu and Menyan. In Xiling

location,altitude is 2,295 m, average air temperature is 4.8°C and climate and experimental equipment are better than the other two locations. In Fuzhu, altitude is 2,488 m, average air temperature is 4°C with half mountain climate condition. Because of no threat of frost, the yield tests of *B. campestris* were arranged in this location. In Menyan, altitude is 3,000 m, average air temperature is -0.5°C, and frost and cold are frequent. So, early maturing and cold tolerance breeding materials were arranged in this location to carry out identification and selection. Areas of experimental plot for early generation were generally 5 x 1.5 = 7.5 m² with 2-3 replications for identifying plant lines, and 5 x 3.6 = 18 m² with 4-6 replications for yield comparision.

Regional trials consisted of two kinds: provincial level and national level. The regional trials of provincial level have 10 locations. Elite lines performing well in the regional trials were directly put into production demonstration in our province or were recomended to regional trials of national level. The spring rapeseed regional trials at national level were mainly distributed in the spring rapeseed regions of eight provinces of north China: Qinghai, Guanshu, Inner Mongolia, Shanxi, Xinjiang, Hebei, Helongjiang and Tibet. The seed company of China entrusted us to be responsible for work of organization and practice of project in spring rapeseed regions of north China.

D. Demonstration and release of new lines : methods and effect

Through the regional trials, for lines where number of locations with increasing production is more than 70% of all locations or own outstanding good characters, we applied to Provincial or National Evaluating Department of Variety for evaluation and registration. Simultaneously, according to adapting range of variety, we organized their demonstration and release. The breeding unit and local releasing station of agricultural technique, together, were in charge of demonstration and release. In accordance with the results of agronomic research, we have drawn up regulation of cultivating techniques. Seed used in popularization were all provided by the seed company of the country or province. Marketable seeds of low erucic acid variety was limited below 1% for erucic acid content.

After Sino-Canadian co-operation project was developed, popularization of single-low and double-low varieties in spring rapeseed regions of China was carried out under the organization and guidance of Qinghai Academy of Agriculture and Forestry. As the organizer of joint test in northern spring rapeseed regions, we dispatched technicians to carry out technical consultation and inspection in every test location. These made rapeseed variety to be replaced smoothly. According to statistics of 1989, the planting areas of spring rapeseed varieties with single-low or double-low were 137,000 ha and accounted for one third of total areas of spring rapeseed all over the country.

E. Study and Improvement for Technique of Quality Analysis

Under the support of our country and aid of IDRC, to coordinate the subject of rapeseed breeding, we set up an analytical laboratory of rapeseed quality equiped with three gas chromatographs, three spectrometers with one U.V. spectrophotometer and a whole set of analytical equipments carrying out both quick qualitative and precise quantitative assay for fatty acid content, fatty acid composition, gloucosinolate level and its composition. A group of technical personnel has been trained to develop research work in accordance with breeding.

a) Introduction and improvement for analytical method of half-seed: Half-seed analysis is a key technique of low erucic acid breeding. Because wet seed is used in original method, seeds which have been treated must be sown at once or stored after drying. To overcome the problem, we replaced wet seed with dry seed, as a result, the process was simplified and efficiency was raised.

b) Research of glucosinolate test: In accordance with default of instrument and reagent in basical unit, we have studied EDTA capacity method determining total

glucosinolate content in rapeseed. It passed provincial level appraisal in 1988. This method analyses glocusinolate by determining sulphate root in glucosinolate molecules. Its advantage is that it does not need expensive apparatus, and the reagent is cheap and easy to purchase. After being released, it was welcomed by the basic unit.

c) Screening of fast test methods for erucic acid: Through the comparison of some methods (such as paper chromatography, heterometric degree, titration, and sampling), we think that the second one is fast and adapted to purchasing department, and the first one is accurate and adapted to screening of seed in the breeding process.

d) Improvement of sample esterification for gas chromatograph: In the introduced method of gas chromatograph determining fatty acids, the sample is esterified by methanol. We experimentally replaced methanol with ethanol, though time of esterification is a little longer, but it may avoid harm of methanol to technicians and protect their health.

PUBLICATIONS DURING THE PROJECT PERIOD

1. Tian Zhengke, Canadian rapeseed in China.
2. Tian Zhengke, Distribution of spring rapeseed in China.
3. Tian Zhengke, The utilization of self-bred slow-witted characters of Rape *B. campestris*) in hybrid production.
4. Tian Zhengke, Breeding of *B. campestris* variety with early maturity, cold tolerance and double-low.
5. Tian Zhengke, Mustared (*B. jun*cea) in China.
6. Zhong Gedong, High yield cultivating practices research of spring-sowing *B. napus*.
7. Zhang Gedong, High yield cultivating technique of spring *B. napus* for mechanized production.
8. Hou Yulan, chemical analysis of erucic acid of rapeseed.
9. Hou Yulan, Testing glucosinolate content of rapeseed with EDTA-capacity method.
10. Tian Zhengke, Technical regulation of elite production for *B. campestris*.
11. Wang Shenghao, Technical research of isolating multiplication and keeping purity in productive base of commodity.
12. Du Dezhi, The research of correlation between quality characters and agronomic characters in *B. napus*.

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4. XINJIANG ACADEMY OF AGRICULTURAL SCIENCES

Zhaomu Wang

The second round of Sino-Canadian rapeseed project was conducted from April 1987 to March 1990. During that period, the rapeseed group of Xinjiang Academy of Agricultural Sciences has successfully completed the breeding objectives.

BREEDING OVJECTIVES

A. Breeding single-low or double-low *B. juncea* cultivars adaptable to Xinjiang and the other spring rapeseed areas in China.

B. Evaluating collected double- or single-low *B. napus*, *B. campestris* and *B. juncea* introductions.

RESEARCH HIGHLIGHTS

1. **Released cultivars**

As a result of previous years work, two *B. juncea* cultivars, Xinyou No.4 and No.5, the first low erucic acid *B. juncea* cultivars, were registered and released by Xinjiang Crop Cultivar Examination Committee in May 27, 1989. Xinyou No.4 (test code, 85 - 1312) is good in one year-one crop areas in the North; and Xinyou No.5 (test code, 85-991) is good to be grown in cold mountain areas or one year-two crops areas. Their yield, quality, maturity and resistance are shown below.

2. **Yield capacity**

2.1 Regional tests: In the provincial trial, Xinyou No.4 yielded 2848.5 kg/ha, and ranked the first. In six out of eight locations, Xinyou No.4 gave the highest yield. Compared with check variety (Xinyou No.1), the range of yield increase was 3.63 - 30.34% for Xinyou No.4. Xinyou No.5 yielded 2649 kg/ha, occupied the third place, 4.59% lower than the check. However, no significance existed in yield difference between Xinyou No.4 and the check or Xinyou No. 5 and the check, Table 1.

In Northern regional co-op trail which covered six provinces, Xinyou No.4 and No.5 were 2.41% and 2.9% lower than check (Xinyou No.1), ranked second and third, respectively. Out of the 10 locations, both cultivars got higher yield than the check at five locations. The yield range increase was 0.84 - 74.14% for Xinyou No.4, and 3.14 - 144.83% for Xinyou No.5, resprectively.

2.2 Performance of yield in farmer's field: While carrying out the regional test, the two cultivars were being demonstrated at two farmer's fields. Results show that both cultivars yielded higher than the check, Table 2. From 1987 to 1989, Xinyou No.5 was grown in 0.42, 1.3 and 20.2 ha in Baicheng county, respectively, and yielded 3210, 2940 and 1980 kg/ha on the average. In 1989, Xinyou No.5 was grown in 218 ha in Xinjiang province. Its yield was 10% higher on the average than the commercially dominant cultivars. Therefore, it is accepted by farmers.

3. **Quality aspects and oil content**

Erucic acid content in the two cultivars is below 1%. Oil content is higher than the check (Xinyou No.1) as well as double-high cultivars, Baicheng Hung and Yili Hung, which are the commercially dominant cultivars in Xinjiang province at present, Table 3.

The breeder seeds of Xinyou No.4 and No.5 is erucic acid-free. Its oleic and linoleic acid content are significantly higher than high erucic acid cultivars. Therefore, its nutrition value is much higher than the high erucic cultivars.

Table 1. Yield of Xinyou 4 and Xinyou 5 in regional test.

Test	Year	Cultivar	Plot Yield kg	Average yield (kg/ha)	+/- over Check (%)	Difference	Rank
Provincial test	1986-88	Xinyou 4	6.33	2848.5	+ 2.60	ns	1
		Xinyou 5	5.89	2649.0	- 4.59	ns	3
		Xinyou 1	6.17	2776.8	-	ns	2
Among province test	1988-89	Xinyou 4	4.19	1883.9	-	ns	2
		Xinyou 5	4.15	1869.5	-	ns	3
		Xinyou 1	4.29	1928.9		ns	1

Table 2. Yield of Xinyou 4 and Xinyou 5 in demonstration (kg/ha).

Location	Year	Test variety	Average yield	+/-	Check (%)	Difference	Rank
Zhaosu	1988	Xinyou 4	3031.5	+	14.8	*	1
	1988	Xinyou 5	2767.5	+	4.6	n	2
Anninqu	1988	Xinyou 4	2446.5	+	5.8	n	1
	1988	Xinyou 5	2163.1	+	1.5	n	2

Table 3. Fatty acid and oil content in *B. juncea* cultivars.

Cultivar	Fatty acid composition (%)							Oil
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	(%)
Xinyou 4	5.27	0.51	44.82	35.57	13.44	0.39	0.00	37.88
Xinyou 5	4.95	-	44.82	36.87	13.44	0.12	0.00	37.56
Xinyou 1	3.63	0.26	24.12	24.25	11.42	13.49	22.83	37.05
Baicheng Huang	3.40	0.20	18.4	23.00	11.27	13.61	30.12	36.21
Hili Huang	3.21	0.21	20.55	21.21	13.64	11.77	29.41	36.34

4 Adaptability

According to the multi-year and multi-location tests, the two low erucic *B. juncea* cultivars have good adaptability to wide areas. Xinyou No.4 is a mid-early cultivar in maturity. Total growth period is 85-90 days. Xinyou No.5 is an early cultivar which has a growth period of 75-80 days, Table 4.

5. Resistance to stress

The plant height of the two cultivars is quite short. Xinyou No.5 is only 1.6 m in height. It is lodging-resistant, and is field favourable to be harvested by combine. Xinyou No.4 is tolerant to whiterust and downy mildew, and its seeds are plump with high oil content.

A number of single- or double-low lines with high yield capacity have been selected, and some male sterile *B. juncea* with low erucic acid content have been bred. These could be the fundamental materials for this breeding project.

6. Introductions

A number of double-low *B. campestris* and *B. napus* and single-low *B. juncea* introductions have been evaluated in field and lab for possible use. A total of 182 varieties have been intro-duced from Canada, France, Sweden, India, Poland and Australia in the last three years, Table 5.

Table 4. Growing period and adaptable areas of Xinyou 4 and Xinyou 5.

Cultivar	Days to maturity	Date of maturity (month/day)	± over check (day)	Suitable growing areas
Xinyou 4	85-90	7/20	+ 1-2	one year-one crop areas in the north
Xinyou 5	75-80	7/10	+ 8-12	cold mountain areas or one year-two crops in plain areas

Table 5. Introduced materials.

Country of origin	No.	B. juncea	B. napus	B. campestris	Others	Year
France	115	96	12	0	7	1987
Canada	7	1	3	3	0	1986
Sweden	50	15	14	16	5	1988
Awstralia	5	4	1	0	0	1988
India	2	0	0	2	0	1989
Poland	3	0	3	0	0	1988
Total	182	116	333	21	12	

7. Utilization

Except a few strong winter types, most of the introductions can ripe normally in Urumoqi with a growth period of 71-96 days.

7.1 Direct use in production: After successfully growing Oro, which was introduced from Canada in 1974, we have tried Tower, Topas, Leijinter recently. Up to 1989, the total acrage of high quality B. napus grown in Xinjiang is nearly 50,000 ha. It has put the rapeseed production in Xinjiang province at a new level.

7.2 Indirect use: Through two years investigation, some high yield and high quality materials such as, spring *B. napus* (Topas Line and Wesbrook), *B. campestris* (Emma and Tobin) etc., have been used in our double-low rapeseed breeding program as the high quality parental forms.

From the economic point of view, some favourable agronomic characters in some of the introductions have been identified. For example, two American *B. juncea* strains, American 1 and 2, bred more pods/plant, were white rust- and downy mildew- tolerant, and the 1000 seed weight of RH was up to 6.75g. A cross of Chinese x *nigra* produced more pods/plant; and a spring *B. napus* gave 30 seeds/pod, etc. All of these materials with good agronomic traits have been used in the recent breeding program as well.

DISCUSSIONS AND SUGGESTIONS

High yield, high quality, disease resistance or tolerance are still the key points in single- or double-low rapeseed breeding program in China. At present, to develop high yielding, disease resistant or tolerant, double-low *B. juncea* cultivars is even more difficult since the gene source of low glucosinolate in *B. juncea* is not available. thus, to exchange breeding materials between Chinese and Canadian scientists will be mutually beneficial and to work out effective selection and evaluation methods in breeding double-low *B. juncea* in the third round of the project is also important.

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PART III

BRASSICA SUB-NETWORK :

COUNTRY PRESENTATIONS

THE FAST DEVELOPING OILCROPS NETWORK A SUMMARY REPORT

Abbas Omran

The Oilcrops Network Project and its constituent projects have an increasing role in enabling large number of small farm families to obtain their daily requirements of oils and fats. The project links together researchers working in a number of IDRC-supported oilcrop improvement projects and national research programmes. These projects are proving increasingly effective in raising edible oil production in several countries of Eastern/Southern Africa and South/South-East Asia

The Network is coordinated by an IDRC Network Advisor, based in Ethiopia at present. The Advisor organizes and facilitates all the Network and Sub-Networks workshops, committees and other meetings, edits and arranges publication of the workshop proceedings, Oilcrops newsletter, reviews, bibliographies and other publications. He visits and provides advice, assistance and encouragement to scientists in national programmes and especially the IDRC-assisted projects attatched to the Network, and also provides assistance to the Ethiopian National Oilcrops Program which is supported by IDRC.

In Phase I (1981-84), initial steps were taken to link IDRC-supported national oilcrop research programmes in Eastern and Southern Africa and the Indian region. Phases II (1984-87) and III (1987-90) continued to strengthen and expand the Network to include additional countries such as China and the Philippines. For a more detailed description of the Network and its various activities please consult previous publications listed in the back.

THE SUB-NETWORKS

Four Sub-Networks were created:

1. Brassica Sub-Network, 1987.
2. Sunflower Sub-Network, 1988.
3. Sesame Subnetwork, 1988.
4. Other Oilcrops ub-Network, 1989.

These Sub-Networks enabled scientists specializing in the various crops to interact more closely and economically in Sub-Network workshop, rather than in the larger Network meetings, and to organize successful collaborative research programmes.

INFORMATION

An effective oilcrops information system was developed to enable scientists to obtain the necessary specializedd information on oilcrops. The Network publications, so far, includes:

- Riley, K.W. (editor). 1984. Oil Crops. Proceedings of a Workshop held in Cairo, Egypt, 3-8 September 1983. IDRC MR93e. 178 pp.
- Omran, A. (editor). 1984-1989. Oil Crops Newsletter Nos. 1-6, Published annually by the IDRC Oilcrops Network.
- ________________. 1985. Oil Crops: Proceedings of the Second Oil Crops Network Workshop held in Hyderabad, India, 5-9 February 1985. IDRC MR105e. 258 pp.
- ________________. 1987a. Oil Crops: Niger and Rapeseed/Mustard. Proceedings of the Third Oil Crops Network Workshop held in Addis Ababa, Ethiopia, 6-10 October 1986. IDRC MR153e. 250 pp.
- ________________. 1987b. Oil Crops: The Brassica Subnetwork. Proceedings of the First Meeting of the Brassica Subnetwork held in Uppsala, Sweden, 7-9 May 1987. IDRC MR168e. 76 pp.
- Basudeo Singh and D.R.C. Bakhetia. 1987. Screening and Breeding Techniques for Aphid Resistance in Oleiferous

Brassicae : A Review. Oil Crops Network, IDRC Canada. 50 pp.
- Omran, A. (editor). 1988. Oil Crops: Sunflower, Linseed, and Sesame. Proceedings of the Fourth Oil Crops Network Workshop held at Njoro, Kenya, 25-29 January 1988. IDRC MR205e. 340 pp.
- Saharan, G.S. 1989. Sesame Diseases. An Annotated Bibliography from 1900-1988 literature. IDRC MR 227e. 75 pp.
- Omran, A. (editor). 1990a. Oil Crops: Proceedings of the Three Meetings held at Pantnagar and Hyderabad, India, 4-17 January 1989. IDRC MR252e. 332 pp.
- ________________. 1990b. Oil Crops: Sesame and Sunflower Subnetworks. Proceedings of the Joint econd Workshop held in Cairo, Egypt, 9-12 September 1989. IDRC MR271e. 222 pp.

COLLABORATION

By encouraging collaborative research between scientists in stronger research programmes (including Canada) with those in weaker programmes, and arranging peer review of research results and programmes in regular workshops and in other ways, the Network helps to raise scientific standards in the member countries. In order to make the most efficient use of limited resources, the advisor is seeking to collaborate in any way possible with other Networks or Institutions involved in oilcrop research and development. In particular, collaboration is sought with IBPGR, the various FAO Networks and activities on oilcrops, as well as with CGIRC and ICRISAT. In order to make the needs for oilcrop research more widely known, and to enhance the resources available to the network and its members, the Advisor and all concerned informs other appropriate donors of the needs of the Network and its members, and invites their representatives to attend Network workshops and meetings.

GERMPLASM

After a slow start due to bureaucratic problems and national reluctance to share germplasm, a useful germplasm exchange programme has been developed through the Network coordination office. The Network Advisor coordinates the collection of germplasm both from member countries and from other sources, makes up nurseries and distributes them in a timely fashion to those members who request them, and who supply germplasm, Table 1. The Advisor also encourages and assists members to exchange germplasm bilaterally, either as part of collaborative research programmes, or in other ways.

Table 1. Oilcrops Network Nursery (up to April - 1990).

Country	Brassica	Linseed	Niger	Safflower	Sesame	Sunflower	Groundnut
Bhutan	4	-	1	-	-	-	-
Canada	-	-	-	3	-	45	-
Cyprus	-	-	-	6	-	-	-
Egypt	-	-	-	10	10	-	-
Ethiopia	5	4	8	3	9	8	-
FAO	-	-	-	-	49	-	-
India	31	2	-	2	-	-	-
Israel	-	-	-	-	21	-	-
Kenya	1	1	-	-	2	2	-
Mexico	1	-	1	7	11	7	-
Nicaragua	-	-	-	-	9	-	-
Nepal	11	2	1	-	1	-	4
Pakistan	-	-	-	3	-	-	-
Philippines	-	-	-	-	16	-	-
P.R.China	8	1	-	2	-	-	-
Somalia	-	-	-	-	13	-	-
Sirilanka	-	-	-	-	3	-	-
Sudan	-	-	-	-	20	-	-
Sweden	8	-	-	-	-	-	-
Tanzania	-	-	-	-	9	-	-
Yugoslavia	-	-	-	-	-	16	-
Total	64	10	11	36	173	78	4

Since this workshop is devoted to Brassica, I shall list all Brassica germplasm lines/varieties received from and dispatched to Network coutnries in the past three years:

BHUTAN

1. Mongar Local
2. Yunkar Serti(local)
3. Bajo Lcoal
4. Baylegphug Local

ETHIOPIA

1. Yellow Dodolla
2. S-67
3. S-71
4. S-115
5. Awassa Population

INDIA

1. Mustard-Kranti
2. " -Krishna
3. " -RH-30
4. " -RK-1467(Vardan)
5. " -JRV-24 (Rohina)
6. " -RK-14 (Vaibhav)
7. " -Durgamani
8. " -Varuna
9. " -Pusa Bold
10. " -B-85 (Seeta)
11. " -RLM-198
12. " -RLM-619
13. " -RLM-514
14. " -RW-351
15. Toria -13-34 (Ayrani)
16. " -PT-30
17. " -TS-29
18. " -T-9
19. " -M-27
20. " -PT-303
21. " -TL-15
22. " -Sangam
23. " -Bhawani
24. Yell. Sarson-YSB-9(Benoy)
25. " " -YSB-24
26. " " -BSH-1
27. Br.Sarson -Pusa
28. " " -Kalyani
29. Taramira -T-27
30. *Eruca sativa*-T-27
31. " " -JR-1

KENYA

1. Linnot

MEXICO

1. SEL-W

NEPAL

1. Sarson, S-4
2. " S-5
3. Toria, Sarlahi Local
4. " , Sindhuli Local
5. " , Bhairhawa Local
6. " ,
7. " , Chilawon Local
8. " , Nijgadh Local
9. " , Navalparasil Local
10. " , Tarahara Local
11. " , Nepal Ganj Local

CHINA

1. Wulumugi (Yellow Rape)
2. Qing You-11
3. Gan You 5
4. 23
5. Bai Cheng (Yellow)
6. Hu You 9
7. Qing You 9
8. Sheng Li Qing Geng

SWEDEN

1. Consul
2. Hanna
3. Emma
4. Tornado
5. Puma-SV-86-27102
6. Sonja-86-37702
7. Topas No. 6050
8. Global No. 4061

TRAINING

The training needs of the Network members are many and varied, ranging from short course training for technicians and scientists at various levels to graduate training. IDRC's Fellowships and Awards Division assists the network to arrange appropriate short-course and degree trainings within member countries and in Canada, according to resources available. So far, short- term training courses- supported by IDRC and organized by the Network- were held as follows:

1. Sesame and Saflowr, held at the Directorate of Oil seeds Research, ICAR, Rajendranagar, Hyderabad, India, February 1987.

2. Rapeseed Methodology in Agronomy and Breeding Rapeseed-Mustard, held at G.B. Pant University of Agriculture and Technology, Pantnagar, India, December 1989.
3. Brassica Quality Training, held in the previous two days (21-22) at the Shanghai Academy of Agricultural Sciences.

Training opportunities are available for more Brassica, Sunflower, and Sesame courses.

SUMMARY OF NETWORK DEVELOPMENT IN CHRONOLOGICAL ORDER

Year	Country	Activity
1981	* India	- Mustard Project,
	* "	- Rapeseed Project,
	* "	- Sesame Project,
	* "	- Safflower Project,
	* Malawi	- Groundnut Project (ICRISAT)
	* Ethiopia	- Highland Oilcrops Project (Nigerseed, Ethiopian mustard, Rapeseed, Linseed, Sunflower)
	* - Oilcrops Network Project started with Dr. Ken Riley, IDRC Network Advisor based in Ethiopia.	
1982	* Ethiopia	- Lowland Oilseeds Project (Sesame, Groundnut, Safflower)
	* Sri Lanka	- Oilseed Project (Sesame, Soybeans, Sunflower, Brassicas)
	* Egypt	- Oilseed Project (Groundnut,, Sesame, Rapeseed, Sunflower)
	* China/Canada	- Project (Brassicas)
	* Mozambique	- Project (Groundnut)
1983	* Sudan	- Oilseed Project (Goundnuts, Sesame, Soybeans, Sunflower)
	* Tanzania	- Project (Pulses and Groundnut)
	**Egypt	- 1st Network Workshop
1984	* - Dr. A.Omran replaced Dr. Riley as Network Advisor.	
	**Italy	- FAO Sesame Consultation Meeting
	**Malawi	- ICRISAT 1st Groundnut Regional Workshop of Southern Africa
	* Network/Canada	- Anther Culture Project (Sesame, Nigerseed)
1985	**India	- 2nd Network Workshop (Sesame and Safflower)
	**Argentina	- 11th Sunflower Conference
1986	**Ethiopia	- 3rd Network Workshop (Niger and Brassicas). Decision was taken to form sub-networks.
	**USA	- American Peasant Research Society
	**Zimbabwe	- ICRISAT 2nd Groundnut Regional Workshop of Southern Africa
	* Pakistan	- Project on rapeseed/sunflower for reclaimed lands
	* "	- Project on Soybean
1987	**Sweden	- Brassica Sub-Network formed
	**Poland	- 7th Rapeseed Congress
	**Austria	- FAO Sesame Project Formulation
	**India	- Sesame and Safflower Training Course
	* "	- Project on Sesame Scholars
	* "	- Project on Sesame Onfarm Research
	* Ethiopia	- Project on general Oilcrops
	* Nepal	- Project on Oilseeds
1988	**Kenya	- 4th Network Workshop (Sesame, Sunflower, Linseed). Sesame and Sunflower Subnetworks formed.
	* "	- Project (Vegetable Oil and Protein Systems).
	* India/Canada	- Project (Brassicas)
	**Malawi	- ICRISAT 3rd Groundnut Regional Workshop of Southern Africa
	**Yugoslavia	- 12th Sunflower Conference
	* Philippines	- Project on Rice-based Sesame

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*New Project or New Phase.

**Meeting, Conference, Workshop, Training..

1989	**India	- 2nd Brassica Sub-network Workshop
	** "	- International Safflower Conference
	** "	- Other Oil Crops Sub-network (Safflower, Nigerseed, Linseed) formed
	** "	- 1st Network Steering Committee Meeting (Network constitution drafted)
	**Turkey	- FAO Sunflower Network meeting
	**Egypt	- 2nd Sesame Sub-network Workshop
	** "	- 2nd Sunflower Sub-network Workshop
	**India	- Brassica Production Training Course
1990	**Sudan	- FAO/UNDP Project Formulation for Sudan, Somalia and the two Yemens
	**China	- International Rapeseed Symposium
	** "	- 3rd Brassica Sub-network Workshop
	**Tanzania	- ICRISAT 4th Groundnut Regional Workshop
	* Kenya	- Sesame Project

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A BRIEF REPORT ON THE BRASSICA SUB-NETWORK

Basudeo Singh

Oil-producing *Brassica* species commonly known as rapeseed and mustard contribute over 13.2 % to the world's total edible oil production. At present, it is the third most important edible vegetable oil source after soybean and palm. These crops have very wide adaptation in terms of soil and atmospheric temperatures, day length, altitude and geographical areas. The important rapeseed/mustard growing countries in the world are China, India, Pakistan, Bangladesh, Nepal in Asia; Canada, in North America; Czechoslovakia, Denmark, France, Germany, Poland, Sweden and U.K. in Europe. Australia and United States and perhaps South America may also become important countries for *Brassicas* in the future if suitable varieties with disease resistance, quality oil and seed meal become available. The oilseed economy of many countries mentioned above is dependent upon oleiferous *Brassicae*.

Oilseeds economy of south Asia with the exception of Sri Lanka is linked with the fate of rapeseed-mustard research, development and production. As per the data reported in FAO year book 1988, the contribution of China, India, Pakistan and Bangladesh to the total area and production of rapeseed-mustard in Asia is well over 99% and to the total area and production in the world is 56.69 and 41.16%, respectively. This shows the importance of rapeseed-mustard as an edible vegetable oil source as well as its weakness in the productivity ranging from the low 694 (Bangladesh) to 1072 kg/ha (China). The relative contribution to the production is not commensurate with area. Hence, greater efforts are needed to enhance the productivity and stability at higher production levels by developing hybrids/synthetics with higher yields and greater resistance to important diseases and pests.

At the third Oilcrops Network Workshop held at Addis Ababa in October 1986, Dr. Geoffrey Hawtin, Director, AFNS, IDRC, raised two major questions:

- "Is the geographic and crop coverage too broad? Would it be better to concentrate on fewer countries and/or fewer crops? Perhaps more focussed "sub-networks" could be considered?"

- "Is it desirable to include crops such as rapeseed, on which much research has been done, together with relatively under-developed crops such as niger, sesame and linseed? Would it be more effective for rapeseed scientists to link more closely with other international associations on this crop?"

As a follow up action of the above two important suggestions made by Dr. Hawtin, a special Brassica Meeting was held at Swedish University of Agricultural Sciences, Uppsala (Sweden) on May 6-7, 1987. Nine scientists from 8 countries participated in the meeting, besides Dr. Hawtin, Dr. Ken Riley and Dr. Abbas Omran from IDRC. The main objectives of the meeting were to identify major rapeseed productivity constraints in Asia and Africa, facilitate germplasm exchange, identify common problems for collaborative efforts, determine the training needs of the member countries and constitute a Brassica Sub-network and its Steering Committee. The details of the deliberations and recommendations made at that meeting are included in the IDRC Manuscript Report - MR 168e. The progress made under each of the items of work plan was reported at the Fourth Oilcrops Network Workshop held at Njoro (Kenya) in January 1988 and included in the Manuscript Report - IDRC MR 205e, pp. 301-303.

The second Brassica Sub-Network meeting was held in india at Pantnagar on 4-6 January, 1989, attended by 29 participants from 10 countries including IDRC officers and local participants. All the country presentations and discussions were divided into 7 sessions. Detailed discussions took place on updating of priority problems from 1st meeting (MR

168e page 57), Sub-Network activities (follow-up on recommendations of 1st meeting), germplasm exchange, nurseries, training (breeding/agronomy, plant protection, quality, post-harvest technology and biotechnology), newsletter, Brassica bibliography, etc. The manuscript report is under preparation[1]. The recommendations made at that meeting are listed below:

- Follow-up of the review on "Screening Breeding Techniques for Aphid Resistance in Oleiferous Brassicae."
- Prepare a review on "Screening and Breeding Techniques for Alternaria Resistance in Rapeseed and Mustard."
- Prepare a review on "Screening and Breeding Techniques for Drought Resistance in Rapeseed-mustard".
- Prepare a "Coloured Technical Bulletin for Diseases and Insects of Rapeseed-Mustard".
- Report on Orobanche from Ethiopia to be shared with Egypt and Nepal.
- One-month training course on research methodology in rapeseed-mustard breeding to be organised at Pantnagar in India in December 1989.
- Seeking nomination for one contact person for Brassica Sub-Network from each country.
- Next Network meeting to be held in in China along with the China International Symposium on Rapeseed during April 1990.

Follow-up Actions on Various Recommendations

A. Reviews:

- "Review on Screening and Breeding for Aphid Resistance" has been written, published and supplied to Oilcrops Network Advisor for distribution.
- The reviewers for "Alternaria Resistance", "Drought Resistance" and "Coloured Bulletin on Rapeseed-Mustard Diseases and Insects" have been contracted and the writing is in progress. The manuscripts are likely to become available by June 1991.

B. Orobanche Report:

Orobanche report of Ethiopia has been shared with Nepal and Egypt.

C. Training:

As per recommendation, a training course on "Research Methodology in Agronomy and Breeding of Rapeseed-Mustard" was organized at Pantnagar, India, 4-29 December, 1989. In all, 17 scientists from China, India, Bangladesh, Pakistan, Nepal, Bhutan, Kenya, Ethiopia and Egypt participated in the training. Future training needs were also discussed.

D. Nomination of Contact Person:

Each member country of the Brassica Sub-Network was requested to nominate a contact person for Brasscia Sub-Network. Nominations have been received from each country.

E. Third Brassica Sub-Network Meeting:

The third Brassica Sub-Network meeting scheduled for April 1990 in China is now being held as per the schedule for which all of us have assembled here today.

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[1] Now published as IDRC Manuscript Report MR 252e, 1989.

RESEARCH PROGRESS ON RAPESEED IN EGYPT

Badr A. El-Ahmar

Abstract

A total of 223 seed samples of varieties and inbreds of Brassica napus, B. compestries, B. juncea, B. carinata and Synapis alba were collected and sown in breeding nursery alongwith 60 segregating samples of F_2, F_3, and F_4. The important vegetative characters were recorded. A comparative yield trial which included 15 entries was carried out in more suitable area for rapeseed. Application of Manganese and Boron to the soil resulted in highest seed yield of 1347 and 1686 kg/fad, respectively (1 feddan = 4200 m^2). The herbicides Treflan at 1 L/fad, Stomp at 1.5 L/fad, Gesagard at 1 kg/fad and Topogard at 1 kg/fad resulted in a high percent of weed control and seed yield.

Rapeseed, as a winter oilcrop, received high interest from both Egyptian government and scientists. The government wishes to cover the gap between actual consumption and national production of edible oil, in order to reduce the import bill which costs about US$ 300 million yearly for edible oil only. The scientists are working to fulfil the Egyptian regulations concerning the chemical and physical properties of rapeseed oil alongwith high seed yield which is more important to the farmers.

In order to co-ordinate the work of the different ministries involved in the production system of oilcrops from sowing to oil extraction, the Council of Oilcrops and Food Legumes started funcutioning since 1988.

The main objectives of rapeseed program are as follows:

1. To incease the seed yield to 2.5-3.0 tonnes/ha.
2. To breed early maturing varieties, resistant or highly tolerant to diseases and broom rape (*Orobanche*).
3. To develop varieties with high seed and oil content and double-zero.
4. Develop nutrient management policies that maximize seed yield.

CROP IMPROVEMENT

Breeding nursery:

Rapeseed breeding nursery contains 223 inbreds which belong to *Brassica napus* (137); *B. campestries* (27); *B. carinata* (5); *B. juncea* (52); and *Sinapis alba* (5), in addition to 60 lines from segregating generations of F_1 to F_4; all were sown at three research stations located in the northern part of the country. Summary of some results appear in Table 1. There is genetic variability among the inbred lines, however, this genetic variability is not wide. A crossing program was started and 60 derivatives (44 F_1, 10 F_2 and 6 F_3) are available.

All selected plants in each segregating generation and inbred lines were covered with cloth bags (100 x 50cm), and tied around the bottom and apex to give chance for self-pollination and maintain or increase purity and homogeneity of the inbreds.

Table 1. Summary of characters of rapeseed at three research stations.

Station	Plant height (cm)		Days to 50% flowering		No. of branches	
	Range	Mean ± S.E	Range	Mean ± S.E	Range	Mean ± S.E
ElSerw	66-204	124.2 ± 3.0	55-140	101.0±2.66	5-14	8.10 ± 0.20
Sakha	114-239	146.2 ± 3.1	74-118	100.8±1.37	6-15	9.96 ± 0.52
Giza	68-194	125.8 ± 2.9	57-132	102.9±2.25	5-14	7.85 ± 0.30

Breeding for disease resistance or tolerance

Sclerotinia sclerotiorum is a serious disease under dry conditions. The fungus attacks rapeseed in that part of the field where there is water logging and causes considerable losses in yield and quality. A diseased field was established and severly infested by the pathogen by sowing rapseed during the winter since 1987. The promising inbred lines of *B. napus*, *B. juncea* and *B. campestris*, in addition to 15 segregating generations were sown in this diseased field since 1987. Some lines are moderatly susceptible and some other lines are tolerant.

Breeding for *Orobanche* resistance

The previous diseased field is also used as *Orobanche* field. The seeds of this parasite were collected and mixed with the soil. Some inbreds from *B. juncea* and some plants from segregating generations showed resistance to *Orobanche*. Since there was no or very limited number of the parasite which grew on rapeseed, the crop appeared healthy and performed equally well as the parasite-free one. This result indicates that *Orobanche*-resistant rapeseed genotypes could be selected, Table 2.

Breeding for shattering resistance

One of the major problems which causes a large amount of seed losses is shattering at harvest time. Therefore, genotype resistant to pod and seed scattering will minimize seed losses at harvest. In order to achieve this, the following procedure was applied at Giza Research Station:

- Keep the plants in the field long after ripening during the hot weather of May so that they could over-mature and completely dry;
- Harvest manualy at mid-day;
- Select the plants which did not shatter and thresh separately;
- Sow the seeds next season in one row; and
- Repeat the same technique to check the progeny of the selected plants.

Breeding for white rust

This disease appeared for the first time during 1986/87 season, when two *B. juncea* inbreds were attacked by white rust. During 1989/90 season, a severe and wide-spread infestation appeared in three research stations located at the northernm part of the country. However, at Giza research station, the disease did not appear, may be due to unfavourable weather during winter time. The observation indicated that the infestation was concentrated in early flowering and maturing inbreds. Furthermore, late flowering and maturing inbreds were not infested, may be due to unsuitable weather for the disease during March to mid April, i.e. the inbred may have escaped infestation under natural conditions. This situation need to be studied under artificial conditions also, to define the nature of resistance of the inbreds.

Table 2. Survey of Rapeseed in Orŏbanche-infested breeding nursery.

Strains	No. of orbanche plants
B. napus	
Altex	9
Midas	17
Cresor (France)	20
Kid	16
Tower	12
Westar	8
Varma	20
I 00 69	23
Canola 101	18
" 102	22
" 103	21
" 104	11
Brown Sarson	6
Mabo	23
Olero	18
Cresor (Sweden)	7
Deakkar	12
Inetta	2
Fisandro	17
Firawell	17
B. campestris	
Torch	24
Candle	19
Rafal	4
Callery	1
Mtedss	18
Wacsway	10
Mnarnoo	3
Dichotoma	8
Topas	9
B. juncea	
R.2456	3
Poorbiraya	5
L.L. 84	0
R.L. 18	5
J 0021	12
B.9	X
B.R.1108	0
Prakash	0
R.C. 751	2
R.C. 781	1
R.I. 18	4
R.L.M. 514	2
R.S. 65	1
T.M. 2	0
T.M. 7	0
T.M. 12	0

AGRONOMIC PROGRAM

Micro-element experiment

This experiment was conducted to study the effect of micro-elements of Zinc(Zn), Iron (Fe), and Manganeze (Mn) either in Sulphate form of Chelate alongwith the effect of Boron (B) in a form of Borax or Boric Acid. The sulphate sources of Zn, Fe and Mn were used as soil application at a rate of 10 kg/fad. The Chelate form was used as foliar application at a rate of 0.5 g/L. Boron was used as Borax form in soil application at a rate of 4 kg/fad and Boric acid as foliar application at a rate of 0.5 g/L, Table 3. This represents the suggested treatments at Sakha and Noubaria.Table 3 reveals that at Sakha, the micro elements, have no effect on days to 50% flowering and plant height. However, soil application of Zn or Zn + Fe + Mn produced high seed yield/plant followed by B as foliar application.

Though, there are considerable effects of some micro-elements, but these have not been reflected on seed yield/ faddan, since there is no significant difference between highest seed yield (831 kg/fad) from Zn application in Chelate form and the control (818 kg/fad), Table 3.

Table 3. Effect of some micro-elements on some vegetative characters and seed yield, Sakha 1978/88.

Treatment	Days to 50% flowering	Plant height (cm)	Seed yield gl	Seed yield kg/fad
Control	98	138	14	818
Zn 10 kg/fad.	99	137	24	589
Fe 10 kg/fad.	101	136	17	339
Mn 10 kg/fad.	99	135	16	448
B 4 kg/fad.	100	150	20	489
Zn + Fe + Mn	99	132	23	638
Zn 0.5 g/L	100	139	13	831
Fe " "	98	134	17	511
Mn " "	103	140	18	387
B " "	98	129	23	442
Zn + Fe + Mn	100	140	20	425
Zn (Sulphate) + Zn (Chelate)	102	141	18	226
			LSD (0.05)	70.40
			C.V. (%)	9.42

At Noubaria, in all the treatments, seed yield/fad increased significantly except in Fe as foliar application, Table 4. Across locations, Mn and B as soil application, resulted in the highest seed yield (1347 and 1686 kg/fad, respectively) followed by Zn as foliar application and Zn (Sulphate) + Zn (Chelate),1174 kg/fad.

Concerning oil and protein contents, the highest oil content (48.4%) resulted from B as soil application followed by Fe, Zn+Fe+Mn (as foliar) and Zn (Sulphate) + Zn (Chelate) which resulted in 47.7, 47.7 and 47.5%, respectively. While Boron either soil or foliar application increased protein content by about 0.5 to 0.6% over the control. Similarly, Zn(Sulphate)+Zn (Chelate) increased protein content by about 0.5% as compared to the control, Table 4.

Weed Control

Weeds are serious competitors to rapeseed for nutritious elements and other physiological factors. Hence, suitable herbicides which do not have a harmful effect on rapeseed will help to increase seed yield and thus farmers' net return. An experiment with 8 herbicides was conducted at Sakha and Noubaria Research Stations using randomized complete block design with 4 replications. The plot size was $12m^2$. At Sakha, Application of Treflan controlled about 69.9% of the weed, and produced the highest seed yield (520 kg/fad) followed by Spectron, Stomp and Gesagard. However, the respective efficiencies of these herbicides to control weed ranged from 60 to 80%, Table 5.

At Noubaria, the survival of rapeseed differ with the herbicide and the rate. The highest percent of survived plants was recorded from Dawco at a rate of 1 L/fad followed by Treflan (0.75 L/fad), Fusilade (1 L/fad) and Pyramine (1.5 kg/fad). On the other hand, the highest degree of weed control was resulted from Byradare (92.5%-2 kg/fad), Topogard (85.7%-1 kg/fad), Stomp (86.25%-1.5 L/fad) Gesagard (85% - 1kg/fad) and Treflan (80%-1L/fad), Table 5.

The highest seed yield was 1011, 849, 834 and 816 kg/fad resulted from application of Dawco (1 L/kg), Igran (1 kg/fad), Treflan (1 L/fad) and Topogard (1 kg/fad), respectively, Table 6.

Table 4. Effect of some micro-elements on rapeseed yield and seed quality, 1987/88.

Treatments*	Seed yield (kg/fad) Sakha	Noubaria	Mean	Oil (%)	Protein (%)
1. Control	818	960	889.0	44.7	27.7
2. Zn 10kg/fad.	589	1425	1007.0	44.2	27.8
3. Fe " "	339	1719	1029.0	45.2	27.3
4. Mn " "	448	1246	1347.0	45.2	25.9
5. Borax 4 kg/fad.	489	1883	1686.0	48.4	28.3
6. Zn + Fe + Mn	638	1162	900.0	46.7	27.6
7. Zn 0.5 g/L	831	1405	1118.0	45.8	27.7
8. Fe " "	511	769	640.0	47.7	27.6
9. Mn " "	387	1098	742.5	46.2	27.1
10.Boric acid "	442	1367	904.5	46.6	28.2
11.Zn + Fe + Mn	425	1677	909.5	47.7	27.7
12.Zn (Sulphate) + Zn (Chelate)	226	2122	1174.0	47.5	28.2
L.S.D (0.05)	70.40	105			
C.V.(%)	9.42	36.85			

*2-6 Soil application, 7-11 Foliar application, 12 Chelate (foliar) and Sulphate (Soil application)

Table 5. Effect of some herbicides on weed control and rapeseed yield at Sakha, 1987/88.

Treatments	Rate	Weed control (%)	Seed Yield (kg/fad)	relative to control (%)
Igran	1 kg/fad.	81.53	-	-
Gesagard	" "	80.82	244	134.0
Topogard	" "	75.59	206	113.2
Treflan	1 L/fad.	69.90	520	285.7
Fusilade	" "	45.40	136	74.8
Dual	" "	70.25	194	106.6
Stomp	1.5 L/fad.	75.00	252	138.5
Spectron	" "	60.50	293	161.0
Hoeing	-	81.60	189	103.8
Unweeded (Control)	-	-	182	100.0
L.S.D (0.05)			90	
C.V. (%)			25.04	

Table 6. Effect of some herbicides on weed control in rapeseed at Noubaria, 1987/88.

Treatments	Rate	Survived plants/plot (%)	Weed control (%)	Seed yield (kg/fad)	Index relative to control (%)
Treflan	0.75 L/fad.	77.50	72.50	669	154.5
"	1.0 L/fad.	21.25	80.00	834	192.6
Stomp	1.5 L/fad.	20.00	86.25	615	142.0
Pyramin	1.5 kg/fad.	70.00	66.25	547	126.3
Igran	1.0 kg/fad.	15.00	78.75	849	196.1
Gesagard	1.0 kg/fad.	25.00	85.00	734	169.5
Topogard	1.0 kg/fad.	38.75	87.50	816	188.5
Byradare	2.0 kg/fad.	1.25	92.50	627	144.8
Dawco	1.0 L/fad.	80.00	78.75	1011	233.5
Fusilade	1.0 L/fad.	76.25	73.75	476	172.3
Hoeing		80.00	72.50	761	175.0
Unweeded (control)		75.00	00.00	433	100.0
L.S.D. (0.05)				262.6	
C.V. (%)				24.2	

DISCUSSION

Singh: How much area, do you have under rapeseed in Egypt?

Elahmar: 400 hectares. next year it will increase upto 4000 hectares.

Ningkang: What should be the quality of oil?

Elahmar: There is strict regulation that the erucic acid should be less than 2% and glucosinolates should be less than 30 μ mol/g.

Islam: What is the yield potential?

Elahmar: For *B. napus* it is 3.0 tons/ha (on research station land). On farm, it is 1.5 - 2.0 tons/ha. For *B. juncea* it is less than 2 tons/ha. For *B. campestris*, is between 1.5 and 2.0 tons/ha.

Islam: Is *Orobanche* a problem only in rapeseed?

Elahmar: It is a problem in all the *Brassica* spp. and other crops as well.

Singh: *Orobanche* is a problem in different regions in Nepal and other countries. *Orobanche* survey has been done by Nepal and ethiopia. Follow their recommendations.

Islam: *Orobanche* is a problem in both *Cruciferae* and *Solanaceae*.

Mundel: What is the rate of seed transmission of *Orobanche* in germplasm exchange?

Elahmar: The seeds are small and can be separated easily.

Mishra: *Orobanche* is also a problem in Nepal so there should be exchange of materials which is resistant to *Orobanche*.

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QUALITY BREEDING IN BRASSICA CARINATA IN ETHIOPIA

Getinet Alemaw and Hiruy Belayneh

Abstract

Yellow-seeded lines of Ethiopian mustard showed more oil, higher protein and lower crude fibre contents than non-yellow-seeded lines from similar genetic background. Recently, zero erucic acid yellow-seeded plants were selected out of interspecific cross between Brassica carinata (BBCC) cv. Yellow Dodolla and B. juncea cv. Zem 2330. Plant-to-row selection from low glucosinolate material has resulted in reduced level genotypes. The ultimate goal is to develop double low genotypes of Ethiopian mustard.

Ethiopian mustard(*Brassica carinata* A. Braun) is the highest yielding oilseed crop in Ethiopian highlands. Improvement programs since the mid 1970's have resulted in improved production packages and high yielding varieties. Seed yields of improved varieties under farmers management reach up to 18 q/ha. Ethiopian mustard is higher yielder, taller, tolerant to pests and diseases than rapeseed. However, under stress environment/bad years rapeseed yields as good as mustard.

In view of the agronomic potential of mustard as an oilseed crop in Ethiopia, we initiated an experiment to increase the oil and meal quality and quantity.

OIL CONTENT

As the altitude increased or growing temprature decreased, oil content increased, Table 1. Also, the soil nitrogen content affected the oil content. In rapeseed and indian mustard, yellow-seeded genotypes gave higher oil content than their brown-seeded isogenic lines.

In Ethiopian mustard, yellow-and brown-seeded lines within accessions were compared for seed weight, oil protein and crude fibre contents. Yellow-seeded sublines gave more oil content and better quality meal, Table 2. The variety "Yellow Dodolla" was selected out of "Mixed Dodolla" for its superior oil and meal quality.

OIL QUALITY

The oil from Ethiopian mustard varieties is high in erucic acid. Low-erucic-acid genes are present in

Table 1. Oil content (%) of three varieties of oilseed Brassica grown at three locations for four years, 1984-88.

	Locations		
Variety	Holetta	Debrezeit	Awassa
Pura*	46.8	43.4	42.0
Tower*	46.5	43.4	41.9
S-67**	42.9	39.4	36.0
Mean	45.4	42.1	39.9
Altitude (m)	2400	1900	1800

*B. napus
**B. carinata

Table 2. The influence of seed coat color on seed weight, oil, protein and crude fibre contents of B. carinata.[1]

Trait	Yellow	Brown	Dif.
1000-seed weight (g)	5.0	4.4	+0.6
Oil (%)	43.3	40.1	+2.6
Protein (%)	33.7	31.1	+2.6
Crude fibre(%)	10.7	13.8	-3.1

[1]Based on 11 genotypes tested for two seasons in two replications at Holetta.

related species *B. juncea* (AABB, n=18) and *B. napus* (AACC, n=19). Development of low-erucic-acid gentoypes was attempted through interspecific hybridization. Low-erucic Yellow Dodolla plants were selected from a cross of *B. carinata* var. Yellow Dodolla X *B. juncea* line Zem. 2330 in BC_1F_2 generation, Table 3. Backcrossing to Yellow Dodolla and reselecting for zero erucic plants has continued. The ultimate goal is to develop double-low Ethiopian mustard.

MEAL QUALITY

The meal of Ethiopian mustard is characterized by high protein and low crude fibre contents. However, it is high in allyl glucosinolate content. Although low-glucosinolate rapeseed genotypes are available, gene source

for low allyl glucosinolate is limited. We started screening for low glucosinolate in 1982 using tase taping. So far, plant-to-row selection has resulted in reduced levels. Also, diallel crosses among promising lines to select desirable recombinants has continued, Table 4.

Table 3. Fatty acid composition of oil from two cultivars and two lines of *B. carinata.*

Genotype	Fatty acids(%)					
	Palmitic	Oleic	Linoleic	Linolenic	Ecosenoic	Erucic
S - 67	2.8	9.5	16.5	14.2	9.8	43.2
Yellow Dodolla	2.6	12.2	18.7	12.1	9.3	42.3
BC_1F_2 24-17	4.0	32.0	42.0	23.0	1.6	0.3
BC_1F_2 51-7	4.0	44.0	33.0	17.0	1.0	0.5

Table 4. Pattern and contents of major glucosinolates in some lines of *B. carinata.*

Genotype	Glucosinolate (µ mole/g of meal)*								
	Allyl	But.	Pent.	Hobut.	Hopent.	Hobenz.	Ind.	Hoind.	Total
208539	75.57	0.30	0.14	1.12	0.12	0.34	0.12	4.30	82.10
208531	68.55	0.32	0.08	1.22	0.08	0.25	0.05	5.52	76.07
S-67	153.60								
Yellow-Dodolla	141.31								

*Allyl = Allyl glucosinolate
But. = 3 - Butenyl glucosinolate
Pent. = 4 - Pentnyl glucosinolate
Hobat. = 2 - Mydroxy 3 - butenyl glucosinolate
Hopent. = 2 - Hydroxy 4 - pentnyl glucosinolate
Hobenz. = P - Hydroxy benzyl glucosinolate
Ind. = Indolglucosinolates
Hoind. = Hydroxy indolglucosinolate

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SOME OF THE CONTRIBUTIONS OF DR. HIRUY BELAYNEH TO OILSEED BRASSICA RESEARCH IN ETHIOPIA

Getinet Alemaw

The following are abstracts of papers authored by the late Dr. Hiruy Belayneh, former Chirman of Brassica Subnetwork.

1. Hiruy Belayneh. 1987. Present status and future strategies of oilseed Brassica research in Ethiopia. Proceedings of the first Brassica Subnetwork Workshop held at Uppsala, Sweden, 6-8 May 1987, (A. Omran ed.). IDRC MR 168e: 15-26.

ABS: The primary objective of the oilseed Brassica improvement program is to increase production both for local consumption and export. The research service is also directed towards that end.

The research achievement has been quite rewarding. As a result of the multi-locational testing, five B. carinata and three B. napus varieties have been released. Progress has been made with quality work. The production technology for oilseed Brassica has also been perfected. The economically important diseases and pests have been identified and the control measures are being developed.

Future strategies of oilseed Brassica research are described. There is a great scope for further improvement.

2. Hiruy Belayneh. 1985. The Current Status of Oilseed Brassica Agronomy Research in Ethiopia. CGIRC Bulletin No. 2: 25-27.

ABS: The Ethiopian mustard (Brassica carinata) is grown in Ethiopia only on a small scale, mainly in fertile, well drained fields often close to house. Contrarily, a sizeable area of B. napus varieties is cultivated on state farms. A number of trails on cultural practices were carried out at Holetta and cooperating stations. Seed yield was generally more influenced by the date of sowing and by NP fertilization than by differences in plant population. Higher yields for both the species were obtained when planted at the onset of main rains, expected in mid-June at most sites. Fertilizer (46/69 kg/ha of N/P_2O_5) signigicantly increased seed yield at several locations. A seed rate of 6-10 kg/ha was found optimal depending upon the location and weather conditions. Cropping sequence studies at Holetta showed that wheat or barley following rapeseed produced good seed yields.

3. Hiruy Belayneh. 1985. Review of Rapeseed/Mustard Breeding and Agronomy Research in Ethiopia. Review of Field Crop Research in Ethiopia. IAR.

ABS: Ethiopian mustard (Brassica carinata) is grown traditionally by Ethiopian farmers as both an oilseed and vegetable crop, while Argentine rapeseed (B. napus) and Turnip rapeseed (B. campestris) were introduced into Ethiopia about 13 years ago.

Coordinated research on oilseed Brassica improvement in Ethiopia started in early 1970's and the research work has progressed through several phases since then, resulting in the release of five B. carinata (S-67, S-71, S-115, Awassa population and Dodolla-1) and three B. napus (Target, Pura and Tower) varieties. Pura and Tower have low erucic acid and low glucosinolate levels. Seed yields of B. carinata approached 4800 kg/ha under ideal conditions where the grain-filling period was prolonged. At sites where maturity was accelerated, yields of B. carinata and B. napus were similar. A breeding program is expected to produce mustard varieties low in both erucic acid and glucosinolate contents in a few years.

4. Hiruy Belayneh, K.W. Riley, Nigatu Tadesse and Getinet Alemaw. 1983. The responses of three Oilseed Brassica species to Different Planting dates and Seed rates in the highlands of Ethiopia. Eth. J. Agric. Sci. 5 (1): 22-31.

ABS: Brassica carinata is grown traditionally by Ethiopian farmers as both an oilseed and vegetable crop, while B. campestris and B. napus were introduced about 10 years ago. The performance of B. carinata (S-67), B. napus (Target), and B. campestris (Torch) cultivars was evaluated at a number of locations in Ethiopia 2000 m above sea level.

See yield of S-67 (B. carinata) approached 3500 kg/ha under ideal conditons, where the grain-filling period was prolonged. At sites where maturity was accelerated

yields of S-67 and Target were similar. Yields of Torch (B. campestris) were low at all sites. Highest yields for all cultivars were obtained when planted at the onset of the main rains, expected in mid-June at most sites. A seed rate from 6-10 kg/ha was found to be optimal, depending on the season and location. Mean oil content of Target (B. napus) was 45%, while both S-67 and Torch average 40% at Holetta in 1981. Mean 1000 seed weight of S-67 was equal to or slightly greater than that of Target, and much greater than that of Torch.

Cultivars of both B. carinata and B. napus can produce good yield in the highlands of Ethiopia, but under long-growing-season conditions, B. carinata may have an advantage. Experimental lines of B. carinata which are earlier and have oil contents equal to those of B. napus have been identified and are being tested.

5. Hiruy Belayneh and Nigussie Alemayehu. 1986. Progress in rapeseed/Mustard Research in Ethiopia. Proceeding of 3rd Oilcrop Network Workshop held in Ethiopia 6-10 October. A. Omran (ed.). IDRC. MR 153e: 12-18.

ABS: Ethiopian mustard (Brassica carinata) has been grown by Ethiopian farmers as both an oilseed and vegetable crop for thousands of years, while the Argentine rapeseed (B. napus) is a recent introduction and is widely grown on state farms.

Since the beginning of coordinated research in oilseed Brasscia in the early 1970's, more than one thousand collections of the two species were evaluated. The research endeavor resulted in the release of five high yielding B. carinata and four B. napus varieties. There is a crossing program now in progress involving high yielding carinata and the introduced B. napus and B. juncea varieties with low erucic and glucosinolate levels to achieve B. carinata cultivars which are both high yielders and low in erucic and glucosinolate contents.

Results of the trials on sowing date, seed rate and fertilizer levels are discussed. Studies on the control of weeds are also reviewed. The on-farm trials showed the possibility of increasing yields when the oilseed Brassica growers follow the recommendation.

Seven diseases and 13 insect pests have been reported on rapeseed and mustard. However, most are minor to date.

6. Hiruy Belayneh, Getinet Alemaw and Nigussie Alemayehu. 1989. Yellow Dodolla-Ethiopian Mustard. Oilcrops Newsletter 6: 58-59.

ABS: Dodolla was collected on land race from a place called Dodolla in Bale region. It was in various states from 1974-79. In 1980 it was released as a mixture of yellow and brown seeds, latter mass selection for yellow seed was initiated and resulted in a new variety "yellow Dodolla" in 1988. Yellow Dodolla is higher in oil and protein and low crude fibre than its parent population.

7. Hiruy Belayneh and Nigussie Alemayehu. 1987. Comparative Performance of Ethiopian mustard (*B. carinata* A. Braun) and Argentine rapeseed (*B. napus* L.) Under Improved and Traditional Farming practices. Proceedings of 7th International Rapeseed Congress held in Poland, May 1987: 1044-49.

ABS: The on-station research work in the past several years has identified improved Ethiopian mustard and Argentine rapeseed varieties. The basic agronomic information was made available at the same time as the new cultivars were released. This package testing was undertaken to acquaint the users with new innovations and appreciate their preference.

The performance of Brassica carinata and Brassica napus varieties was tested for two seasons at four sites under both improved management practices developed on the research centres, and traditional farming practices. The Late B. carinata entries were able to use the longer growing season in the highlands of Ethiopia and yielded much better than the earlier maturing B. napus types. The researchers' package resulted in substantial seed yield increase. Variety and fertilizer were the two most important factors for higher seed yields. Among the varieties tested, S-67 performed best across site under both management practices. B. napus was more responsive to better management.

8. Getinet Alemaw and Hiruy Belayneh. 1990. Influence of seed coat color on seed weight,oil, protein and crude fibre content of Brassica carinata A. Braun. Proceedings of the 3rd Brassica Subnetwork Workshop held at Shanghai, China on 23 April. (A. Omran ed.) IDRC Manuscript Reports (under publication).

ABS: The influence of seed coat color on seed weight, oil content of the whole seed as well as protein and crude fibre contents of the meal of B. carinata was studied using 11 accessions and one cultivar for two seasons at Holetta. The mean seed weight, oil, protein and crude fibre contents were 4.6 g, 41.6%, 32.2% and 12.3%, respectively. Yellow seeded lines produced heavier seed weight (0.6 g), higher oil (3.2%), and protein (3.1%) contents than brown seeded lines from similar genetic background. The crude fibre content of the meal from yellow seed was lower than that of the meal from brown seeds by 3.1%. This relationship suggested that the alleles controlling seed coat color in B. carinata exibit plieotropic effects on seed quality traits. The study demonstrated that selection for yellow seed coat color could result in higher oil and improved meal quality in B. carinata.

9. Getinet Alemaw and Hiruy Belayneh. 1988. Influence of seed coat color on seed quality in "Gomenzer" (*Brassica carinata*). SEBIL 1(1):20.

ABS: Yellow and brown seeded sublines from similar backgrounds were planted at Holetta in split-plot. Yellow seeded sublines showed more oil and heavier seed weight than their non-yellow counter parts. In

similar studies with B. campestris, it has been shown yellow seeded sublines had a thinner seed coat. Yellow seed coat color has also been associated with increase in proportion of the embryo to the total seed weight resulting in higher oil, higher protein and less crude fibre than their brown seeded isogenic lines. A similar relationship appears to occur in gomenzer.

This relationship suggests that the alleles affecting seed coat color also exhibit pleiotropic effects on seed quality. In brown seeded gomenzer cv. S-67 the seed color was controlled by a single gene pair with brown being incomplete dominance over yellow. The homozygous recessive condition resulted in yellow seed.

10. Getinet Alemaw and Hiruy Belayneh. 1989. Variation of Alkenyl Glucosinolates in "Gomenzer" (*Brassica Carinata* A. Braun). SEBIL 2 (No. 1 and 2): 7.

ABS: "Gomenzer" germplasm was screened for glucosinolate pattern and content. Allylglucosinolate contributed about 90% of the total amount. Cultivars S-67 and Yellow Dodolla contained 164 and 150 micro-moles of glucsoinolate per gram of defatted dry meal, respectively. Two populations, PGRC/E 208531 and 208539, showed reduced levels. As a result of plant-to-row selections out of these populations, low glucosinolate lines were identified.

11. A. Haile, E. Zerfu and H.Belayneh. 1988. Performance of Local and Improved mustard (*B. carinata* A.Br.) and linseed (*Linum usitatissimum* L.) cultivars under improved and traditional farming practices. J. Crop Research. 1: 43-49.

ABS: Local and improved cultivars of mustard and linseed were grown for two growing seasons, 1986 and 1987 in the Central and North Western zones at two levels of management, improved and traditional. The improved linseed varieties (CI 1525 and CI 1652) and mustard selection (S-67) recorded very high yeilds over the years. Accross locations, the local and improved varieties of both crops gave higher yields where the improved technologies were properly implemented as compared to the traditionally managed fields. Hence, management is critical in mustard and linseed production areas. The marginal rate of returns were 29.4 and 139.1 (birr/q) for the Agricultural Market Corporation and local prices, respectively.

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STRATEGIES IN RAPESEED AND MUSTARD DEVELOPMENT IN KENYA[1]

M.J. Mahasi

Rapeseed is a relatively recent crop in Kenya having been introduced in the late 1970's. Most of the germplasm available has been introduced from various European countries, Canada, China, India and Pakistan. A total of 80 introductions are now available at the National Plant Breeding Research Centre (NPBRC), Njoro. These represent the three species *Brassica. napus*, *B. campestris* and *Brassica juncea*. Fifty of the introductions are of the *B. napus* type and are low in erucic acid, though some are high in glucosinolates. Many of the *B. campestris* and *B. juncea* types do not have the required characteristics such as low erucic acid (5% maximum in the oil) and glucosinolates (less than 3 mg/g of meal).

Commercially, rapeseed production in Kenya is not extensive mainly due to poor pricing. The cost of the oil crop products is controlled at a level that the farmers' returns can not compensate for the production costs and his expected earnings. The result of this is a national dependence on importations to meet the country's requirements for edible oils. The importation is estimated at approximately 60-80 million US$ annually.

The potential for commercial production of oil crops in Kenya is high and the market is readily available. If the government formulates a policy on oil crop production in the country, then rapeseed production will increase rapidly. Meanwhile, research on various aspects of crop production is in progress. The work reported here was started to ensure that rapeseed varieties suitable for growing under Kenyan conditions are made available either through introduction of commercial varieties, screening of various introduced lines or development of new varieties bred locally

1) Paper received but not presented.

SCOPE OF WORK

The following work is conducted on the germplasm lines:

1. Screening and testing.
2. Interspecific crossing.
3. Evaluation of varieties in different agro-ecological zones.
4. Variety description and maintenance breeding.
5. Rapeseed and mustard observation plots.
6. Selection for early maturity, drought tolerance, low erucic acid, high yields, high oil and protein contents and non-shattering yellow seeded types.

ADAPTABILITY TRIALS

Rapeseed adaptability trials are carried out on the introduced seed stocks in the various agro-ecological zones to determine the most suitable areas for production. The *B. napus* varieties are highly adaptable to the Upper Highland zone (UH), while *B. campestris* and *B. juncea* perform well in the Lower Highland zone (LH). The last two species are lower yielding with low oil content, but their early maturity enables them to escape late-season-drought.

SEED YIELDS AND QUALITY

Although rapeseed research at NPBRC was initiated in the mid 1970's, it was not until 1982 that it became mandatory to test and release only low erucic acid types. The low erucic rapeseed varieties being determined by the level of fatty acids. The maximum amount of erucic acid considered safe in edible oil products is 5%. Tests have been carried on in both high-and medium-

potential zones and the results over the years, Table 1, show that *B. napus* varieties can yield upto 2 tons/ha while that of *B. campestris* ranges between 1.0-1.3 tons/ha. These averages being considerably higher compared to other countries where rapeseed is widely grown.

Table 1. Maturity days, Average seed yield and Oil yield/ha for some of the recommended varieties over the years.

Variety/ Line	Days to Maturity	Mean seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)
B. napus				
Willi	170	3591	45.0	1616.0
Christa	162	3190	44.0	1403.6
Gulliver	164	1972	47.0	926.8
Topaz	121	1873	48.0	899.0
Oro	141	1831	47.0	860.6
Tower	150	1914	44.0	842.1
Niklas	160	1706	48.0	818.9
B. campestris				
Sv. 73/604	107	1389	43.4	602.8
Sv. 72/1002	105	1377	43.0	592.8
Sv. 68/420	106	1246	45.5	566.9
Sv. 73/617	105	1215	42.4	515.2
Sv. 73/10063	105	1174	43.0	505.4
Sv. Torpe	109	1174	42.5	499.5
Candle	105	1067	43.4	463.1
Sv. Belle	106	1072	43.0	461.5
Tobin	105	1001	45.4	454.4

The *B. napus* varieties are better adapted to the cooler high altitudes. As one moves to the warmer medium zones, insect pests and diseases become more severe. The major ones include the sawfly, aphids, flea beetles and lepidopterous larvae. The common diseases are Alternaria black spot, blackleg, sclerotinia stem rot and staghead.

Some introductions have been identified as suitable for growing in Kenya. Others have been found to possess some good characteristics such as earliness, high oil content, high yields, disease resistance and good oil quality; though lacking in some other desirable characteristics. These can be used in the breeding program to improve commercial varieties.

Although only a small acreage is grown, the future of rapeseed will be promising once the market constraints have been removed.

INTERSPECIFIC CROSSING PROGRAMME

The hybridization programme was started in 1988 with the objectives that included the development of varieties that are: disease-resistant; high-yielding with high oil-protein contents; shattering-resistant; yellow seed colour; early maturing; drought-tolerant; and low in erucic acid.

The species used in the interspecific crossing program were *B. napus, B. campestris* and *B. juncea*. These three species vary greatly in oil content, maturity days, seed yields, disease and insect pest susceptibility, quality, plant morphology, drought tolerance, seed characteristics, etc.

The following varieties were chosen:

B. napus	*B. campestris*	*B. juncea*
Karat	Tobin	T59
Topaz	Candle	RH30
WW1307	SV.73/604	?ZEM 1

Interspecific hybrids in *Brassicas* have not successfully been produced in the past due to serious barriers to gene-exchange which hinder successful transfer of genes in both wild and cultivated species.

Procedure:

1. Fourty to sixty plants were planted in 1m rows at a spacing of 30 cm between rows;

2. Ten plants were selected and emasculated when the flower buds had just turned yellow to produce the F_1. The same plants were selfed to maintain the parents; and

3. The racemes that were manually cross-pollinated were bagged to prevent pollen contamination.

B. campestris is self-incompatible and hence maintenance is by sib-mating. *B. napus* and *B. juncea* are self-compatible, therefore, maintenance was accomplished by bagging and shaking together two or three plants of the same variety. The deviation in flowering and maturity was determined using Westar (*B. napus*) as a standard variety. The Main observations made were:

1. In the crossing program done in 1988, crossability was lower between than within species.
2. Greater success was achieved when parents had a common genome e.g. *B. napus* x *B. juncea* (AA genome)
3. In some instances, pods were formed without any seed in them. While some had rudimentary seeds.

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STATUS OF BRASSICA CROPS IN PAKISTAN

Mohammed Hanif Qazi and Parvez Khaliq

Pakistan is in short of edible oils. This shortage started developing during early 70's due to increase in population and per capita consumption and stagnant local production. The deficit of edible oil in the country is met through imports. During 1988-89, about 869 thousand tonnes of edible oil costing Rs. 8.10 billion were imported, Table 1.

Table 1. Share of domestic production and import in the total edible oil in Pakistan and value of imported oil.

Year	Domestic production ('000t)	Import ('000t)	Total* ('000t)	Import as % of total	Value of ** imported edible oil (Rs. million)
1970-75	234	119	352	33.5	531
1975-80	196	330	527	62.4	1865
1980-81	246	471	717	65.7	2625
1981-82	260	624	884	70.6	3450
1982-83	275	657	932	70.5	3670
1983-84	200	730	930	78.5	6516
1984-85	262	684	946	72.3	6954
1985-86	340	825	1065	70.8	6128
1986-87	371	740	1111	66.6	4062
1987-88	400	961	1361	70.6	7229
1988-89e	407	869	1276	68.1	8100
AGR(%)**	+3.5	+13.2	+8.1	-	-

e = Estimated, * = Total does not include the opening stock,
** = Annual growth rate.
Sources: 1. Basic Facts of Pakistan, 1980-81 and 1984-85
2. Economic Survey of Pakistan, 1986-87 and 1988-89
3. Agricultural Statistics of Pakistan (many issues)

Oilseed crops grown in the country are classified in the two groups, viz., conventional or traditional and non-conventional or new crops. Rapeseed-mustard, groundnut and sesame are conventional crops and are being grown in Pakistan since a long period of time. Sunflower, safflower and soybean have been introduced recently. Cotton which is primarily a fiber crop, contributes a major portion towards the total domestic edible oil production. It is, therefore, also considered as a conventional edible oil crop. The area, production and share of different oil seed crops in Pakistan are given in Tables 2,3 and 4.

The area and production of conventional crops remained almost stagnant for the last 19 years rather declined over time, while the area under non-conventional crops is negligible and has not increased as rapidly as planned. The present local production of oilseeds meets only about 30% of the total edible oil requirement of the country. The major portion, more than 71%, comes from cottonseed. As an average of the last four years (1986-1989), rapeseed-mustard contributed about 21%, non-conventional oilseeds (sunflower,soybean and safflower) 4.8% and other crops (sesame, corn and rice barn) 2.5%. Groundnut which is the second important oilseed crop of the country is used as roasted nut, and no oil is extracted from it. Sesame is mostly used for confectionery purpose, however, some of it is crushed for oil, as well.

Rapeseed-mustard oil is not used in Ghee industry as it contains high level of erucic acid and traces of sulphur compounds (glucosinolates). This oil is mostly used for deep frying, preserving pickles, lubrication, anointing the body and as hair oil.

Table 2. Area under oilseed crops in Pakistan.

Year	Conventional Crops				Non-conventional Crops				Grand
	Brassica	Sesame	Grd.nut	Total	Soybean	Sunfl.	Saffl.	Total	Total
	-----('000 ha)-----								
1970-75	518.6	31.5	36.2	586.3	1.19	0.76	0.07	2.04	588.30
1975-80	448.7	36.4	43.3	528.4	2.50	0.39	0.16	2.99	531.30
1980-81	417.8	44.1	46.5	507.6	3.16	4.68	2.38	10.22	517.80
1981-82	390.9	28.5	72.6	483.3	3.69	7.24	4.23	15.16	508.50
1982-83	385.5	28.5	69.3	483.3	4.10	8.13	4.13	16.36	499.60
1983-84	313.3	22.4	72.6	408.3	4.30	8.46	2.87	15.66	424.00
1984-85	346.9	34.2	59.1	440.2	4.54	9.59	2.88	17.01	457.20
1985-86	350.6	37.5	54.9	443.0	4.21	19.80	2.70	26.71	469.70
1986-87	302.8	33.2	62.8	398.8	6.50	44.53	3.00	54.03	452.83
1987-88	269.9	18.0	66.5	354.4	1.43	56.85	1.14	59.42	413.82
1988-89e	333.5	20.0	63.3	416.8	1.70	37.34	-	-	-
AGR(%)*	-2.2	-2.2	+3.9	-1.6	-1.90	+26.00	-23.90	+16.10	-1.20

e = Estimated
* = Annual growth rates are calculated on the basis of 19 years for conventional oilseed crops.
Sources: Agricultural Statistics of Pakistan, 1987-88; Personal Communication, Ministry of Industries.

Table 3. Production of oilseeds in Pakistan.

Year	Conventional crops				Non-conventional crops				Grand
	Brassica	Sesame	Groundnut	Total	Soybean	Sunfl.	Saffl.	Total	Total
	-----('000 tonnes)-----								
1970-75	279.5	10.9	51.50	341.90	0.89	0.47	0.05	1.41	343.31
1975-80	259.0	14.7	58.70	332.40	1.08	0.23	0.17	1.48	333.88
1980-81	252.5	18.3	57.40	328.20	1.34	3.49	2.16	6.99	335.19
1981-82	238.8	16.6	72.20	327.60	1.54	5.86	4.40	11.80	339.40
1982-83	246.4	10.8	84.10	340.90	1.35	6.31	4.13	11.12	352.02
1983-84	217.0	8.8	88.00	313.80	1.45	6.80	3.07	11.34	325.14
1984-85	234.8	13.6	69.10	317.40	1.80	7.79	3.14	12.73	330.13
1985-86	249.4	14.9	63.10	327.40	1.50	17.60	2.90	22.00	349.40
1986-87	213.2	12.5	75.00	358.70	2.50	37.80	2.80	43.10	401.80
1987-88	204.2	7.2	52.10	163.50	0.31	42.57	0.47	43.35	206.85
1988-89e	250.6	8.3	53.80	312.70	0.68	33.61	0.11	34.40	347.10
AGR (%)*	-0.38	-1.0	+0.98	-0.19	-7.35	+28.60	+8.80	+19.40	

e = Estimated
* = Annual growth rates are calculated on the basis of 19 years for conventional oilseed crops
Sources: Agricultural Statistics of Pakistan, 1987-88; Personal Communication, Ministry of Industries.

Table 4. Share of different oilseed crops in the total production of edible oil in Pakistan.

Year	Production of edible oil ('000 tons)					Oil as % of total		
	Rape-Mustard	Cotton-seed	Other*	N.C. Oilseeds	Grand total	Cotton-seed	Rape Mustard	Other** & NC oilseeds
1970-75	79	140	16	-	235	59.5	33.8	6.7
1975-80	73	109	14	-	196	55.2	37.5	7.3
1980-81	71	160	13	1.7	246	65.0	28.9	6.1
1981-82	72	117	13	3.7	260	65.8	27.7	6.5
1982-83	74	186	11	3.9	275	67.6	26.9	5.5
1983-84	65	117	10	4.0	200	58.5	34.5	7.0
1984-85	76	169	12	5.2	262	64.5	27.0	6.5
1985-86	82	238	12	8.3	340	70.0	24.1	5.9
1986-87	74	271	11	15.5	371	73.0	19.9	7.0
1987-88	71	286	10	32.6	400	71.5	17.8	10.8
1988-89e	88	294	10	14.9	407	72.2	21.6	6.1

e = Estimated
* = Sesame, rice barn and corn
** = Non-conventional oilseeds: sunflower, soybean and safflower.
Source: Agricultural Statistics of Pakistan (many issues).

RAPESEED-MUSTARD (*Brassica* spp.)

Crop Situation

Rapeseed-mustard are important species of Brassica grown as oilseed crops in Pakistan. These have remained one of the major sources of oil in the subcontinent for centuries. Its nutritional potential remained neglected for a long time due to its undesirable smell and non-availability of a package of production and processing technology. Plant breeding work and improvement in processing and refining techniques have now enabled us to extend the use of rapeseed-mustard oil for cooking, salad shortening and margarines.

Presently, five Brassica species are cultivated in Pakistan as field crops. Among them, the cultivation history of "sarson" (*B. campestris*), "raya" (*B. juncea*) and and "taramira" (*Eruca sativa*) goes back to centuries. Introduction of *B. napus* "gobhi sarson" is rather recent and its cultivation as a seed crop is confined to NWFP and some areas of Punjab. Another newly introduced species, *B. carinata* (Ethiopian mustard) is fastly coming up as a high yielding, aphid and drought tolerant type which is suitable for growing under irrigated as well as rainfed conditions.

Total area under these crops in the country is almost equally distributed among all the species grown. Twenty five to thirty percent of rapeseed-mustard is grown under barani (rainfed) conditions and about 70% of the crop is grown under irrigated conditions. Rapeseed-mustard are grown in winter except toria and poorbi raya (sown in late August to September and harvested in December).

Sarson and raya are planted both in irrigated as well as barani areas. Province-wise area and production of rapeseed-mustard crops is given in Table 5. Among four provinces, Punjab has the largest (53%) share of the total area, whereas Sindh, NWFP and Baluchistan contribute 25, 15 and 7%, respectively.

In the districts of Pubjab: Sargodha, Faisalabad, Mianwali, Kasur, Multan, D.G. Khan, Bahawalnagar, Bahawalpur and Rahimyar Khan, Brassica crops are grown on more than 20,000 ha each. Sukkur, Nawabshah and Sanghar are the main growing districts in Sindh. In the province of NWFP, D.I. Khan district is the main rapeseed-mustard growing area. In Baluchistan, cultivation is concentrated in Nasirabad district with an area of around 20,000 ha.

Varieties

The varieties of rapeseed-mustard along with their agronomic characters are given in Table 6.

Table 5. Province-wise area and production of rapeseed-mustard, from 1980-81 to 1988-89.

Year	Area('000 hectares)					Production ('000 tons)				
	Punjab	Sindh	NWFP	Balu-chistan	Total	Punjab	Sindh	NWFP	Balu-chistan	Total
1980-81	244.7	104.9	41.6	25.8	417.0	157.3	63.8	21.1	10.3	252.5
1981-82	223.6	102.0	46.9	18.4	390.0	145.7	61.2	22.5	9.4	238.8
1982-83	221.3	100.6	44.2	19.4	385.5	153.7	60.4	21.2	10.7	246.0
1983-84	150.6	96.1	43.0	23.0	313.3	127.8	57.6	18.2	13.4	217.0
1984-85	185.6	86.2	50.9	24.2	346.9	149.3	49.7	21.9	13.9	234.8
1985-86	206.8	83.1	36.4	24.3	350.6	171.7	48.0	16.1	14.1	249.4
1986-87	158.2	83.1	40.6	20.9	302.8	135.0	48.1	17.8	12.3	213.2
1987-88	136.3	80.4	33.4	18.9	269.0	132.2	46.3	12.7	13.0	204.2
1988-89	177.4	80.6	41.3	34.3	333.6	162.1	46.6	17.7	22.6	249.0

Source: Agricultural Statistics of Pakistan (many issues).

Table 6. Rapeseed-Mustard varieties cultivated in Pakistan.

Cultivar	Species	Yield Potential (kg/ha)	Days to Maturity	Method of breeding and pedigree	Status	Recommended areas
Toria selection A	Brassica campestris	1844	90-110	Mass selection from local material	Approved	Canal colonies of Punjab and Sindh
Brown sarson A. selection A(BSA)	-do-	2100	150-170	-do-	Improved	-do-
Yellow sarson line-1 (YSL-1)	-do-	2065	150-170	Pure line selection from local material	Approved	Irrigated areas of Punjab and Sindh
Raya line 18(RL-18)	B. juncea	3226	180-200	-do-	-do-	Throughout Pakistan
Poorbi raya	-do-	2765	100-110	Intervarietal hybridization (T21xDacca raya)	-do-	Canal colonies of Punjab
S-9	-do-	2760	120-130	Mass selection from local material	-do-	Irrigated areas of Sindh
Early raya	-do-	2565	120-140	Pure line selection from Bangladesh rape	-do-	Irrigated and Dobari areas of Sindh
LGL	B. napus	1200	185-195	Pure line selection from Japan rape	-do-	Mainly for fodder in mixture with berseem
DGL	-do-	1270	185-195	-do-	Improved	Irrigated and Rokhobi areas of NWFP
PR-7	-do-	1235	190-200	-do-	Approved	-do-
Oro	-do-	1235	170-185	Introduction from Canada	-do-	-do-
Ganyou-5	-do-	2426	180-200	Selection from China material	-do-	-do-
Peela raya	B. carinata	3600	200-210	Selection from local material	-do-	Rainfed & irrigated areas of NWFP
Taramira selection	Eruca sativa	800	190-200	Mass selection from local material	-do-	Throughout Pakistan under marginal and barani conditions

Average Yield

The yield per hectare of rapeseed-mustard in Pakistan is very low as compared to other countries of the world, as is evident from Table 7. However, there has been a slight increase in yield per hectare from 526 kg in 1970-71 to 751 kg in 1988-89, registering an average annual growth rate of (+) 2.11%.

Table 7. Comparison of average yields of rapeseed-mustard in the major growing countries of the world.

Country	Yield(kg/ha)
Canada	1162
China	1072
France	2854
India	748
Japan	2000
Pakistan	758
Poland	2549

Source: FAO Production Year Book, vol. 42,1988.

Cropping Pattern

a) Existing cropping pattern:

1. Rape/Mustard - (Oct. - April) Groundnut - (April - Nov.) Wheat - (Nov. - May)

2. Rice - (June - Oct.) Rape/Mustard - (June - Oct.)

3. Wheat - (Nov.- May) Guar - (May - July) Rape/Mustard (Sept.- April)

Proposed cropping pattern

1. Rape/mustard (Oct.-April) - Maize (July-Oct.) -Wheat (Nov. - May) - Soybean (July-Oct.).

2. Wheat - (Nov.-May) - Guar - (May-July) - Rape/mustard (Sept.-Dec.) - Sunflower - (Feb.-May) - Maize (July -Sept.).

3. Cotton (May-Nov.) - Sunflower (Feb.- May) - Mungbean/soybean (July-Oct.) - Rape/mustard (Oct.-April).

4. Rape/mustard (Oct.-April) - Sunflower (July-Oct.) - Gram/wheat Oct.- April) .

5. Rape/mustard (Oct.-April) - Soybean (July-Sept.) - Gram/wheat (Oct.- April).

Constraints for high Yield

A number of biological and socio-economic constraints have been identified to limit the production of these crops. These include:

Biological:
- Use of marginal land;
- inadequate seed bed preparation;
- non-availability of improved varieties;
- damage of insects (mainly by aphids) and diseases;
- low plant population;
- lack of judicious and potimum fertilizer application;
- damage by cold water at higher altitute of NWFP;
- critical forage shortage in winter encourages harvesting rape and mustard for fodder; and
- lack of proper seeding, harvesting and threshing equipments.

Socio-economic:
- Lack of floor price fixed by the government;
- non-use of oil in hydrogenated products because of sulphur products in the oil;
- consumer bias against use of the oil;
- competition with other winter crops i.e. wheat, gram, lentil and forages etc.; and
- lack of understanding by planners and administrators on the potential of these crops.

Current Status of Rapeseed-mustard Research

- Research institutes/stations currently working on Brassica crops are:
- National Agricultural Research Centre (NARC), Islamabad.
- Oilseed Research Institute, Faisalabad, Punjab.
- Barani Agricultural Research Institute, Chakwal, Punjab.
- Agricultural Research Station, Mingora, Swat, NWFP.
- Agricultural Research Institute, Ratta Kulachi, D.I. Khan, NWFP.
- Agricultural Research Institute, Tandojam, Sindh.
- Agricultural Research Institute, Sariab, Quetta, Baluchistan, and
- Brassica Research Center, NWFP Agricultural University, Peshawar.

The main objectives of the National Coordinated Research (NCR) program are:

- to coordinate and develop linkages with relevant international research centres for exchange of germplasm and scientific literature.
- to coordinate the provincial institutes and facilitate the exchange of germplasm, segregating material and improved lines, implements, amchinery, training and funds, etc.
- collection, evaluation, maintenance and increase of germplasm of oilseed crops.
- conduct basic and applied research of national improtance on oilseed crops.

National Coordinated Yield Trials (NCYT) is the core activity of NCR under which candidate varieties are tested in different agro-ecological zones of the country, Table 8.

Table 8. Yield (kg/ha) of Brassica entries in NURYT from 1984-85 to 1988-89.

ENTRY	1988-89 (10)*	1987-88 (16)	1986-87 (10)	1985-86 (17)	1984-85 (9)	MEAN
B. campestris:						
B.S.A.	-	-	1256	1278	1037	1212
K-953	-	-	1261	1348	1061	1252
K-41	-	-	1325	1339	-	1334
Tobin	-	-	1017	1073	729	971
K-794	-	-	1335	1363	-	1353
Torch	-	-	-	1353	785	1156
K-946	-	-	-	-	835	835
K-1071	-	-	-	-	1222	1222
Candle	-	-	990	-	990	
B. napus:						
PR-7**	1048	1262	1407	1229	1032	1208
NARC-82	-	-	1337	1195	894	1159
Westar	993	1037	1322	1095	698	1042
Marnoo	1133	1118	842	1152	-	1080
Ganyou-5	1138	1275	1475	1399	728	1240
Altex	847	-	866	1010	910	924
Tower	-	-	-	-	713	713
Tatyoon	1090	1056	-	-	-	1069
Muluka	1103	-	-	-	-	1103
Shirlee	1287	-	-	-	-	1287
Henna	806	-	-	-	-	806
DGL	1103	-	-	-	-	1103
S-28	1155	-	-	-	-	1155
B. juncea:						
Varuna	-	-	-	1485	1438	1469
PR-269	1142	1459	1566	1349	1448	1393
Early Raya	-	-	-	1237	-	1287
S-9**	1044	1394	1601	1403	1333	1365
K-318	-	-	-	1394	1407	1399
P-61-25	-	1544	1565	1429	-	1503
RL-18**	-	1525	1578	1205	-	1411
BM-1	1257	1726	1843	1602	1338	1607
Yellow Raya	-	-	-	1479	1243	1397
77-1300	-	-	-	1412	1038	1283
PR-129	-	-	-	-	1557	1557
PR-123	-	-	-	-	1248	1248
DIC-111	-	-	-	-	1331	1331
PR-84	-	-	-	-	1237	1237
SM-83001	1083	1461	-	-	-	1316
ORI-50-6	-	1638	-	-	-	1638
P-6-1	1066	1512	-	-	-	1340
P-98	1134	1564	-	-	-	1399
UCD-1202-6/2	1188	1664	-	-	-	1481
UCD-1202-9/2	1078	1313	-	-	-	1223
SPS-23/1	1093	1462	-	-	-	1320
V-30	1113	-	-	-	-	1113
L-85	-	-	-	1256	-	1256
P-53	-	-	-	1205	-	1205

* = Figures in parenthesis show number of locations from which the average was taken.
** = Check variety.

The main objectives of the provincial research program on rapeseed-mustard are:

- evaluation of new varieties possessing high yield potential with other desirable characters such as short duration, high oil content and double-zero traits.
- assessment of crop production and protection requirements for newly developed strains.
- multi-location on-farm testing of newly developed strains within the province to seek the approval of provincial seed council, and
- seed multiplication of newly developed varieties/cultivars.

A CIDA Project, entitled'BARD' is also actively working on rapeseed-mustard. The main objectives of this project are:

- to breed/introduce high quality, high yielding cultivars and suitable technology for use in barani (rainfed) areas with special emphasis on increasing production of traditional oilseed crops (rapeseed-mustard and groundnut).
- assist in developing appropriate machinery by providing financial and professional support to the local manufactures.
- develop, verify and extend new production technology suitable for use to farmers on barani lands.

The project was commenced in November 1982, since then quite a few achievements have been made under this project which are as follows:

- Tremendous strides have been made in the outreach programs for rapeseed-mustard. Yields have become more consistent, even in the face of adverse weather conditions. Advances have been made in mechanization of planting and harvesting. The introduction of a prototype drill for small seeded crops was highly successful. It helped to obtain optimum plant stand of rapeseed-mustard over a range of soil conditions in the Punjab and NWFP, Provinces.

- An analytical oilseed laboratory has been established. The laboratory is capable of analysing oil content, fatty acid composition of oil, glucosinolate content and composition of the meal. This laboratory is a valuable asset for improving oil contents and quality in different breeding programs in the country.

- Progress in improvement of rapeseed-mustard varieties is promising. Breeding material with desirable quality traits, high yield and good plant vigour has been identified.

- Canola type rapeseed varieties such as Tower, Regent, Altex (*B. napus*), and Candle, Torch (*B. campestris*) were introduced from Canada for the first time in the variety testing program at NARC in 1979-80. In the following year, Tower and Candle were planted in Mingial area of Rawalpindi on the farmers' fields.

In 1984, a new Canadian rapeseed variety "Westar" (*B. napus*) was introduced. This variety has shown a yield potential of 1170-1427 kg/ha on the farmers' fields. On a semi-commercial scale, 250 acres in 1987-88, 225 acres in 1988-89 and 350 acres in 1989-90 of Westar variety were successfully grown on farmers' fields in Rawalpindi, Attock and Swat districts.

Seed produced from this crop was processed and Canola oil was made available in the local market. In 1988, 13275 litres of refined oil was obtained. During 1989 the same experience was repeated and 12.5 tones of refined canola oil was marketed through Utility Stores. Consumers were asked to assess the acceptability of the taste and was found to be highly favourable.

Breeding work on Rapeseed-mustard at Brassica Research Center, NWFP Agricultural University, Peshawar

a) <u>Resistance to cabbage aphid (*Brevicoryne Brassicae L.*)</u>: Cabbage aphid or Mustard aphid is a serious pest of the genus *Brassica*. Resistance to cabbage aphid has been reported in *B. oleracea* cultivars. Some tolerance at vegetative stage has been observed in *B. juncea*. Resistance has been transferred from *B. oleracea* to *B. napus* and now line 3 (AR) can serve as a bridge for gene interchange between *B. oleracea*, *B. napus* and *B. campestris*.

A cross between *B. napus* cultivars 'Tower' (2n=38) and *B. oleracea* cultivar 'Rawara' (2n=18) was made. The F_1 hybrid obtained was back crossed with *B. napus* cultivar

'Rangi' biennial (2n=38). The F_1 and F_2 generations from this cross was called as 'Line -3'. This line is still a segregating line for aphid resistance and is usually biennial. Resistance to cabbage aphid was confirmed by planting this line at Peshawar and Abbotabad.

Line 3 (AR) was crossed with different cultivars of *B. napus* (Westar, NARC-82, Kiwi Salam and Altex) and *B. juncea* (LL-84, and P53-48-2). The cross of Altex x AR gave successful results. The F_1 seeds of this cross were raised at Brassica Research Station, NWFP Agriculture University, Peshawar during 1986 while the F_2 generation was planted in Kaghan. Selection for aphid resistance was not done in Kaghan becuase of inavailability of aphids and other facilities. Selection of Altex x AR hybrids for aphid resistance was carried out in the F_3 generation.

The F_3 generation of the cross Altex x AR along with its parents were planted in green house of Peshawar during 1989. Both the cross and parental material were inoculated with cabbage aphid after 10 days of emergence. Data on seedling reaction to aphid was recorded at weekly interval for four weeks, Table 9. Statistical analysis of the data showed that the parent Altex and the cross Altex x AR exhibited high degree of susceptibility while line 3 showed resistance to cabbage aphid, Fig 1.

Table 9. Development of resistance to cabbage aphid by crossing line 3 (AR) with Altex (Observations are based on 100 plants for each treatment).

Parents & Generations	1st Week			2nd Week			3rd Week			4th Week		
	R*	I*	S*	R	I	S	R	I	S	R	I	S
Altex(P_1)	0	64	36	0	20	80	0	1	99	0	0	100
Altex x AR (F_3)	14	69	17	0	28	72	0	19	81	0	8	92
AR** (P_2)	62	35	3	59	33	8	58	34	8	58	34	8
Total	76	168	56	59	81	160	58	54	188	58	42	200
	X^2= 119-95			X^2= 171.77			X^2= 204.05			X^2= 231.89		

* R = Resistant, I = Intermediate, S = Susceptible.
** Aphid Resistant line 3.

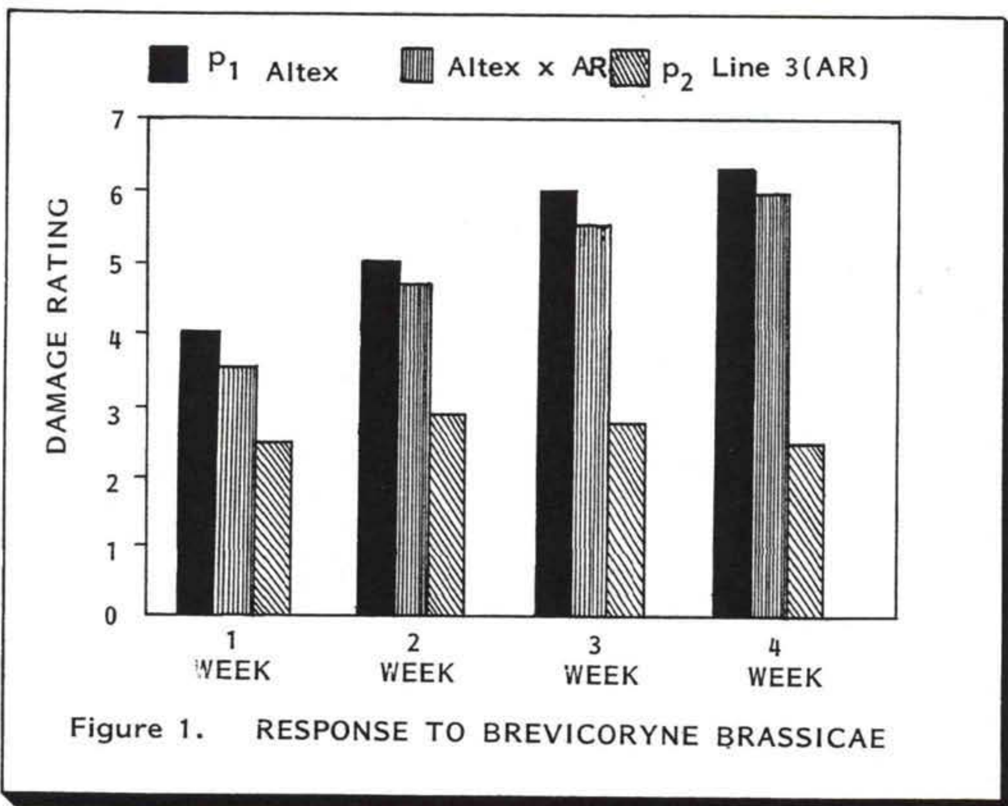

Figure 1. RESPONSE TO BREVICORYNE BRASSICAE

From Table 9, the source of resistance to aphid was confirmed in line 3. Scientists from USA and East Germany have also confirmed this line as resistant to cabbage aphid. However, these studies showed that transfer of the resistance is a complex process and may be controlled by more than one gene.

Seed from F_3 (Altex x AR) plants, having variable degrees of resistance were harvested. The F_4 seeds from the selected resistant plants were sown at Kaghan for further studies. Some of these plants were back crossed with Line-3. Both the back crossed and F_3 (Altex x AR) plants are being tested at Brassica Research Station, Peshawar. Ten-days old seedlings of both the parents were inoculated with cabbage aphid and selection for resistance to aphid was made on the basis of damaging rate and dry weight of the seedlings. Data were recorded four times at weekly intervals. The resistant material was transplanted in the field during January, 1990. Seeds from these resistant lines will be harvested during May 1990. These harvested seeds crop will be planted at Kaghan for further field testing.

b) <u>Intraspecific and interspecific crosses</u>: Different intraspecific and interspecific crosses of *B. napus, B. juncea*, and *B. carinata* were also planted during 1988-89 at Brassica Research Station, Peshawar. The hybridity of these crosses was confirmed by using marker gene and chromosome counts. Details of the crosses are given in Table 10.

The main objectives of the crossing program are:

- to transfer drought tolerance from *B. juncea* cv.LL-84 and P53-48-2 to *B. napus* cultivars.
- to transfer yellow seed coat colour from *B. juncea* and *B. carinata* to *B. napus* cultivars.
- to transfer low glucosinolates and erucic acid characters from *B.napus* cv. Westar to *B. juncea* cv.LL-84 and *B. carinata* cv. Yellow Raya.

To achieve the above mentioned objectives in the shortest possible time, three generations of breeding material are produced in only one year i.e., two generations from autumn to spring at Peshawar and one generation at Kaghan during Summer. Six intraspecific and nine interspecific F_2 crosses were planted at Kaghan during May 1988. About 200-300 individual lines were selected in each cross for oil quality, yield and seed coat colour. For drought tolerance, interspecific crosses between *B. napus* and *B. juncea* were planted at Banda Dawood Shah, District Kark, Table 11. In each cross, about 50-200 individual lines were selected for drought tolerence on the basis of their yield potentials.

Table 10. Different intraspecific and interspecific F_3 and F_4 crosses planted at NWFP Agricultural University, Peshawar during October, 1988.

Cross		Brassica Species
Marnoo x Ganyou-5	(F_3)	*B. napus* x *B. napus*
Marnoo x Westar	"	" " "
Ganyou-5 x Westar	"	" " "
Westar x Salam	"	" " "
Ganyou-5 x Altex	"	" " "
Ganyou-5 x Salam	"	" " "
Marnoo x Salam	(F_4)	" " "
Westar x Salam	"	" " "
Altex x Salam	"	" " "
P53-48-2 x Salam	(F_3)	*B. juncea* x *B. napus*
LL 84 x Salam	"	" " "
LL 84 x Westar	"	" " "
Westar x LL 84	"	*B. napus* x *B. juncea*
Altex x P53-48-2	"	" " "
Westar x Yellow Raya	"	*B. napus* x *B. carinata*
Altex x Yellow Raya	"	" " "
P53-48-2 x Y.Raya	"	*B. juncea* x *B. carinata*
P53-48-2 x Y.Raya	(F_4)	" " "
Yellow Raya x Salam	(F_3)	*B. carinata* x *B. napus*

Table 11. Six interspecific F_3 crosses planted at Banda Dawood Shah for drought tolerance-selection.

Cross		Brassica species
P53-48-2 x Salam	(F_3)	*B. juncea* x *B. napus*
LL 84 x Salam	"	" " "
LL 84 x Westar	"	" " "
Westar x LL 84	"	*B. napus* x *B. juncea*
Altex x P53-48-2	"	" " x
Westar x Yellow Raya	"	*B. napus* x *B. carinata*

The material planted at Kaghan was harvested during September, 1988. The selected lines were again planted at NWFP Agricultural University, Peshawar during October, 1988 for further test of the F_3. These materials were harvested during April, 1989. Again 200-300 individual lines were selected in each cross on the basis of seed yield oil quality and seed coat colour.

The harvested F_4 and F_5 seeds of different intra and interspecific crosses were planted at Kaghan during May and harvested in September, 1989.

From each cross, 50-300 lines were selected for plantation at Peshawar. All the F_5 and F_6 materials harvested in Kaghan were sown at Brassica Research Centre, Peshawar, as well as at NARC, Islamabad for further observation. Data pertaining to average pod and stem angles, seeds per pod, pods per main stalk and pod length (based on 100 observations) are given in Tables 12 and 13.

Table 12. Average number of seeds/pod of 5 interspecific F_5 population along with their parents. Data is based on 100 observation.

Cross	No. of seeds/pod			No. of pods/main stalk		
	P_1	F_5 Cross	P2	P_1	F_5 Cross	P_2
B. napus x B. juncea cv. Altex cv. P53-48-2	20.09	21.75	15.15	77.73	89.20	27.15
B. juncea x B. napus cv. P53-48-2 cv. Salam	15.15	17.68	27.73	27.15	67.48	82.97
B. juncea x B. napus cv. LL84 cv. Salam	13.32	22.28	27.73	50.23	86.64	82.97
B. napus x B. carinata cv. Altex cv. Yellow Raya	20.09	21.13	13.83	77.73	86.83	23.12
B. carinata x B. napus cv. Yellow Raya cv. Salam	13.83	22.64	27.83	23.12	68.78	82.97
L.S.D. (5% level) =		1.63			7.33	
S.E. =		±0.59			±2.64	
C.V. =		21.26%			28.24	

Table 13. Average pod, stem angle and pod length of 5 interspecific F_5 crosses compared with their parents. Data is based on 100 observation.

Cross	Pod and stem angle (cm)			Pod length (cm)		
	P_1	F_5 Cross	P_2	P_1	F_5 Cross	P_2
B. napus x B. juncea cv. Altex cv. P53-48-2	76.04	61.06	13.70	6.48	5.75	4.45
B. juncea x B. napus cv. P53-48-2 cv. Salam	13.70	48.70	71.29	4.45	5.98	7.33
B. juncea x B. napus Cv. LL84 cv. Salam	23.26	53.50	71.29	4.42	6.53	7.33
B. napus x B. carinata Cv. Altex cv. Yellow Raya	76.04	68.70	19.44	6.48	6.20	5.00
B. carinata x B. napus Cv. Yellow Raya cv. Salam	19.44	50.72	71.29	5.00	6.79	7.33
L.S.D. (5% level) =		3.99			0.28	
S.E. =		± 1.44			±0.10	
C.V. =		20.86			12.24	

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NATIONAL UNIFORM RAPESEED-MUSTARD YIELD TRIALS AND THEIR ROLE IN VARIETY SELECTION

Mohammed Hanif Qazi and Masood A. Rana

National Uniform Rapeseed-Mustard Yield Trials (NURYT) are conducted at various research stations through out Pakistan under the direct supervision of researchers. The basic rational behind these trials is selection of varieties which are outstanding in yield production and other desirable characters. The multi-environmental trials (NURYT) are a vital link between genetic improvement and the production environments. In this context, specific problems of adaptation are most likely to be confronted within genetic improvement programs, and this most often provides a chance for research workers to address some important aspects of plant improvement such as inter-specific responses, genotype x environment interaction, genotype stability and optimum management strategies.

Selection of improved genotypes of rapeseed-mustard generally proceeds at three levels involving initial germplasm screening at experimental stations, preliminary testing of selected lines in replicated trials and final evaluation or pre-release testing of potential commercial varieties in the National Uniform Trials.

The national uniform testing of potential commercial varieties of rapeseed-mustard is an important activity of the Coordinated Research Program whose coordinator is based at the National Agricultural Research Centre (NARC), Islamabad. Candidate lines contributed by the breeders of various provincial and federal rapeseed-mustard improvement programs are tested for their yielding ability, adaptation to various agro-ecological zones of Pakistan, disease resistance, oil content, and other agronomic characteristics. This report is based on data from these trials which form the basis of recommendations by the Variety Evaluation Committee (VEC) regarding the approval and release of promising new varieties.

The objectives of the NUYT are to provide an opportunity to research workers who are developing new varieties to assess the performance of their advanced breeding lines over a wide range of climatic, cultural and disease conditions in the various agro-ecological zones of the country. It also allows local research and extension workers to compare the performance of new varieties from other provinces and institutes.

HISTORY

The NURYT was initiated in 1984-85 for the first time. Twenty three advanced lines/varieties of four *Brassica* species were included which were planted at nine locations in three provinces, Table 1. Among them, six entries were from each of *Brassica campestris* and *B. napus*, ten entries from *B. juncea* and one from *B. carinata*. In the following year, the NURYT were conducted at 17 locations in all the four provinces with 24 entries. For a better comparison, the cultivars which were early and performed better in autumn were grouped together. For this group, an additional NURYT was initiated from 1986 in Zaid Kharif season (when crop is planted in August or September and harvested in November/December). The Zaid Kharif NURYT was conducted only for two years (1986-87) and then it was terminated.

Testing of the *B. campestris* entries was discontinued in 1987-88 because there was no regular breeder working for this species anywhere in the country, and other breeders lost their interest in the material of this species.

PROCEDURES

Seed set preparation and planting:

Breeders of different research stations and institutes send their candidate varieties to the coordinator at NARC, to be included in the NURYT. Seed sets are prepared at NARC and sent back to

Table 1. Number of locations and entries of Brassica included in each species in the NURYT, 1984-85 to 1988-89.

Year	No. of locations	Species	No. of entries
Rabi Season			
1984-85	9	B. campestris	6
		B. napus	6
		B. juncea	10
		B. carinata	1
			23*
1985-86	17	B. campestris	6
		B. napus	6
		B. juncea	11
		B. carinata	1
			24*
1986-87	10	B. campestris	6
		B. napus	6
		B. juncea	6
			18*
1987-88	16	B. napus	6
		B. juncea	11
			17*
1988-89	10	B. napus	11
		B. juncea	11
			22*
Zaid Kharif			
1986	5	B. campestris	1
		B. napus	6
			7*
1987	4	B. campestris	2
		B. napus	6
			8*

* Total number of entries included in the NURYT.

the cooperating unit in the provinces along with a booklet containing field lay out, brief description on how to take data on different variables and data sheets. This is done to achieve uniformity in the recording of data and to make the results comparable.

Observations are recorded for many traits such as plant stand, days to 50% flowering, days to maturity, plant height, seed yield, oil content, 1000-seed weight, aphid infestation, shattering, lodging and disease attack. The trials are planted according to the planting time of the respective location which usually varies from October 15 to November 15 for winter crop and August 15 to September 15 for Zaid Kharif crop. Entries are coded at the time of seed preparation. This is done to avoid the possible bases of the cooperating research workers that could be involved during the evaluation of their varieties. These are decoded after the receipt of data.

Monitoring:

A travelling seminar is arranged in the last week of February or beginning of March every year when the crop is in the stage of maturity in the Sind province, late seed development in Punjab and flower completion in NWFP province. Participants of the seminar make a multi-discipline team consisting of breeders, plant pathologists, entomologists, agronomists and extension workers. The group visit the NURYT at different locations and try to cover most of them. Specialists take notes on the respective aspects and also group discussions take place in the field at each location. At the termination of travelling seminar the scientists group spend one day at NARC to put their observations together and formulate their recommendations.

Review of results: After the harvest of trials, each cooperating scientist sends his result to the Coordinator at NARC, who compiles the results in the form of a report. Every year, in the month of July or August, the scientists working on Brassica crops gather at NARC, Islambad to participate in the Annual meeting where the NURYT results are presented and discussed. In the same meeting, the scientists plan, number and names of entries and locations of trial for the next NURYT. This opportunity is availed to plan research programs to be undertaken during next year as well.

SUMMARY OF NURYT RESULTS UPTO 1988-89

The cultivars of *B. juncea* produced higher seed yields than other three species: *B. campestris, B. napus* and B. carinata in the NURYT of 5 years, 1984-85 to 1988-89, Table 2. *B. juncea* cultivars PR-269, BM-1 and S-9 included in the uniform trials for five years and thus as an average of 62 locations, BM-1 was the highest yielding cultivar with a mean seed yield of 1607 kg/ha. BM-1 exceeded both check varieties in seed yield, by 20% from S-9 and 16.1% from RL-18. The other high yielding cultivar was P-61-25 with 1503 kg/ha mean seed yield over 43 locations in 3 testing years, 1985-86 to 1987-88. It exceeded the check varieties S-9 and RL-18 by 10.1 and 6.5%, respectively. Varuna, another high yielding cultivar, was tested for the first two years only and then dropped because it had similar

Table 2. Yield (kg/ha) comparison of Brassica entries in NURYT, 1984-85 to 1988-89.

Entry	1988-89 (10)*	1987-88 (16)	1986-87 (10)	1985-86 (17)	1984-85 (9)	MEAN
B. campestris						
B.S.A	-	-	1256	1278	1037	1212
K-953	-	-	1261	1348	1061	1252
K-41	-	-	1325	1339	-	1334
Tobin	-	-	1017	1073	729	971
K-794	-	-	1335	1363	-	1353
Torch	-	-	-	1353	785	1156
K-946	-	-	-	-	835	835
K-1071	-	-	-	-	1222	1222
Candle	-	-	990	-	-	990
B. napus						
PR-7**	1048	1262	1407	1229	1032	1208
NARC-82	-	-	1337	1195	894	1159
Westar	993	1037	1322	1095	698	1042
Marnoo	1133	1118	842	1152	-	1080
Ganyou-5	1138	1275	1475	1399	728	1240
Altex	847	-	866	1010	910	924
Tower	-	-	-	-	713	713
Tatyoon	1090	1056	-	-	-	1069
Muluka	1103	-	-	-	-	1103
Shirlee	1287	-	-	-	-	1287
Hanna	806	-	-	-	-	806
DGL	1103	-	-	-	-	1103
S-28	1155	-	-	-	-	1155
B. juncea						
Varuna	-	-	-	1485	1438	1469
PR-269	1142	1459	1566	1349	1448	1393
Early Raya	-	-	-	1237	-	1287
S-9**	1044	1394	1601	1403	1333	1365
K-318	-	-	-	1394	1407	1399
P-61-25	-	1544	1565	1429	-	1503
RL-18 **	-	1525	1578	1205	-	1411
BM-1	1257	1726	1843	1602	1338	1607
Yellow Raya	-	-	-	1479	1243	1397
77-1300	-	-	-	1412	1038	1283
PR-129	-	-	-	-	1557	1557
PR-123	-	-	-	-	1248	1248
DIC-111	-	-	-	-	1331	1331
PR-84	-	-	-	-	1237	1237
SM-83001	1083	1461	-	-	-	1316
ORI-50-6	-	1638	-	-	-	1638
P-6-1	1066	1512	-	-	-	1340
P-98	1134	1564	-	-	-	1399
UCD-1202-6/2	1188	1664	-	-	-	1481
UCD-1202-9/2	1078	1313	-	-	-	1223
SPS-23/1	1093	1462	-	-	-	1320
V-30	1113	-	-	-	-	1113
LL-85	-	-	-	1256	-	1256
P-53	-	-	-	1205	-	1205

* Number of locations from which the average was taken.
** Check variety.

agronomic characters to that of BM-1 which belongs to the same origin. All the varieties of *B. juncea* are high in erucic acid and glucosinolates (double high).

In *B. napus*, PR-7 (check variety), Westar and Ganyou-5 were tested for five years whereas Marnoo was tested for four years and NARC-82 and Altex for three years. The most promising variety among these was Ganyou-5 which, as an average of 62 locations, produced higher yield than the check PR-7. But the difference in yield was not much (2.6%). All other entries included and

tested in NURYT were low yielding than the local check. Ganyou-5 is a double high variety which was introduced from China. The double-low varieties from Canada, Australia and Sweden are considerably lower yielding than the local check PR-7 which is a double-high.

In *B. campestris*, two Canadian double-low varieties, Tobin and Torch were

included in the NURYT but both were lower yielding than the local double-high material. K-794, K-41 and K-953 were higher yielding than the check variety, BSA.

The above results indicate that the cultivars belonging to *B. campestris* and *B. napus* were comparatively lower yielding than those of to *B. juncea*. The resons for lower yields can be listed as follows:

i) These species are highly sensitive to temperature which affects the seed-filling process and seeds do not develop completely,

ii) Both species shatter very heavily,

6iii) They are susceptible to pests and diseases,

iv) *B. campestris* cultivars are mostly short-seasoned and mature much earlier than *B. napus* and *B. juncea*,

v) No regular breeding program exists in any province and thus, much work is not done for the improvement of this species in Pakistan.

B. juncea is the species which performed best throughout all the agro-ecological zones of the country where all the species were tested in NURYT. It is most probable that this species is a native of Indo-Pak sub-continent and a high genetic diversity in the germplasm of the species exists in this part of the world. In addition, strong breeding programs for *B. juncea* are being operated in the Punjab and Sind provinces which did a good job in the improvement of the species. This species also has a non-shattering characteristic and is more hardy than *B. campestris* and *B. napus*. It is hoped that if double-zero character is introduced in this species, it will be a great break-through for increasing oilseed production in Pakistan.

CONCLUSION

National Uniform Rapeseed-Mustard Yield Trial is an effective vehicle for variety evaluation. As a result of these trials, BM-1 and P-61-25 have been identified as outstanding candidate varieties for registration and to be released for general cultivation. NURYT is recognized by the National Seed Council as the only acceptable variety evaluation mode for recommendation and registration of varieties in Pakistan. Provincial Seed Council of NWFP use NURYT results for registration and variety recommendation. In the other provinces, there is an increasing trend of acceptability of NURYT results though do not totally rely upon it for variety registration and recommendation.

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PRESENT STATUS AND FUTURE STRATEGIES OF OILSEED BRASSICA RESEARCH IN INDIA

P.R. Kumar and P.S. Bhatnagar

In India oilseed Brassica is the second important oilseed crop after groundnut. In recent years (1986-87 to 1988-89) production and productivity of Brassica oil seed have increased substantially to 4.4 million tones and 909 kg/ha from the levels of 2.61 million tones and 708 kg/ha, respectively, Table 1 & Fig. 1. By 2000 AD, additional production of 4 million tonnes is to be achieved to provide 30 g fats per person per day (1). The task is stupendous and challenging, yet achievable. The strength of recently developed technology-tests on farmers' fields has led to the optimism of achieving the desired productivity increase under both rainfed and irrigated conditions. However, stability in production and productivity is still elusive. The paper highlights the current status of research activities and future research strategies in achieving envisaged targets.

Table 1. Area, Production, Productivity of Rapeseed/ Mustard in India during 1966-67 to 1988-89.

Year	Area (Million ha)	Productivity (kg/ha)	Production (Million Tonnes)
1966-67	3.01	408	1.23
1967-68	3.24	483	1.57
1968-69	2.87	469	1.35
1969-70	3.17	493	1.56
1970-71	3.32	594	1.98
1971-72	3.61	396	1.43
1972-73	3.32	545	1.81
1973-74	3.46	493	1.70
1974-75	3.68	612	2.25
1975-76	3.34	580	1.94
1976-77	3.13	496	1.55
1977-78	3.58	460	1.65
1978-79	3.54	525	1.86
1979-80	3.47	411	1.43
1980-81	4.11	560	2.30
1981-82	4.40	541	2.38
1982-83	3.83	577	2.21
1983-84	3.87	673	2.61
1984-85	3.99	771	3.07
1985-86	3.98	674	2.68
1986-87	3.72	700	2.61
1987-88	4.62	788	3.46
1988-89	4.87	907	4.41

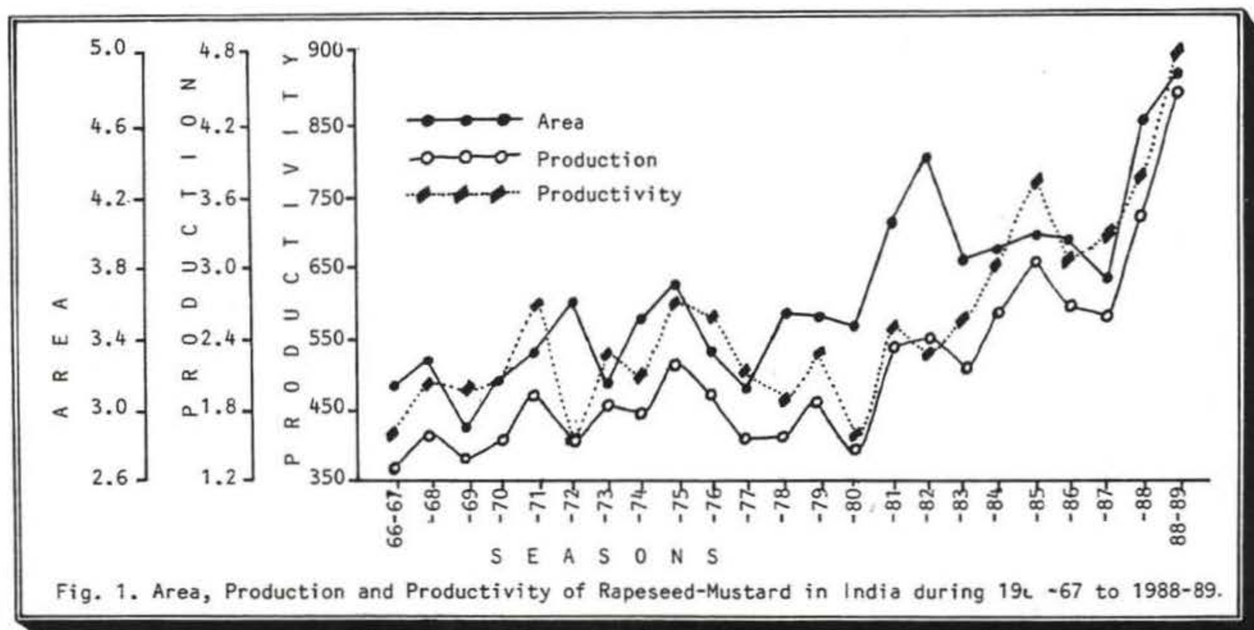

Fig. 1. Area, Production and Productivity of Rapeseed-Mustard in India during 19ι -67 to 1988-89.

PRESENT STATUS

Management of genetic resource

India is considered the primary centre of diversity; distributed over eight phytogeographical zones in the country. During 1981 to 1989, 2164 land races have been collected from different parts of the country. During this period, 1634 accessions were introduced from 18 countries (Canada, USA, Sweden, France, Holland, Australia, Pakistan, West Germany, Bangladesh, Nepal, Hungary, USSR, U.K., Poland, Bulgaria, New Zealand, Zambia and Spain). the lines have been evaluated for qualitative and quantitative characters on the basis of descriptors prepared. The promising ones have been identified for different biotic and abiotic stresses and other economic attributes.

Utilization of genetic resource:

The spectrum of genetic diversity available has helped in bringing improvement of oilseed Brassica The promising accessions have been subjected to selection pressure and/or utilized in recombination/mutation breeding for developing high yielding and stable varieties. The promising high yielding varieties identified/released through different breeding approaches are given below:

(i) Through selection

- *Brassica juncea* L. Czern & Coss: T-11, T-16, BR-13, BR-40, Patan-67, Varuna, Kranti, Krishna, Durgamani, Laha-101, Shekhar, Rohini, RL-18, Seeta, RH-30 and NDR-8501.
- *Brassica campestris* L. var. Toria: M-2, M-3, M-18, M-27, TS-29, B-54, BR-23, BR-29, BR-36, RAUTS-17, T-36, DK-1, T-9 and ITSA.
- *Brassica campestris* L. var. Brown Sarson: BSA, BSG, BSH-1, BS-2, BS-70, B-65 and KOS-1.
- *Brassica campestris* L. var. Yellow Sarson: YS-66-197-3, PYS-6, K-88, Patan-66, T-10, T-42 and YST-151.

(ii) <u>Through recombination</u>

- *Brassica juncea* L. Czern & Coss: Sanjucta Asesh, Prakash, Pusa bold, RW 85-59, RW-351, Vaibhav, Vardan, RH-819, RH-8113, RH-785, RH-781, RL-1359, DIRA-367 and DIRA-247.
- *Brassica campestris* L. var. Toria: PT-303, TH-63 and Panchali.
- *Brassica campestris* L. var. Yellow Sarson: YSB-19-7-C.

(iii) <u>Through mutation breeding</u>

- *Brassica juncea* L.: RLM-198, RLM-514, RLM-619, TM-2, TM-4, TM-18, TM-19, TM-21 and RH-7859.

<u>Insect-resistance</u>:

Among different insects, Mustard Aphid (*Lipaphis erysimi* Kalt.); Painted Bug (*Bagrada cruciferarum* Krik.); Mustard sawfly (*Athalia laucens proxima* Klug.); Leaf minor (*Phytomyze* horticola Gouraceu) and Flea bettle (*Phyllotreta cruciferae* Goeze.) are of economic importance. The chemical control is quite effective but most of the chemicals are lypophilic in nature. It is, therefore, considered essential to develop varieties with inbuilt resistance and to adopt biological control measures.

Hybridization using T-6342 as a source of aphid resistance was undertaken and genotypes RH-7846 and RH-7847 with fair degree of tolerance have been developed. The genetic material generated through hybridization is under different stages of testing under multilocation trials.

There are more than twelve natural enemies of mustard aphid. Two insects, lady bird beetle (*Coccinella septumpunctata*) and syrphid fly (*Syrphid* sp.) are important predators. Unfortunately, their population remains low at the peak time of aphid infestation, as the predators require higher temperature for survival and multiplication, whereas low temperature in December-January is conducive for multiplication of aphids. The efforts are underway to develop a predator race which can multiply rapidly at the peak period of aphid infestation.

<u>Disease resistance</u>

Alternaria blight, white rust, downy mildew and phyllody are some of the major diseases of oilseed Brassica in India. Hybridization program has resulted in developing the variety RH-8113 with field resistance to Alternaria blight and white rust diseases. In addition, strains RH-8114,

KRV-Tall, PR-8701, and PR-8705 have been identified showing field tolerant reaction. The gene(s) responsible for resistance to white rust have been successfully transferred from *Brassica carinata* to *B. juncea* (3). The material developed through interspecific crosses between *juncea* and *carinata* is under advanced stages of testing.

Frost tolerance

Frost is not a common phenomenon but it leads to considerable losses in seed yield in unpredictable frosty years. Four *juncea*-(RH-781, Rh-8574, RH-8886 and RW-175) and one *Eruca sativa*-(TMH-52) frost tolerant (<10% damage) genotypes have been identified.

Drought tolerance

More than 53% of the area under oilseed Brassica is rainfed. A number of varieties/strains of Indian mustard (RLM-514, RH-7361, RH-30, RH-819, Vardan, RH-781) have been identified drought tolerant under rainfed conditions.

Quality improvement

Intervarietal and interspecific crosses have been made to eliminate/reduce concentrations of erucic acid and glucosinolates and to balance fatty acid composition in the oil. The lines with low erucic acid (<5%) and low glucosinolates (<30 μmoles/g) have been developed by crossing exotic *juncea* cultivars (Zem-1 and Zem-2) possessing low erucic acid with Indian cultivars (RLM-619, RL-1359 and RH-30) possessing high erucic acid. Likewise, exotic *campestris* cultivar Tobin possessing low erucic acid and glucosinolate was crossed with Toria cultivars TL-15, TLC-1, and T-9. The exotic sources used for zero erucic acid content possess Fig. 1 some undesirable agronomic characters, viz., late maturity, poor yield and susceptibility to downy mildew. The generated breeding material is in different segeregating generations. Interspecific hybridization between *juncea* (cv. RLM-198) x *napus* (cv. Oro) gave a large number of progenies with chromosome number 2n=36; some of them possessing lower erucic acid concentration and good agronomic characters in the F_1s.

Development of hybrids

The cytoplasmic male sterility (cms) in Indian mustard generated interest in the development of hybrids. Initial efforts through intraspecific hybridization between indigenous and exotic lines of *juncea* and cms sources did not result in pollen fertility. Parental ancestor monogenomic species (*campestris* AA and *nigra* BB) constituting digenomic *juncea* species (AABB) reverted to partial fertility restoration. Complete fertility restoration was, however, brought when genes from both AA ($RF_1 RF_1 RF_2 RF_2$) and ($RF_3 RF_3 RF_4 RF_4$) genomes were brought together in a single genotype. The restorer genes were considerably influenced by modifier genes and the adverse climatic conditions resulting into male sterility in hybrids. Efforts are underway to diversify cms system for developing superior hybrids as well as for purification of restorer gene(s). The hybrids are under trials at different locations in the country.

Salt tolerance

The problem of salinity and alkalinity is gradually increasing with the increase in ground water irrigation facilities. A large number of strains/germplasm is screened at the Central Soil Salinity Research Institute, Karnal. Strains DIRA-337, DIRA-343, RH-851, NDR-8604 and 8501 have been found to be high yielding and promising in salt affected soils.

Shattering resistance

The dehiscence of siliquae at the time of harvesting causes considerable loss in seed yield. Efforts made in the past resulted in identification of Indian mustard genotypes like RH-30, Pusa bold, RH-8130, RLC-1021 and RH-8131 as resistant/moderately resistant to shattering. Some resistant lines have also been developed through introgression.

Varieties for late sown conditions

In order to bring a sizeable area under intensive cropping system, efforts have been made to identify suitable varieties for late sown and limited irrigation conditions in different agro-climatic situations. A number of promising varieties of Indian mustard, viz., RH-7859, Vardan and RLM-619 have been found performing well under such conditons.

Varieties for non-traditional areas

Results are quite encouraging in improving productivity of rapseed/mustard in non-traditional

areas. The varieties like Seeta, RLM-619, Pusa bold, Pusa Barari, RLM-514 and Kranti are performing exceedingly well in southern and western states.

Varieties for high altitudes

Summer cultivation of rapeseed-mustard at high altitudes on hills and valleys has become a reality. In Himachal Pradesh, a promising and better adaptable toria variety, TL-15 has been developed recently in 1988.

PRODUCTION TECHNOLOGY

Soil test calibrations for targetted yields:

Soil test calibration for recommending fertilizer doses for targetted yields of oilseed Brassica has been developed. The results have been verified subsequently on farmers' fields. Results of trials conducted on research farms and farmers' fields to verify soil test calibrations are presented in Table 2. Data on the average values indicate that the yields are within ± 15% deviation from the targetted yields.

Production under high intensity crop rotation:

Production potential of high intensity crop rotations involving Indian mustard as a winter crop and relative economics have been worked out under the All India Coordinated Research Project, Table 3. It is evident that crop rotations involving rice-mustard-green gram, rice-mustard-black gram, guar-mustard-bajra resulted in higher productivity and net returns. This is particularly significant in areas where assured irrigation and intensive fertilizer application are the major constraints in improving productivity of oilseed Brassica.

Potential of improved technology

The relevance and effectiveness of improved technology package have direct bearing on its application and adoption on extensive scale. Large-scale field demonstrations on farmers' fields have been conducted to demonstrate the potentials of the improved technology.

The results of field demonstrations established, beyond doubt, the potentials of improved agro-production technology in realizing increased yields of oilseed Brassica, Table 4.

FUTURE RESEARCH STRATEGIES

1. Development of varieties for higher seed and oil yields with in-built resistance/tolerance to:
   a. Mustard aphid,
   b. Alternaria blight and white rust,
   c. frost,
   d. drought, and
   e. salinity/alkalinity.

2. Emphasis on increasing oil content *per se*.

3. Increase emphasis on hybrid Brassica for increased productivity.

4. Application of biotechnological approaches for breaking yield barriers for increased and stabilized production.
5. Improvement of oil and seed meal qualities.

6. Development of varieties for late sown conditions.

7. Introduction and improvement of rapeseed-mustard in non-traditional areas.

8. Acceleration of researches on integrated nutrient management.

9. Intensification and diversification of rapeseed-mustard based cropping system.

10. Intensification of researches to develop integrated pest management practices.

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Table 2. Soil-test based fertilizer recommendation for mustard: Results of verification trials (2).

Location, Soil (type)	No. of trials	Average soil test values (kg/ha) N	P	K	Average Fertilizer (kg/ha) *	N	P_2O_5	K_2O	Mean yield target	Mean yield obt. (kg/ha)	Response Ratio (kg/ha)	% Deviation
Dhali, Alluvial (YS. 66-197-3)	1	200	20.0	100	a	68	7	23	1500	1680	-	12.0
Jabalpur, Black (Varuna)	7	287	10.7	381	a	122	72	51	2000	1912	6.1	-4.4
					b	60	30	20		1157	6.8	
					c	0	0	0		413		
New Delhi, Alluvial (Varuna)	1	223	23.7	235	a	88	94	96	2500	2167	4.2	-13.3
					c	0	0	0		1010		
New Delhi, Alluvial (Pusa Bold)	1	228	33.0	342	a	78	106	55	2500	2110	4.1	-15.6
					c	0	0	0		1140		
New Delhi, Alluvial (Pusa Kalyani)	2	202	28.7	335	a	100	41	4	2250	1927	5.0	-14.4
					c	0	0	0		1208		
Ludhiana, Grey-Brown (RLM-619)	6	90	14.7	143	a	38	60	18	1500	1362	-	9.2

* a = Soil test based fertilizer recommendation for specific yield target.
b = General recommended dose.
c = Control.

Table 3. Production potential of high intensity crop rotations and their relative economics from selected locations involving mustard as a rabi crop.*

Centre, STATE	Crop rotation Kharif	Rabi	Summer	Total fertilizer (kg/ha) N	P	K	Average yield (kg/ha) during Kharif	Rabi	Summer	Tot. yield (kg/ha) including pulses & oilseeds	Gross return (Rs/ha)	Net return (Gross minus fert. cost)
Pantnagar, UP	Rice	Wheat	Greengram	260	175	37	3826	4204	711	8741	11875	9669
"	Rice	Mustard	Greengram	227	162	37	3676	899	954	5529	10971	8985
Mashodha, UP	Rice	Wheat	Greengram	250	152	120	4435	4796	865	10096	13805	11604
"	Rice	Mustard	Blackgram	210	140	100	4420	1578	569	6567	13273	11358
Varanasi, UP	Rice	Wheat	Greengram	310	160	140	4748	3494	1000	9242	13147	10592
"	Rice	Mustard	Greengram	310	160	120	4757	1427	813	6997	13862	11347
S.K. Nagar, GUJARAT	Bajra	Wheat	Greengram	225	142	40	1930	2009	565	4504	6439	4425
"	Guar	Mustard	Bajra	140	120	0	714	1476	1654	3844	9866	8576
Navsari, GUJARAT	Rice	Wheat	Greengram	365	195	120	5680	2242	1438	9360	14012	11508
"	Rice	Mustard	Guar	185	150	60	3783	559	837	5179	9097	7320
Banswara, RAJASTAN	Rice	Wheat	Greengram	215	115	0	3935	3196	159	7290	8949	7156
"	Rice	Mustard	Greengram	175	150	0	4000	490	134	4624	7024	5414

* Three years pooled data, AICORPO, ICAR.

Table 4. On-farm testing of rapeseed-mustard improved technology in different states.

State	Crop	No. of demonstrations	Average Yield (kg/ha)	State Average seed yield (kg/ha)
Punjab	Mustard	27	1492	922
"	Toria	25	1516	
"	Gobhi Sarson	10	1901	
Haryana	Mustard	826	1882	783
Rajasthan	Mustard	6	2562	812
Gujarat	Mustard	5	2561	1293
"	Yellow Sarson	1	2430	
Uttar Pradesh	Mustard	244	1393	612
"	Toria	6	1299	
Bihar	Mustard	2	1950	684
West Bengal	Mustard	2	1751	600
"	Yellow Sarson	5	1768	
Assam	Toria	171	1022	481
All India		1330	1809.76	

* Includes entire rapeseed-mustard crop commodity.

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RAPESEED-MUSTARD IN NEPAL

B. Mishra

Agriculture is the predominant occupation of Nepalese people which engages more than 90% of the population. Agriculture contributes more than 60% of the total export earnings, along with the forest which provides nearly 82% of the total raw material for industries. The total area of the country is 147,181 km^2 out of which only 18% (26533 km^2) is used for agriculture.

Oilseed crops are most important cash crops of the country which have vital role in the Nepalese economy. Oilseeds, particularly rapeseed-mustard provide the main cooking oil. However, other oilcrops like groundnut, sesame, niger and sunflower are also being grown in one or another part of the country.

In the past, Nepal was exporter of rapeseed-mustard. But in recent years it becomes importer of edible oil. The national annual imports and exports are presented in Table 1.

Table 1 Total national imports of oil and exports of oilseeds for the period 1984/85-1987/88.

Type of Oil	Year			
	1984/85	1985/86	1986/87	1987/88
Imports ('000 Rs)				
Butter oil	10340	14228	16238	22142
Palm oil	39979	42069	52263	44504
Soybean oil	11266	12145	72255	232468
Vegetable oil	1445	5083	96	4481
Coconut oil	8256	7685	3182	11320
Total	72401	82929	177222	345412
Exports ('000 Rs)				
Niger seed	4251	15160	27977	93681
Linseed	-	-	103079	141315
Total	4251	15160	131056	234996

Source:- Nepal overseas trade statistics from 1984/85 to 1987/88, Trade Promotion Centre, Kathmandu.

AREA/PRODUCTION/ PRODUCTIVITY OF RAPESEED-MUSTARD

Rapeseed-mustard are the most important edible oil crops of Nepal. They cover nearly 90% of the total oil crops growing area. The national area, production and productivity of rapeseed-mustard are given in Table 2.

It is clear from the Table that there were little increases in area and production except for the last five years. Productivity was always fluctuating up and down around the 600 kg/ha. Out of 75 districts, only 20 produce more than 80% of the national rapeseed-mustard production, Fig. 1.

Table 2. National area, production and yield of rapeseed-mustard, 1970/71 to 1988/89.

Year	Area ('000 ha)	Production ('000 M.T.)	Yield (kg/ha)
1970/71	105.51	54.91	520
1971/72	111.36	57.44	516
1972/73	112.26	59.58	531
1973/74	113.92	63.62	558
1974/75	112.00	65.85	588
Average	110.01	60.28	542.6
1975/76	112.99	68.49	606
1976/77	107.75	61.38	570
1977/78	133.06	78.45	590
1978/79	143.98	92.50	642
1979/80	118.13	61.87	524
Average	123.18	72.53	586.4
1980/81	122.28	77.14	631
1981/82	113.90	79.12	695
1982/83	110.34	69.59	631
1983/84	110.70	73.35	663
1984/85	127.82	84.03	657
Average	117.00	76.64	655.4
1985/86	138.46	78.66	568
1986/87	142.89	82.50	577
1987/88	151.49	94.37	623
1988/89	154.86	99.19	640
Average	146.92	88.68	602.0

Source:- Hand Book of Agricultural statistics of Nepal, H.M.C., Ministry of Agriculture, Department of Food and Agricultural Marketing services, Agricultural Statistics Division, Lalitpur, Nepal, December 1988.

CONSTRAINTS IN RAPESEED-MUSTARD PRODUCTION

- Being grown mostly in rainfed areas.
- Lack of sufficient high-yielding

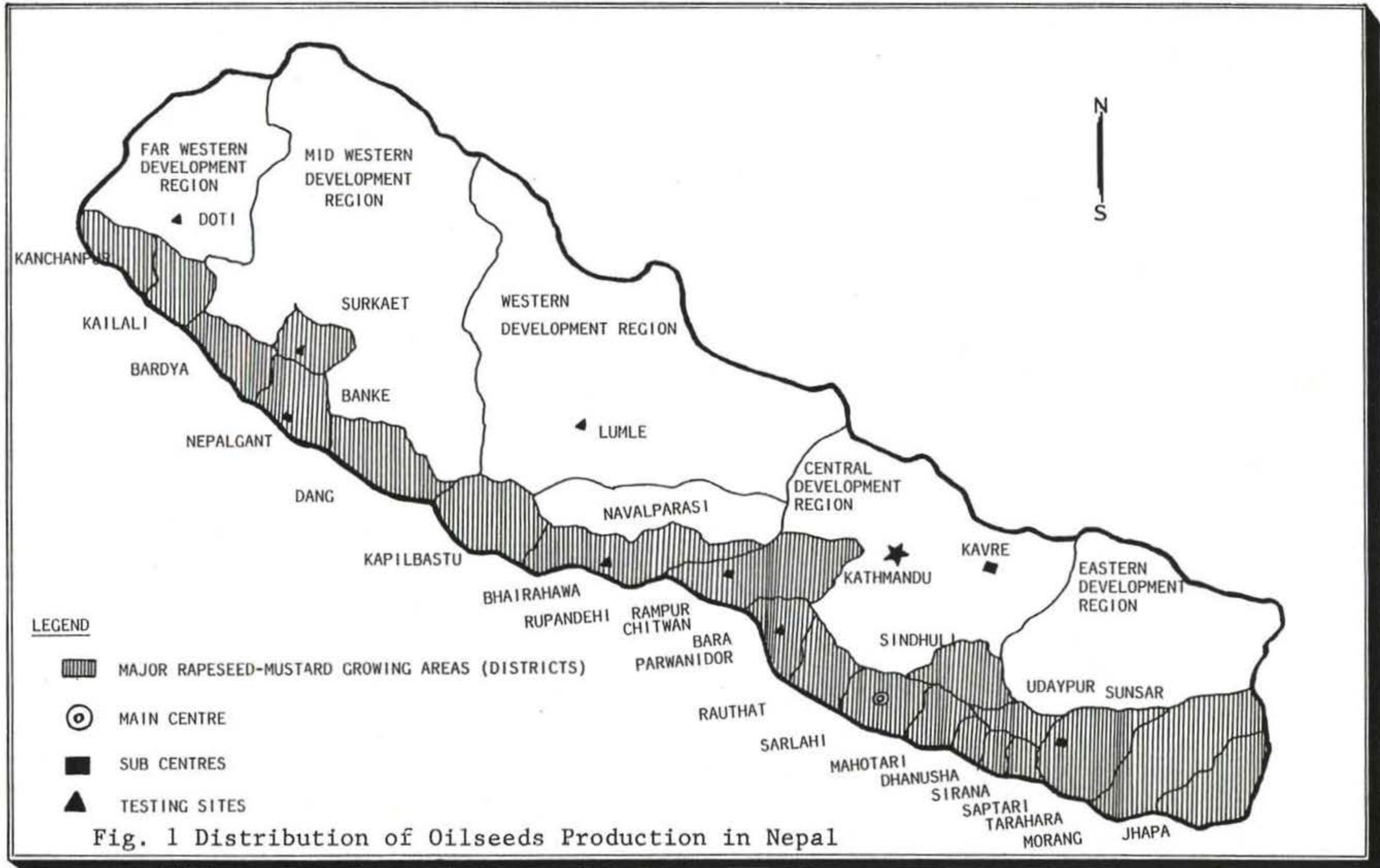

Fig. 1 Distribution of Oilseeds Production in Nepal

varieties suitable for different agro-climatic conditions.
- Inadequate quality seeds.
- Grown mostly under low input conditions.
- Poor management practices.
- Farmers do not give required plant protection measures.
- Lack of organized marketing system.

VARIETAL IMPROVEMENT WORK

In *Brassica campestris* var. toria the highest seed yield (1319 kg/ha) was produced by PT-303. Flowering of all the tested materials was found to be 26-32 days in Terai and 30-39 days in Hills. All the materials matured in 3 months in Terai and 104-121 days in Hills.

In *Brassica campestris* var. Yellow Sarson, S-4 produced the highest seed yield (635 kg/ha) under Terai condition whereas at Pakhribas (Hill) PYS-6 produced 1078 kg/ha.

Brassica juncea var. Krishna produced 1078 kg/ha. It matured in 115 days under Terai condition.

Materials from SAARC member countries (India, Pakistan, Bangladesh, Sri Lanka) were tested and good results have been recorded; Pusa bold gave the highest seed yield.

RELEASED VARIETIES

The rapeseed variety T-9 of Indian origin was released before many years and was popular among growers. Recently (1989) PT-303 from Pantnagar, India has also been released for commercial cultivation.

Two mustard varieties 'Pusa bold' and 'Krishna' also from India, have been recommended for cultivation in 1989.

MAJOR DISEASES OF RAPESEED-MUSTARD

Alternaria blight (*Alternaria brassicae*) is the most important disease of rapeseed-mustard. However, some other prevalent diseases are: white rust -(*Albugo candida*); Downy mildew (*Perenospora parasitica*); and Sclerotinia rot (*Sclerotinia sclerotiorum*).

IMPORTANT INSECT PESTS OF RAPESEED-MUSTARD

The most serious and common insect is mustard aphid (*Lipaphis erysime*). Also, mustard sawfly (*Althelia legens proxima*) causes severe losses to this crop. Some other insects are hairy caterpillar (*Diacrisia* spp.), flea beetle (*Phylotreta cruciferae*) and painted bug (*Bagrada cruciferearum*).

OROBANCHE

Broomrape (*Orobanche* spp.) has become a serious problem in many parts of the country. So far, No solution has been suggested for this serious root parasite.

DISCUSSION/COMMENT

Downey: In relation to a slide showing adjacent fields for seed production, Toria next to *B. juncea*, one must be careful to watch isolation distances between seed production fields of the same species in order to maintain purity.

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CONSTRAINTS AND OPPORTUNITIES OF BRASSICA OILSEED PRODUCTION IN BANGLADESH

M.A. Islam, M.A. Khaleque, K.P. Biswas and M.R.I.Mondal

Oilseeds in Bangladesh constitute the commercial crops of Bangladesh. Oils and fats, apart from forming an essential part of human diet, serve as important raw material for the manufacture of soaps, paints, varnishes, hair oils, lubricants, textile auxiliaries, pharmaceutical, etc. Oil cakes and meals are used as animal feeds and as manures.

The bulk of vegetable oil production in Bangladesh is derived from six oilseeds (mustard, groundnut, sesame, safflower, niger and sunflower) forming the edible group, and (linseed and castor) forming the non-edible group. In addition, cotton seeds, seeds from some oil bearing tree species, etc. are also being exploited as vegetable oils. About 70% of the total oilseed area is covered by rapeseed and mustard.

Development of oilseeds and vegetable oils holds an important place in Bangladesh's economy. There has been big gap between supply and demand of vegetable oils which has been met through annual imports, Table 1. The per capita consumption of vegetable oils in the country is very low as compared to the world average. In this context, the increase in production of oilseeds has been given priority. Every possible effort is being made to accelerate the production of oilseeds in the country.

Table 1. Oilseed production and requirement of oil in Bangladesh.

Year	Population (million)	Requirement of Oil (000 mt)	Oilseed production (000 mt)	Production of Oil (000 mt)	Deficiency of Oil (000 mt)	Requirement of Oil/Cap/ annum(g)	Requirement of Oil/ha day(g)
1974-75	78.0	142	168	56	86	1821	4.99
1979-80	87.7	160	246	82	78	1824	5.00
1984-85	98.0	178	270	90	88	1816	4.98
1989-90	111.0	202	300	112	90	1820	4.99

Source: Third Five Year Plan, Planning Commission, People's Republic of Bangladesh.

CONSTRAINTS IN INCREASING PRODUCTION OF OILSEEDS

Fluctuations in production of oilseeds from year to year could be attributed to the following constraints and bottlenecks:

1. Socio-economic constraints: Most of our farmers are small and marginal, they can not afford to invest on various inputs. Oilseeds mostly grown under rainfed conditions, become high-risk crops. As a result, oilseed crops are grown mostly under poor crop management resulting in low yield. The non-realization of the benefit of improved crop production technology is, therefore, more due to poor economic condition of the farmers.

2. Environmental constraints: About 97% of the area under oilseed crops is rainfed comprising mostly marginal and sub-marginal lands with soils of poor fertility (9). Substantial production losses were found to occur due to pests and diseases.

3. Technological constraints:
-Lack of high yielding varieties, particularly those which could give high stable yield under rainfed and short winter conditions and resist pest and diseases especially against *Alternaria* and *Orobanche*.
-Shortage of improved farm implements,

-Low cost technology for the control of pests and diseases,
-Inappropriate post-harvest technology to prevent post harvest losses,
-Less extraction and deterioration in quality of oil, and
-Non-availability of shattering tolerant variety in *Brassica napus*.

4. Organizational and infrastructural constraints:
-Inadequate arrangements for production and distribution of quality seed,
-Supply of other inputs in time like credit and irrigation.
Lack of transfer of technology from researcher to farmers,
Insufficient storage and grading, and
-Poor marketing of oilseeds coupled with wide fluctuation in price.

, BRASSICA OILSEEDS DEVELOPMENT PROGRAM

Status prior to 1975

Area, production and yield of different oilseeds in 1972-73 are shown in Table 2. Acute shortage of edible oilseeds prevailed since long time in Bangladesh. In 1972-73, the production of oil was only 51,359 mt which met only 29% of the requirement for 75 million people leaving a deficiency of 71%. This shortage was met by import every year at the cost of foreign exchange. At that time, high yielding varieties of oilcrops of improved production technologies of oilseeds were lacking. Scientists were engaged in research for main crops like rice, jute, sugarcane, etc. and least attention was given to oilseeds. So, the oilseeds improvement program remained unattained. The importance and necessity of oilseed improvement program was realized during the late 1970's and Oil Seeds Research and Development Project was initiated in 1971. But the project could not function due to liberation war. So, a new project for the development of oilseeds especially *Brassica* oilseeds was taken up in 1975 with few scientists only.

Status after 1975

Intensive research on oilseeds was initiated in 1975 when the Accelerated Winter Oilseed Improvement and Development Program (AWOIDP) started functioning with the assistance of Swedish International Development Agency (SIDA) during the First Five Year Plan (FFYP) of Bangladesh. Scientists were trained in Sweden, India, UK and Philippines under the program. Equipments were procured and an oilseed laboratory was established at Bangladesh Agricultural Research Institute (BARI). At the same time, other Institutes of Bangladesh like Bangladesh Agricultural University (BAU), Bangladesh Institute of Nuclear Agriculture (BINA), Rajshahi University (RU), Mennonite Central Committee (MCC), etc. also started research on oilseeds.

Status after 1980

During the Second Five Year Plan (SFYP) period, BARI developed two high yielding varieties of mustard namely: SS-75 (Sonali Sarisa) and TS-72 (Kalyania) and BAU developed one variety, M-12 (Sampad), Table 3. As a result, the oil production started increasing and rose to 57.7 thousand tons in 1981-82. *Brassica* oilseeds, being the major oilseeds in the country contributed a total production of 41,700 tons (13).

Extension Officers, like subject matter officers and subject matter specialists of the Department of Agricultural Extension (DAE), were trained during

Table 2. Area, production and yield of different oilseeds, 1972-73.

Crop	Area (acre)	Production (mt)	Yield (kg/ha)	Oilseeds available available for oil purpose (mt)	Oil equivalent
Rape and mustard	473,155	106,160	591.88	104,858	34,953
Winter and Summer Til (Sesame)	117,925	27,460	590.63	27,135	9,045
Winter and Summer Groundnut	56,570	31,000	1,256.25	29,443	7,361
Total	647,650	164,620		161,436	51,359

Source: Accelerated Winter Oilseed Improvement and Development Program (AWOIDP), Govt. of Bangladesh.

Table 3. Different rape and mustard cultivars (including HYV) in Bangladesh, December, 1982.

Crop/variety	Days to maturity	Yield (kg/ha)	Oil content (%)	Year of recommendation
Tori-7	70-80	950-1100	39-40	Traditional
TS-72 (Kalyania)	75-85	1500-1600	41-42	1979
SS-75 (Sonali Sarisha)	90-100	1800-2000	43-44	1979
M-12 (Sampad)	90-100	1600-1800	42-44	1982
Rai-5	90-100	950-1100	39-40	Traditional

Source: Oilseed Research Centre, BARI

1981-84. The newly developed technologies of oilseeds and demonstration of high yielding varieties were demonstrated at the farmers' fields by the newly trained officers. The coverage of High Yielding Variety (HYV) of mustard increased from 12 to 17% during 1982-85, Table 4. The productivity also increased from 591 kg/ha in 1972-73, Table 2, to 733 kg/ha in 1984-85, Table 4. Bangladesh Agricultural Development Corporation (BADC) initiated mustard seed production and the Department of Agriculture Extension (DAE) conducted intensive demonstration of HYV in farmers' field. Special publications like "Krishikatha" (Oilseed Issue, November 1983) and leaflets on the developed varieties and other technologies were brought out. As a result, oil production continued to increase upto 91,712 mt in 1985, Table 4.

Table 4. Production statistics of rape and mustard in 1982-1985 cropping season.

Year	Variety	Area (ha)	% of Total	Production (mt)	Yield (kg/ha)	Total oil (mt)	Source
1982-83	HYV	34,491	11.3	30,515	885	-	BARC
	Local	269,982	88.7	182,433	676	-	
	Total	304,473	100.0	212,948	699	70,649	
1983-84	HYV	43,927	14.0	-	-	-	
	Local	269,838	86.0	-	-	-	DAE
	Total	313,765	100.0	225,000	717	75,000	
1984-85	HYV	62,371	17.0	62,075	995	-	
	Local	313,031	83.0	213,063	681	-	DAE
	Total	375,402	100.0	275,138	733	91,712	

Source: Third Five Year Plan, Planning Commission, People's Republic of Bangladesh.
* NA= Not available

Status after 1985

A program was taken up in the Third Five Year Plan (TFYP) to produce 112 thousand tons of oil for 111 million people in 1990 against the target of 202,000 mt at the rate of 5 g/h/d, Table 1.

In 1988, the number of HYV of rape and mustard has been increased to seven, Table 5. Other technologies have also been developed to grow oilseed crop without affecting rice and wheat. Practice of inter-cropping, relay-cropping and mixed cropping with pulses, sugarcane, maize, etc. were followed with improved technologies. As a result, production continued to rise steadily. Accordingly, the edible oil production went up to around 106,000 mt in 1987-88, Table 6, against the set target of 202,000 mt, leaving a deficiency of over 90,000 mt of oil. *Brassica* only contributed 73,000 mt of oil.

Recently, attempts have been made to introduce B. *napus* in Bangladesh by resynthesis and introgression. As a result, three advance lines of *B. napus* are now under regional yield trial. Two

Table 5. Different rape and mustard cultivars (including HYV) in Bangladesh December, 1988.

Crop/Variety	Days to maturity	Yield (kg/ha)	Oil content(%)	Organization	Year of recommendation
Tori	70-80	950-1100	39-40	ARI	Traditional
TS-72 (Kalyania)	75-85	1500-1600	41-42	BARI	1979
SS-75 (Sonali Sarisha)	90-100	1800-2000	43-44	BARI	1979
M-12 (Sampad)	90-100	1600-1800	42-44	BAU	1982
Rai-5	90-100	950-1100	39-40	ARI	Traditional
M-248 (Sambal)	100-110	1050-1150	38-39	BAU	1984
RS-81 (Daulat)	95-105	1100-1250	38-39	BARI	1988

Source: Oilseed Research Centre, BARI.

Table 6. Area and production of oilseed crops for the year 1987-88.

Crop	Area (ha)	Production (mt)	Yield (kg/ha)	Oil equivalent @233% (Ton)
Rape and mustard	317,863	222,008	699	73,263
Sesame	81,985	49,027	598	16,179
Groundnut	38,472	47,753	1,241	15,758
Other Oilseeds	310	190	612	63
Total/Average	438,630	318,978	788	105,263 (52%)

Source: Bangladesh Bureau of Statistics.

problems, shattering and sterility, have been detected in *B. napus*. Attempts are being made to overcome these problems.

OPPORTUNITIES FOR FUTURE BRASSICA OILSEED DEVELOPMENT

During the coming years, more quantity of edible oil will be required for consumption. Therefore, a larger program has been set up for oilseeds under the Crop Diversification Program to accelerate the pace of oilseed production.

The major strategy comprises increasing the production and productivity of oilseeds. The experience so far gained from the results of demonstration in farmers' fields in several districts namely: Jessore, Jhenaidah, Magura, Kushtia, Pabna, Brahmanbaria, etc. indicated that concentrated-area-crop approach with irrigation and fertilizer can pay rich dividends. This approach should be incorporated in the National Oilseed Development Program, (10).

__The broad strategy will be__:

a) To increase the productivity through varietal replacement by available HYV, use of quality seed, large scale use of fertilizer and organic manures, effective plant protection measures and other improved agronomic practices.

b) To expand irrigated area under oilseed crops especially for rape and mustard.

c) To increase area under rape and mustard by double/multiple cropping program.

d) To distribute large number of minikits.

e) To strengthen seed production and distribution program.

f) To increase [illegible] and popularization of fertilizers in potential areas.

g) To use more power expeller for efficient extraction of oil instead of "Local Ghani", and

h) To organize village demonstration, on-farm training, provision of incentive price through government procurement policy etc.

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DISCUSSION

<u>Kumar</u>: Did the variety of mustard shown which had a compact pod structure had Been looked at for aphid reaction?

<u>Islam</u>: No.

<u>Mundel</u>: What species are used for intercropping with sugarcane and how this was done?

<u>Islam</u>: Both *B. campestris* and *B. juncea* are intercropped with 2 rows of sugarcane adjacent to 2 rows of Brassica. The Brassica rows are harvested when the Sugarcane is only 20 cm tall.

<u>Singh</u>: The mustard protects sugar cane from frost injury.

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PROGRESS IN RAPESEED-MUSTARD RESEARCH IN BHUTAN

Tayan Raj Gurung

The domestic production of edible oil in Bhutan is low compared to the requirement and hence, the deficit is met by imports, Table 1. The national requirement of oil is increasing every year with the growth of population and enhanced per capita demand with the increase in family income. To curtail foreign exchange expenditure on edible oil, the Royal Government has accorded high priority to the development of oilcrops in the country.

Table 1. Present food situation in Bhutan.

Item	Production (MT)	Import (MT)
Rice	43,200	25,000
Wheat	13,000	7,564
Oil	1,041	2,740

The importance and high value of oilseed was realized by the Royal Government since the introduction of "Development Plans" almost 28 years ago. With the assistance from different projects/agencies, the Department of Agriculture made numerous efforts to popularize different oilcrops in the country. For instance, in Bumthang, sunflower was introduced as an oilcrop in 1974, pearl lupine in 1977 and *Brassica napus*, mustard and soybean in early 1970's. Most of these crops could not be sustained due to various field problems, such as: pest and disease incidence, soil fertility or high nitrogen fertilizer requirement, needs for special equipment for oil extraction and small and marginal farmers. However, rapeseed-mustard continued to be the predominant oilcrop in Bhutan.

Production

Rapeseed (*B. campestris* var. toria) is the principal species grown in Bhutan. It is cultivated from 200 to 3000m elevation in approximately 5000 ha every year, both as irrigated and rainfed crop after rice and maize (1984 survey). It has higher potential in respect of area expansion and productivity. The existing area and potential area is depicted in Figure 1.

It is estimated that the national average seed yield is 700 kg/ha which is low mainly due to the poor technologies in use. Therefore, a major emphasis is being placed on finding appropriate production technologies for higher yields. The potential of improved variety has been demonstrated in Wangdi-Punakha Valley, Table 2.

Table 2. Yield comparison of improved (T9) and Local (Toria) in Wangdi-Punakha Valley.

	Yield (mt/ha)			
Variety	1986-87	1987-88	1988-89	Mean
T9	1.22	1.20	1.10	1.17
Local	0.76	0.67	0.54	0.66

PROSPECTIVE

Rapeseed production can be increased provided the essential inputs and technologies, including all the operations from seed bed preparation to final disposal of the produce are made available to the farmers.

Center for Agricultural Research and Development (CARD) has established several definite objectives to achieve self-sufficiency in edible oil. The following approaches clearly indicate the future prospective of rapeseed-mustard as an important oilseed crop:

1. Varietal improvement

Varietal improvement is geared towards the identification of high yielding/ early maturing varieties with reasonable resistance to major diseases and insect pests. Collections from national and international sources were evaluated in Preliminary Observation Nursery. Several lines with desirable characters have been identified and are being included in the present testing program, Table 3.

With few years of intensive testing, two high yielding varieties, T9 and M27 of Indian origin, were released in 1988

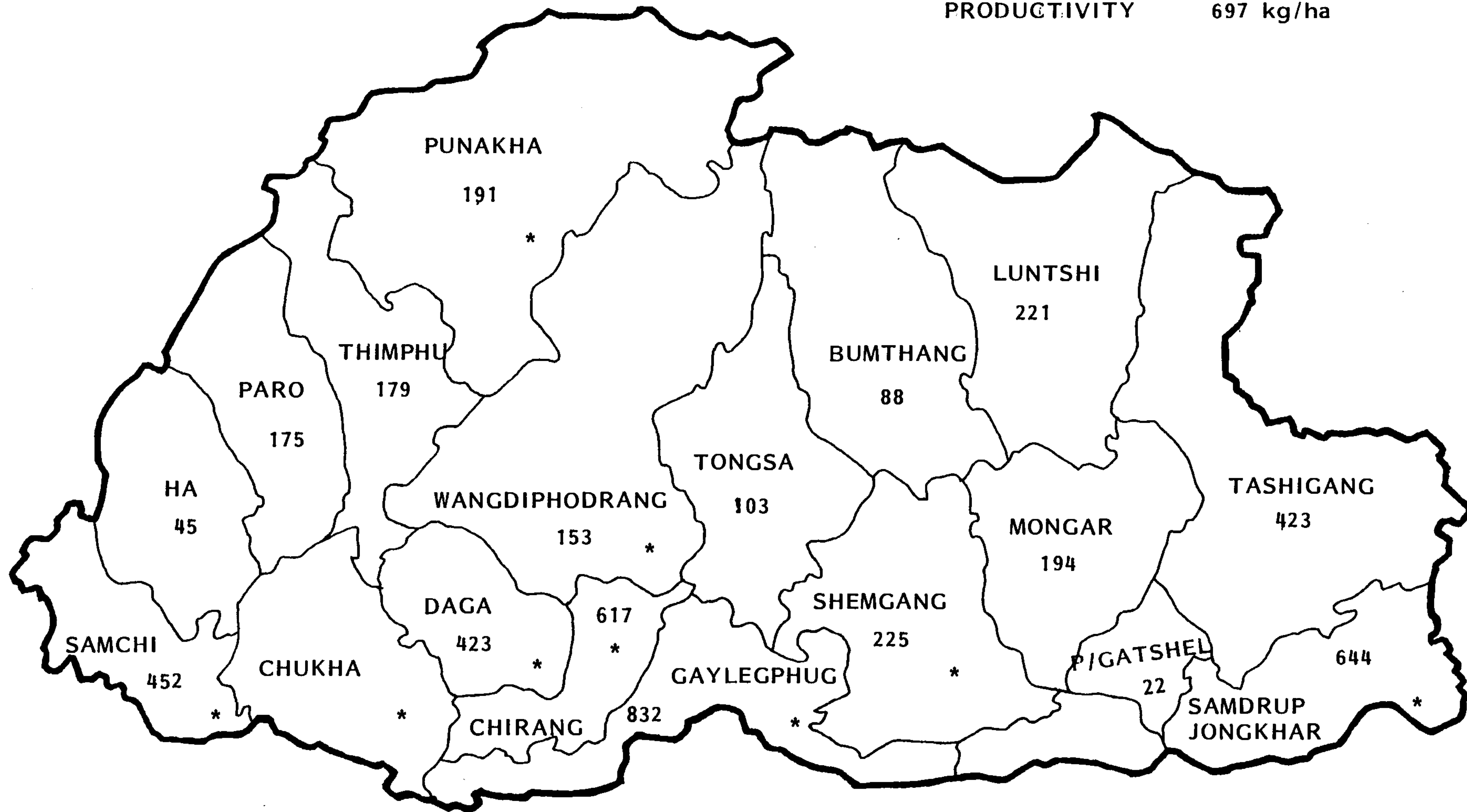

Fig. 1. BHUTAN Rapeseed Acreage (Ha) (1984 Survey, DOA). * : Potential Area

Table 3. Selection from Mustard Preliminary Observation Nursery (PON) CARD, Wangdiphodrang 1988.

No.	Variety	Origin/ Source	Days to flowering	Branches Primary	Branches Secondary	Siliquae /plant	1000 seed wt.(g)	Remarks
1	BT 9	India	51	5	15	14	82.0	Ap.M
2.	Pusa Kalyani	"	65	4	13	179	2.0	Ap.M-S
3.	Varuna	"	77	4	12	147	4.0	Ap.M;WR.L
4.	Krishna	"	72	3	9	179	3.0	Ap.M; WR.L
5.	PT 303	"	50	3	74	3	.0	Ap.L
6.	Kranti	"	75	4	13	74	4.0	Ap.M; WR.L
7.	Toriya A	Pakistan	52	3	15	182	2.0	Ap.M-S
8.	DR 7	"	49	3	6	45	2.0	Ap.M-S
9.	Local Sano	Nepal	47	2	8	75	3.0	Ap.M
10.	Local Thulo	"	46	2	7	91	2.0	Ap.M-S
11.	Shershog Local	Punakha/ Bhutan	44	2	9	70	2.0	Ap.M
12.	Khuru Local	"	44	2	6	52	2.0	Ap.M
13.	Baap Local	Thimphu/ Bhutan	45	3	7	57	3.0	Ap.M
14.	Memba Serbu	Mongar/ Bhutan	46	2	10	65	3.0	Ap.M-S
15.	Local	Gaylegphug/ Bhutan	43	2	6	48	3.0	Ap.M-S
16.	Pachu Local	Chukha/ Bhutan	43	3	8	62	2.0	Ap.M-S
17.	Tala Local	"	50	2	6	73	2.0	Ap.M-S
18.	Dolechin Local	"	46	2	7	63	2.0	Ap.M-S
19.	Bhalujhora Local	"	49	1	8	69	2.0	Ap.M-S
20.	Bitheykha Local	Paro/Bhutan	45	1	6	43	2.0	Ap.M-S
21.	Limshi Local	"	45	2	10	58	2.0	Ap.M-S
22.	Rashigang Local	"	45	3	12	105	2.0	Ap.M-S
23.	Tashipjee Local	"	43	3	6	56	2.0	Ap.M-S
24.	Tshigen Local	"	42	3	7	52	2.0	Ap.M-S
25.	Bargkha Local	"	42	3	6	59	2.0	Ap.M-S
26.	Dogakha Local	"	42	3	12	98	2.0	Ap.M-S
27.	Lemokha Local	"	44	3	8	69	2.0	Ap.M-S
28.	Tshongkha Local	"	44	3	8	76	2.0	Ap.M-S
29.	Type 9 (Std. Check)	India	47	4	9	90	2.0	Ap.M-S
30.	Bajo Local (Check)	Bhutan	46	3	8	66	3.0	Ap.M-S

Note: Seeding date : 24.10.88, Fertilization : 80:40:20 NPK Kg/ha.

for cultivation. The improved varieties have higher yield potential than the local varieties, Table 4.

Further, the selected rapeseed-mustard varieties received through SAARC-MLTR were evaluated in Advanced Evaluation Trial, Table 5. The selected entries from 1989-90 trials will be advanced to on-farm testing.

Table 4. Yield comparison (t/ha) of improved and local rapeseed/mustard varieties, 1988.

Sites	VARIETIES T9	M27	Varuna	Local
Wangdi-Punakha	1.1(124)*	1.0(125)	1.3(140)	0.9(125)
Chirang	0.7(137)	0.5(137)	1.0(178)	0.6(137)
Tashigang	0.3(113)	0.5(113)	0.5(154)	0.4(107)
Gaylegphug	0.2(124)	0.4(125)	0.2(140)	0.4(125)

*Numbers in parenthesis are maturity days.

Table 5. Selection from Initial Evaluation Trial, CARD, 1988.

Variety	Days to Flowering	Days to Maturity	Branches Primary	Branches Secondary	Siliquae per branch	1000-Seed Wt.(g)	Mean Yield (t/ha)
Krishna	98	149	3	6	10	4.0	1.04
Pusa Bold	93	155	3	4	6	4.0	1.03
Vaibhav	94	145	3	6	8	4.0	1.02
PT303	94	136	3	7	8	3.3	1.01
Toria 7	93	136	3	6	7	3.0	1.00
TS27	91	133	3	6	9	3.3	1.00
CHLS	94	136	3	5	8	3.6	0.98
PT30	93	135	3	6	7	3.0	0.95
M27	96	130	4	6	9	2.7	1.00
T9	93	135	3	6	9	3.0	1.00
Local (Check)	92	120	3	6	7	2.7	0.96

Note: Sown: 16.11.88 Fertilizer: 80:40:20: NPK kg/ha

2. Agronomy

Current cropping systems demand growing rapeseed in sequence with major crops like rice and maize. This brings the question of possibility of early sowing and timely harvesting of rapeseed.

Research has confirmed that October-mid November sowing in rice-based cropping system and September-October in maize-based cropping system are necessary for higher seed and oil yields, Tables 6 and 7. Later sowings were wiped out by polyphagous caterpillar and aphids.

As per the available information, the recommended rate of fertilizer is 75:50:0 NPK kg/ha. In a separate trial, the highest yield was reported from application of 80:40:30 NPK kg/ha, Table 8. As this crop follows the major cereal crops, studies on integrated use of plant nutrients was felt necessary.

Such studies have been initiated from the last crop season with an objective of formulating a sustainable nutrient management system for a cropping pattern rather than a crop-wise recommendation.

Table 6. Yield response (t/ha) and growth duration (days) of popular rapeseed varieties at different sowing dates.

Sowing Dates (S)	VARIETIES (V)@ T9	M27	Varuna	Local	Mean yield
Sept.30	1.01(126)*	1.16(134)	1.48(138)	1.01(134)	1.17
Oct. 30	1.21(120)	1.12(120)	1.34(138)	1.18(120)	1.21
Nov. 15	1.13(124)	0.99(120)	1.32(140)	1.12(120)	1.14
Nov. 30	0.84(125)	0.73(125)	0.92(145)	0.63(125)	0.78
Mean	1.05	1.00	1.27	0.99	

Statistical analysis	Significance	C.V.	LSD (0.05)
Sowing date (S)	*	6.09	0.104
varieties (V)	**	8.28	0.093
S x V	**		

@ Numbers in parenthesis are maturity in days.

Table 7. Yield (t/ha) and growth duration of varieties at different sowing dates at observational plot, CARD-Bhur.

Sowing Dates	VARIETIES* T9	M27	Local	MEAN YIELD
Sept 30	0.16 (93)	0.35 (97)	0.34 (95)	0.28
Oct 15	0.24 (90)	0.40 (98)	0.38 (94)	0.34
Mean	0.20	0.38	0.36	0.31

* Number in parenthesis are maturity days.

3. Technology Transfer

The major step towards promotion of rapeseed-mustard cultivation was "Mustard Intensification Program, 1988-89" with the objective of acquainting the farmers with the improved package and thereby achieving self-sufficiency in edible oil. Under this program, 88.2 ha (764 households) was planted to T9 by Punakha-Wangdi Valley Development Project. The improved package gave a substantial yield increase over the local package, Table 9.

Table 8. Rice-based fertilizer trial on mustard, Chirang, 1988.

Rates (kg/ha) N P K	FYM	Yield (t/ha)
0: 0: 0	-	0.432
0:40:30	-	0.448
40:40:30	-	0.454
80:40:30	-	0.754
40: 0:30	-	0.341
40:80:30	-	0.631
40:40: 0	-	0.545
40:40:60	-	0.567
0: 0: 0	5000	0.370
0: 0: 0	10000	0.406
	CV% :	16.9
	LSD(0.05) :	0.143

Table 9. Mustard intensification program 1988-89, PWVDP.

Gewog/Block	Average Yield (t/ha) T9	Local
Chubbu	1.923	0.453
Talow	0.708	0.370
Ghuma	1.883	0.910
Tewang	0.558	0.743
Kabji	0.320	0.270
Shengana	1.215	0.490
Bjimi	1.315	0.570
Mean	1.132	0.544

FUTURE DIRECTION

Present experience indicates that it is necessary to solve the field problems which are varied and often complex with proper strategy. Accordingly, the fields which need future attention are as follow:

1. Varietal improvement;
2. Diversification of oilcrops;
3. management/integrated nutrient and pest
4. Production of quality seed;
5. Field demonstration;
6. Training of field-level extension staff and farmers;
7. Possibility of area expansion;
8. Post harvest/processing; and
9. Marketing.

DISCUSSION

Kumar: What percentage of the total crop area in Bhutan is for oilseed production?

Gurung: 9% of the total area of the country is agricultural land. Of these 50,000 hectares are sown to rapeseed; rapeseed is 90% of the total oil crops. The altitude of the cropping areas in Bhutan ranges from 200 - 3000 m all *Brassica campestris.*

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OVERVIEW OF RAPESEED PRODUCTION AND RESEARCH IN CHINA

Yan Zhang

In the recent 10 years, rapeseed production showed a tremendous expansion in China with nearly 2-fold increase of cultivation area and over double increase of the total output, Table 1. As a main oil crop in China, rapeseed spreads almost all over the country and occupies 40-45% of the total area of oil crops -except soybean- and 37-39% of the total production of edible vegetable oil. Rapeseed meal is traditionally used as fertilizer and feed. In addition, it is also very important in crop rotation because of a lot of nitrogen left-over from the rapeseed leaves, petals and roots.

Table 1. Area, production and yield of rapeseed in China.

Year	Area ('0000 ha)	Production ('0000 t)	Yield (Kg/ha)
1980	2841.4	238.40	838.3
1981	380.1	406.50	1,069.5
1982	412.2	565.60	1,372.1
1983	366.9	428.70	1,168.4
1984	341.3	420.50	1,232.1
1985	449.4	560.70	1,247.7
1986	491.6	588.10	1,196.3
1987	526.7	660.51	1,260.0
1988	493.6	504.40	1,020.0
1989	533.0	540.00	1,013.0

With the improvement of the living standard of the Chinese, we have a great potential market for edible vegetable oil. This year, the purchasing price of rapeseed will be increased. It will give farmers a big encouragement to expand their rapeseed production effectively.

ACHIEVEMENTS AND PROGRESS IN RESEARCH

1. Double-low quality breeding

There are four institutions involved in the Sino-Canadian Rapeseed Breeding Project: Wuhan and Shanghai located in winter rapeseed area, and Qinghai and Xinjiang situated in spring rapeseed area. The general objective of the project is to breed new varieties of the three *Brassica* species for:

a) high yield,
b) maturity adaptable to each cropping system,
c) high oil content with erucic acid below 1%, and
d) good quality meal with glucosinolates content below 30% µmol/g for animal feed.

Materials from Canada and Europe with low erucic acid and glucosinolates have been used for crossing several cultivars and new lines. Three low erucic varieties of winter *B. napus*, Zhong Guo Di Jie No.1, 2, and 3 have been developed by Institute of Oil Crops Research, Wuhan (IOCR, CAAS) which can grow in different ecological regions of the upper and middle Yangtze River Valley with maturity suitable for 2-3 cropping sequences. They have been registered by several south-western provinces and listed as released varieties, Table 2.

Shanghai Academy of Agriculture Sciences (SAAS) has successfully selected, demonstrated and produced commercially sufficient quantities of the double-low *B. napus* line 84-24016. The oil with low erucic acid has already been tested in some consumer products (salad oil, margarine and cookies). The meal containing low glucosinolates is being used for broiler feeding trial in comparison with high glucosinolates rapeseed and soybean meals. But the seed yield of 84-24016 is 10% less than that of the normal varieties, so it is still being improved. Single-low strain, 8701 was put into the National Variety Performance Test in the lower reach of the Yangtze river in 1989. Its seed yield has reached the production levels of local commercial varieties. The oil content is 41.0% with erucic acid less than 1%. Symptoms of virosis and sclerotinosis developed slightly.

Table 2. Fatty acid and oil contents of low-erucic acid cultivars.

Cultivar	Composition of Fatty acid(%) C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	Oil Content(%)
Zhongyou 1	5.49	2.93	58.61	21.24	10.63	1.11	0.0	39.1
Zhongyou 2	3.56	1.19	63.24	22.13	9.09	0.76	0.0	42.6
Zhongyou 3	3.25	1.51	64.94	20.56	8.66	1.08	0.0	41.0
24016	4.24	-	52.20	27.25	13.52	1.92	0.87	39.2
8701	4.87	-	59.71	22.01	11.97	1.18	0.25	41.0
Ganyou 5CK	3.57	-	15.15	13.95	9.49	10.87	47.06	39.4
Xinyou 4	5.27	0.51	44.82	35.57	13.44	0.39	0.0	37.8
Xinyou 5	4.95	-	44.62	36.87	13.44	0.12	0.0	37.6

Two varieties of *B. campestris*, Qingyou Nos. 11 and 13, low in erucic acid have been bred and released in Qinghai Academy of Agricultural Sciences (QAAFS). They are adapted to high altitude areas with short growing seasons. It has proved that they are suitable to other areas of north-western China.

Xinyou 4 and 5 are the two *B. juncea*, with low erucic acid content which have been developed by Xinjiang Academy of Agricultural Sciences (XAAS).

2. Substantiation for Genic Male Sterility (GMS) Hypothesis

A. Inheritance of heterozygous GMS ,Table 3:

Genetic model of heterozygous GMS

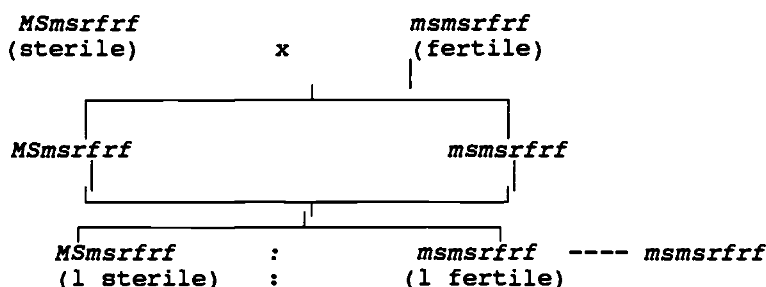

Table 3. Segregation of heterozygous 9AB sib-mating and 9B selfing.

Year	Sib-mating (9A x 9B) fertile	sterile	x^2 (1:1)	9B Selfing fertile	sterile
1984	491	503	0.1217	282	0
1985	317	290	1.1140	479	0
1986	218	207	0.2353	305	0
1987	162	159	0.0125	204	0
1988	239	237	0.0020	300	0
1989	169	148	1.2680	203	0

B. Inheritance of homozygous GMS,Table 4:

Genetic model of homozygous GMS

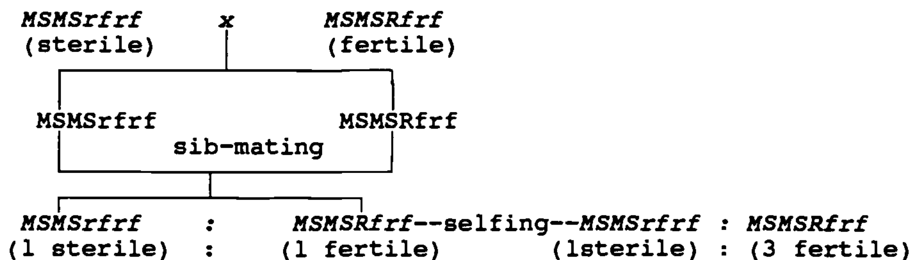

Table 4. Segregation of homozygous 6AB sib-mating and 6B selfing.

Year	Sib-mating (6A x 6B) fertile	Sib-mating (6A x 6B) sterile	X^2 (1:1)	6B Selfing fertile	6B Selfing sterile	X^2 (3:1
1984	16	17	0.0000	16	5	0.0159
1985	40	36	0.1184	94	22	1.9425
1986	316	329	0.2232	658	223	0.0306
1987	600	628	0.5936	952	318	0.0000
1988	842	813	0.4737	1154	376	0.1255
1989	325	340	0.2940	503	158	0.3676

C. Inheritance of temporary maintainer, Table 5:

Genetic model of temporary maintainer

Table 5. Sterility of homozygous 6A x temporary maintainer 9B.

Year	Total plants	Sterile plants	Sterility %
1985	157	154	98.1
1986	614	614	100.0
1987	11,371	11,207	98.6
1988	1,233	1,227	99.5
1989	778	773	99.4

D. Restoration test:

Three different genotypes of sterile lines were crossed with the two restorer lines and the F_1 was fully restored, Table 6. This indicates that the materials have *RfRf* gene responsible to develop the restorer line.

Table 6. Restoration Test.

Type of sterility	Cross	Total plants	Fertile Plants	Restoration (%)
heterozygous	9A/4190	150	150	100.0
	9A/Ribao	121	121	100.0
homozygous	6A/4190	132	132	100.0
	6A/Ribao	38	38	100.0
fully sterile	fully st./4190	70	70	100.0
	fully st./Ribao	157	157	100.0

3. Rapeseed biotechnology

Anther-and pollen-cultures were carried out in Shanghai (SAAS) from 1987 to 1989. The anthers of F_1-F_4 are from single or double-low winter *B. napus*. The result shows:

a) Anthers from different genotypes have different induction frequencies of pollen embryoids,
b) 10% sucrose in the medium is the best one, and
c) B5 medium added with 0.1 mg/l 2,4-D and 0.1 mg/l NAA performed good.

Fifty-eight haploids were planted in the field for selection and crossing in 1990.

4. Studies on diseases

Three main diseases which severely affect rapeseed in China are virus, *Sclerotinia* and white rust. SAAS has carried out experiments, for several years on the isolation and identification of virus as well as the selection of resistant materials in rapeseed. Four kinds of viruses (TuMV, TMV, CMV and Ribgrass mosaic virus) have been found. TuMV in rapeseed could be differentiated to 3 strains and CMV to two strains. There is no variety or material which has been found immune or resistant to viruses. However, some materials tolerant to viruses were selected and utilized in breeding. IOCR uses a number of *B. napus* and their crossing progenies to study tolerance to *Sclerotinia*. The selection for resistance and tolerance has been made under artificial inoculation in the greenhouse and natural infection in the fields. So far, they have already obtained a number of single-and double-low *B. napus* with high tolerance, and also provided good methods of identification and selection for high yield, good quality and resistance breeding.

DISCUSSION

Mundel: What stage the *B. napus* varieties were at when winter temperatures dropped to - 15°C.

Zhang/Fang: *B. napus* which survives these temperatures is at the 7-10 leaf seedling stages. (Some discussion followed which ensured that - 15°C was the extreme of cold and that in general water temperatures would be warmer than this).

Kumar: Which varieties were low?

Zhang/Fang: Xin You 4, Xin You 5 (*B. juncea*) and Qin You 11, Qin You 13 (*B. campestris*).

Qazi: Where did the restorers for the genetic male sterility system came from?

Zhang/fang: The restorer lines came from Japan, from Shiga's material via the Chinese germplasm collection at the Oilseed Crops Research Institute in Wuhan.

Islam: What was the reason for the low production levels in 1988?

Zhang/Fang: This was due to bad weather conditions. There is no shattering tolerance in *B. napus* and the *napus* varieties are harvested by hand when 80% of the plants are mature.

Downey: In order to judge correctly, one must not look at pods but at the seeds, and determine the proportion of the seeds which are changing colour. The exact stage at which the crop should be cut will vary from area to area but it should be based on seed colour change.

Singh: How much area was presently sown to F_1 hybrids in China?

Zhang/Fang: About 16% of the total area is presently sown to F_1 hybrids. this is 0.8 million hectares. The main hybrid is Qing You 2.

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ANALYSIS OF EIGHT HIGH-QUALITY RAPESEED (Brassica napus L.) STRAINS FOR HIGH AND STABLE SEED YIELD

Chaocai Sun, Guanghua Fang, and Hua Zhao

The development of high quality rapeseed varieties is a key project in China. In the last several years, a number of high quality rapeseed varieties/strains have been bred and put into the National Rapeseed Variety Performance and Production Tests. This paper highlights the analyses of eight high quality strains in the national trial of the lower reach of the Yangtze river for high and stable seed yield.

MATERIALS AND METHODS

The tested materials were four single-low strains (8701, 4039, 1026, 75-01-1) and four double-low strains (126, 135, D89, Jian 7) selected by Shanghai, Jiangsu, Anhui and Zhejiang Academies of Agricultural Sciences, and Zhenjiang Agricultural Research Institute. The commercial variety Ningyou-7 was used as a check. The experiment was conducted in 1988-1989 at seven locations situated in the lower reach of the Yangtze river. A randomized block design was used with three replications. The net plot size was 20 m^2. The data were subjected to analysis of variances. The stability of seed yield of the varieties was analyzed by interaction variances among the varieties and locations and their coefficient of variations (3).

RESULTS AND DISCUSSION

A. Analysis of the high quality rapeseed strains for high seed yield:

The results were valid because the seven locations lie in the different ecological conditions and production levels in the lower reach of the Yangtze River. Highly significant differences among the varieties, locations and their interactions were noted, Table 1. The seed yields of Jian 7, 1026 and 8701 showed no significant differences as compared with CK Ningyou 7. The seed yields of 4039, 75-01-1, D89, 126 and 135 were highly significantly different from that of CK, Table 2.

Table 1. Mean square of seed yield for high-quality rapeseed strains.

Source of variation	df	MS
Blocks	14	0.17
Locations	6	21.18**
Varieties	8	7.19**
Interactions	48	0.48**
Residual	112	0.07

** Significant at 0.01 level.

Table 2. Mean seed yield and stability analysis of eight high-quality rapeseed strains.

Strains	Seed yield* kg/ha			Stability parameters I.V.**	C.V.(%)
Ningyou 7(CK)	1859	a	A	0.273	14.05
Jian-7	1824	ab	A	0.090	8.22
1026	1824	ab	A	0.218	12.79
8701	1799	ab	A	0.039	5.49
4039	1759	b	B	0.021	4.12
75-01-1	1649	c	B	0.090	9.10
D89	1464	d	C	0.131	12.34
126	1154	e	D	0.137	16.02
135	1124	e	D	0.105	14.40

* indicates that the means followed by the same letter are not significantly different at 0.05 (small letters) or 0.01(Caps) probability levels by Duncan's new multiple range test.
** I.V.= Interaction variance among varieties and locations.

B. Analysis of the high-quality rapeseed strains:

It is difficult to combine the traits of high and stable seed yield into a rapeseed variety. The seed yield of CK was the highest, but not stable, because there were large interactive variance (IV) and coefficient of variation (CV), Table 2. The smaller IV and CV of 8701 (0.039 and 5.49) suggest that this variety is more

stable. The seed yield of 8701 was higher than that of the CK at four locations but slightly lower at three locations. The IV and CV of Jian-7 were 0.09 and 8.22, respectively, with medium stability. The seed yield of 1026 was high, but not very stable. Its IV and CV were larger than those of 8701 and Jian-7.

Although IV and CV of 4039 were the smallest of all the strains and hence the most stable, its seed yield was not high. The other strains did not have characteristics of both high and stable seed yield.

The low-erucic strain 8701 and low erucic and glucosinolate strain Jian-7 had the characteristics of both high and stable seed yield. They could be widely cultivated in the lower reach of Yangtze river.

ACKNOWLEDGEMENT

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CANOLA RESEARCH IN AUSTRALIA

Gregory Buzza

In the last three years, the annual Australian production of canola has been about 50,000 - 70,000 ha with a yield of 1.0 - 1.4 t/ha. The best crops yield 2.5 - 3.0 t/ha. In the past, some *B. campestris* was grown but at the moment only *B. napus* is grown. All varieties are canola-quality.

The canola crop is grown in the wheat belt. It fits in well in the rotation system between pastures and cereals. The crop is sown in late Autumn or early Winter at about the same time as wheat - often a little earlier. It flowers in the early Spring (September) and is harvested in late Spring (Oct., Nov. and Dec.). It can usually be harvested before wheat. The varieties are "Spring" varieties but are grown in the Winter and Spring i.e. similar to India and Pakistan.

There are four breeding programs. Two commercial companies are breeding hybrids, pacific seeds and Ag seeds and two public programs are breeding conventional open-pollinated varieties. These are conducted by the N.S.W. Department of Agriculture at Wagga Wagga by Neil Wratten and by the Victorian Department of Agriculture at Horsham by Phil Salisbury. In addition, a breeding program for *B. juncea* is conducted by Division of Plant Industry, CSIRO, Canberra by Rex Oram. Other major centres of research on canola breeding, physiology and agronomy are the Department of Agronomy, University of Western Australia (Noel Thurling) and the Department of Agronomy, University of Tusmania (Neville Mendham).

The development of methods of quality analysis has been researched at the Department of Chemistry, University of Wollongong (Roger Truscott) and by Rod Mailer in association with the N.S.W. Department breeding program at Wagga Wagga.

The co-ordination of research and the testing of new cultivars is organized through a biennial meeting of ARAB (Australian Rapeseed Agronomists and Breeders). This meeting is also a research conference where papers are delivered on recent research. Proceedings are published, the most recent meeting was in Toowwooba in September 1989. The next will be in Horsham (Southern) in the Spring of 1991.

In future, it is expected that the area of canola in Australia will soon steadily increase and that the yield per hectare will also increase.

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GOALS FOR 1989-1991 AND PROGRESS OF THE BARANI AGRICULTURAL RESEARCH AND DEVELOPMENT PROJECT (BARD) IN PAKISTAN, PERTAINING TO BRASSICA.

Hans Henning Muendel

BARD is a Pakistan-Canada cooperative project, headquartered at the National Agricultural Research Center (NARC) in Islamabad, for research and development in mainly rainfed (Barani) areas. The Pakistan Agricultural Research Council is the Pakistani partner; and the Canadian International Development Agency (CIDA) is the Canadian partner, with Agriculture Canada being the executing agency on behalf of CIDA.

The project was initiated in 1982 and is due for completion, with phase-in to the NARC, by June 1991. One of the major crop development thrusts is on the rapeseed/mustard complex. The main emphasis here is on canola-quality, variety introduction, testing and breeding.

For the rapeseed/mustard component of BARD, our work is organized towards the following seven goals, with current developments briefly indicated:

1. **To identify early-generation and advanced lines of *Brassica napus*, low in erucic acid (<5%) and glucosinolates (<40 μmoles/g), earlier maturing and/or higher yielding than Westar and at least equal to Ganyou-5 (Pak-China 88); and to generate backcrosses of high oil and high yielding lines with canola-quality parents.**

Eighty-one advanced-generation lines are planted in preliminary yield trails, being harvested from late April to early May. A summer nursery in the hills (at Kaghan) was used to select the parental lines.

2. **To coordinate the National Uniform Rapeseed/Mustard Yield Trial (NURYT); Include BARD-developed 'canola'-quality *B. napus* lines selected from preliminary yield tests, as well as 'canola'-quality lines from NARC and other centers, and introductions from abroad.**

This 16-entry test is grown at 11 locations throughout Pakistan and Azad Jammu/Kashmir, from high rainfall locations and irrigated fields to commonly very dry locations. Eight of the entries are from the BARD-breeding program; three are introductions from Australia (Shiralee, Maluka and Tatyoon), one introduction from Canada (Westar, the current 'canola' standard used by BARD); two introductions from Sweden (one very early, short and upright, the other late but also very upright); Ganyou-5 (a non-canola introduction from China, released last year for its high yield in the North West Frontier Province); and a local non-canola check, DGL.

3. **To convene the Annual Technical Program and Work Planning Meeting of Rapeseed/Mustard Scientists in Pakistan. Organize a Rapeseed/ Mustard Travelling Seminar.**

Twenty-nine scientists from 14 establishments throughout the country participated in the August 29-30, 1989 Annual Technical Program and Work Planning Meeting.

From 25 to 30 scientists participated in the week long Fourth BARD Rapeseed/ Mustard Travelling Seminar, held from March 4 to 10, 1990. Research institutes, farmers' fields, seed fields and processing facilities of the Punjab Seed Corporation, oil crushers, solvent extraction and refining factory, soap manufacturing plant - all in Punjab Province - were some of the stops on our way.

4. **To increase production of seed 'grown-from' Westar in Punjab and NWFP through contract research, maximization and outreach programs and in cooperation with the Agronomy Component, at O.R. sites.**

For the 1989/90 rabi season, approximately 130 ha were seeded for this 'canola' outreach, with seed supplied or sold from BARD.

5. To determine diverse optimum agronomic practices related to weed control, fertilizer use and planting dates, for effective rapeseed/mustard production:

5.a. Determine effective and economical dose of herbicides for the control of broadleaf and grassy weeds in rapeseed/mustard.

Application of 0.5 l/ha of Fusilade gave good results for annual and perennial grassy weed control. Use of Treflan increased *Brassica* yields.

5.b. Determine most economical dose of fertilizers (N & P) for optimizing yield in rapeseed and mustard under Barani and irrigated conditions.

Two years' data indicate that 100 N and 30 P kg/ha broadcast, followed by 75 N and 30 P kg/ha side-banded at seeding, gave best results.

5.c. Determine the effect of planting date on yield of *Brassica* species.

Mid-October plantings, in the Islamabad area, gave the highest yields.

6. To promote the production of canola oil from production of the crop on farmers fields. Encourage private industry involvement in the procurement and processing of canola seed and the sale of canola oil.

BARD purchased farmers' canola seed and arranged to have it crushed and refined commercially, both the 1987/88 and 1988/89 crops. In each case, around 13 tons of refined oil were available for sale through commercial stores and through the BARD project. Sales were made at farmers' field-days and various other fairs, open houses, etc. In the first year, questionnaires were given with each 2.5 L tin sold. As a refund, Rs 5 was assured for filled out questionnaires, a good return of over 1000 was achieved. Responses on use of the oil were overwhelmingly positive.

7. To train Senior Scientific Officer in operation of a plant breeding program and two Senior Scientific Officers in rapeseed agronomy and extension.

One person was sent for post-graduate studies to Saskatoon. One Ph.D holder was hired for becoming involved in the breeding aspects. One BARD Scientific Officer, along with another one from NARC, was sent to India for 4 weeks to attend *Brassica* breeding/agronomy training session sponsored by IDRC Brassica Sub-Network in December 1989. BARD is financially supporting Ph.D. studies of the same Scientific Officer, with the Oilseed Advisor being accepted by the Qaid-i-Azam University in Islamabad as Co-Advisor for the Ph.D program.

8. To evaluate and provide supervision as Scientific Authority on research contracts with other agencies, funded by BARD.

The Oilseed Advisor currently acts as Scientific Authority on seven contracts involving various aspects of rapeseed/ mustard research in three provinces.

The BARD Agricultural Engineering group, in collaboration with ourselves and the Farm Machinery Institute of NARC is involved in:

- modification of tillage and tractor-mounted seeding equipment for rapeseed/mustard: for improved stand establishment by improving furrow openers, packing wheels and delivery systems; and
- procuring and evaluating rapeseed reaping and threshing equipment (e.g. refer to experimental/small plot reaper from Swift Machine and Welding Ltd.)

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PART IV

BRASSICA SUB-NETWORK:

DISCUSSIONS AND RECOMMENDATIONS

COLLABORATIVE PROGRAMMES

MINUTES OF MEETING FOR SCIENTIFIC EXCHANGE AND INSTITUTIONAL COLLABORATIVE PROGRAMMES AMONG MEMBER COUNTRIES OF BRASSICA SUB-NETWORK SHANGHAI, CHINA, APRIL 28, 1990

1. This meeting was organized by the Network Advisor as recommended by the Brassica Subnetwork committee.

2. The scientists from member countries of Brassica Sub-Network and officials from Science and Technology Exchange Division, Ministry of Agriculture, P.R. China, participated in the deliberations.

A. Germplasm Exchange:

3. It was mutually agreed hat member countries may exchange germplasm among themselves. The contact persons for requests as well despatch of material are detailed below:

Country	Contact Person	Copy to
P.R. China	Mr. Guanghua Fang Associate Professor Crop Breeding and Cultivation Institute, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China	Mr. Ma Jinhui Deputy Chief Scientist Exchanges Division Ministry of Agriculture 11, Nongzhanguan Nanli Beijing, China
India	The Director National Bureau of Plant Genetic Resources, Pusa, New Delhi 110012	Dr. P.R. Kumar Project Coordinator (Rapeseed:Mustard) Haryana Agricultural University, Hissar 125004
Pakistan	The Director BARD, NARC GP. O.Box 1785 Islamabad	Prof. Mohammad Hanif Quazi Member(Crop Sciences) Pakistan Agricultural Research Council, P.O.Box 1031, Islamabad
Bhutan	Mr. Tayan R. Gurung National Oilseeds Coordinator, Center for Agricultural Research and Development, Department of Agriculture, Royal Government of Bhutan, Wangduphodrang	
Nepal	Mr. G.P. Koirala, Oilseeds Coordinator, National Oilseeds Development Program, NAWALPUR, SARLAHI	
Bangladesh	Mr. M.A. Khaleque, Director (Oilseeds), Bangladesh Agriculture Research Institute, JOYDEBPUR, GAZIPUR	

Ethiopia — Mr. Getinet Alemaw,
Institute of Agricultural Research
P.O.Box 2003, ADDIS ABABA

Egypt — Dr. Badr El-Ahmar, Oilcrops Section,
Agricultural Research Center,
GIZA 12619

4. All materials must be accompanied with phytosanitary certificate.

5. Detailed description of materials with special features, background and pedigree should also accompany the material, with a copy to Dr. Omran for inclusion in the Oil Crops Newsletter.

6. Copies of requests/despatch should be sent to the following for expediting action and coordination.

   Dr. Abbas O.Omran, Technical Advisor,
   Oilcrops Network, P.O.Box 23464,
   Addis Ababa, ETHIOPIA

   Dr. Basudeo Singh, Chairman,
   Brassica Sub-Network, IDRC Intern Scientist,
   G.B. Pant University for Agriculture and Technology, Pant Nagar - 263145, INDIA

B. Scientist Exchange

7. It is considered essential for reciprocal exchange of scientists among member countries under Brassica Sub-Network.

8. The Chinese side renewed the invitation of 1987 for Indian Scientists to visit research institutes in China.

9. The Indian side appreciated the offer and requested for a fresh formal offer addressed to:

   Dr. R.S. Paroda, Deputy Director General (Crops),
   Indian Council of Agricultural Research,
   Krishi Bhawan, New Delhi - 125 004 and

   Dr. N. Mateo, Associate Director,
   IDRC, Tanglin, P.O.Box 101,
   SINGAPORE 9124.

10. It was agreed that local hospitality in terms of local travel, lodging and boarding shall be met by host institution/country. The international travel and incidental expenses are to be met by sponsoring countries or from some other source. Dr. Abbas O.Omran agreed to explore the possibilities of supporting travel expenses for such exchanges.

C. Sino-Indian Collaboration On Brassica Hybrid Research.

11. The Chinese and Indian Scientists agreed to meet in a separate session to identify mutual areas of interest and to develop a draft project proposal for consideration of both sides. The minutes of this separate session follows.

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INDIA/CHINA COLLABORATION

MINUTES OF MEETING OF COUNTERPART SCIENTISTS FOR INTERNATIONAL COLLABORATIVE RESEARCH BETWEEN CHINA AND INDIA

SHANGHAI, CHINA, APRIL 28, 1990.

1. This meeting was organized as recommended by the special meeting for scientific exchange among Brassica Sub-Network member countries.
2. The following scientists participated in the discussions:

Prof. Zhang Yan
Director, Agriculture Commission of Shanghai Municipality, Shanghai, P.R. China; and
Co-Chairwoman, Brassica Sub-Network (IDRC)

Dr. Basudeo Singh
PG.B. Pant University for Agriculture Technology, Pantnagar - 263145, India; and
Chairman, Brassica Sub-Network (IDRC)

Dr. R.K. Downey
Agriculture Canada Research Station,
Saskatoon, Sask. Canada

Dr. M.I. McGregor
Agriculture Canada Research Station,
Saskatoon, Sask. Canada

Dr. Abbas O.Omran
Technical adviser, IDRC Oilcrops Network,
Institute of Agriculture Research,
P.O.Box 23464, Addis Ababa, Ethiopia

Mr. A.D.R Ker
Senior Program Officer, IDRC
Regional Office for Eastern and Southern Africa,
P.O. Box 62084 Nairobi, Kenya

Prof. Tingdong Fu
Head, Chinese Cooperating Research Group on Rapeseed Heterosis,
Huazhong Agriculture University,
Wuhan 430070, P.R. China

Mr. Yunchang Li
Lecturer, Institute of Oil Crops,
CAAS, Wuhan, 430062, P.R. China.

Ms. Xiuzhen Qian
Associate Professor, Institute of Oilcrops,
CAAS,Wuhan 430062, P.R. China

Ms. Ping Shi
Research Scholar, University of Western Australia,
Nedlands 6009 Australia

Mr. Hunqing Si
Associate Professor,
Yunnan Academy of Agriculture Sciences,
Kunnming 650205, P.R. China.

Prof. Dianrong Li
Science and Technology Educational Centre of State Farms and Land Reclamation, Shanxi Province, Shanxi, P.R. China.

Dr. P.R. Kumar
Project Coordinator (Rapeseed-mustard),
Haryana Agriculture University, and
Principal Scientist, Indian Council of Agricultural Research, Krishi Bhawan, New Delhi - 110001, India

Dr. Badr A. Elahmar
Director Oilcrops Research Section,
Field Crops Institute, GIZA 12619, Egypt

Mr. Getinet Alemaw
Institute of Agriculture Research Centre,
Holetta Research Center,
P.O.Box 2003 Addis Ababa, Ethiopia

3. Prof. Zhang Yan referred to the discussions in the special Meeting held in the forenoon and desired future collaboration on the following major issues:

   a. Heterosis breeding and utilization
   b. Germplasm exchange for evaluation

4. Dr. Singh mentioned that research work on identification of different male sterility systems is continuing at six research institutions in India. He welcomed the proposed collaboration under IDRC Brassica Sub-Network in the light of Dr. N. Mateo's discussions with ICAR officials in New Delhi in Jan. 1990 for such a collaboration.

5. Dr. Kumar specifically outlined research activities on heterosis breeding in *B. juncea, B. campestris* and *B. napus*. He mentioned that the Indian side would welcome collaboration on hybrid program in *B. juncea, B. campestris* and *B. napus* (spring type). There is a need for male sterile lines (CMS and GMS) of the 3 species.

6. Prof. Fu introduced that China has a heterosis research group involving nine institutions. They are interested in Indian *B. juncea* heterosis breeding and germplasm resistant to insects and diseases. Chinese scientists welcomed the cooperation with Indian scientists in exchanging germplasm, information, male sterile line and F_1 hybrids for yield testing.

7. Prof. Zhang Yan considered that the collaboration among Canada, India and China will benefit each counterpart and promote rapeseed research effectively. She agreed to make proposal and submit to IDRC and respective governments for approval and support.

Collaboration

8. After critical evaluation and assessment of status of researches, both sides agreed to the following issues for future collaboration.

   a. Researches on the development of hybrids of *B. campestris, B. juncea* and *b. napus* (spring type) simultaneously at the collaborative institutions.
   b. Supply of F_1 hybrid seed of the three types by china to India and *B. juncea* type by India to China for multi-location testing.
   c. Exchange of CMS and GMS maintainer and restorer lines of the three types. For effective exchange, Dr. Downey volunteered to supply CMS lines of *B. campestris* and *B. juncea* to both Chinese and Indian Institutions.
   d. At the outset, exchange of 30 released varieties (100 gm each), in cultivation by July 15, 1990 along with their salient features. In addition, supply 40 g each of the 30 varieties from India and China to Dr. Downey for quality evaluation in Canada.
   e. Exchange of germplasm of the three types for simultaneous evaluation on the basis of the descriptors, by corresponding institutions in both countries.

Project Formulation

9. Both sides agreed to develop 3-year draft project proposals highlighting status, objectives, institutions, plan of work (year-wise), targets, exchange of material and visits (man-month), financial component, respective country institutions support, etc.

10. Both sides agreed to exchange informally the proposals by July, 1990 for preparation of a joint project document for formal clearance of the respective governments. During the project formulative stage, the contact persons will be prof. Zhang Yan and Dr. P.R. Kumar.

Collaborative Institutions

11. The two sides identified institutions (tentatively) for collaboration, on the basis of heterosis breeding program in progress:

Areas	Chinese Institutions	Indian Institutions
B. campestris	Sichuan Academy of Agri. Sci. Qinghai Academy of Agri. Sci. Ms. Dongyu Ling/Dr. Z.K.Tian	GBPANT, Pantnagar NDUAT, Faizabad Dr.Sachan/Dr.Chauhan
B. juncea	Yunnan Academy of Agr.Sc. Xinjiang Academy of Agr. Sc. Mr. Hunqing Si/D. Z.M. Wang	IARI, New Delhi PC Unit (R M), Hisar Dr.Chopra/Dr.Kumar
B. napus	Hauzhang Agri. Univ. Institute of Oilcrops CAAS Prof. T.D. Fu/Prof. C.Q.Lu	PAU, Ludhiana HAU Hissar Dr.Labana/Dr.H.Singh
Germplasm exchange	Institute of Oilcrops CAAS Ms. X.Z.Qian (Assoc. Prof.)	NBPGR, New Delhi Dr. R.S. Rana

Financial Support

12. The two sides would carry out researches from their own resources.

13. For reciprocal exchange of scientists, it was agreed that local hospitality in terms of local travel, lodging and boarding would be met by host institution/country. For international travel and per diem expenses, it was proposed to seek the possibility of financial support from IDRC under Net Work resources.

14. In his concluding remarks, Dr. Downey expressed happiness for the initiatives of both sides for scientific collaboration. These are immense opportunities for exploitation on both sides. Dr. Downey hoped for fruitful discussion and closer interaction at the governmental levels. Finally, Dr. Downey offered his full support for a successful Sino - Indian collaboration.

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GENERAL DISCUSSIONS AND RECOMMENDATIONS

A. POTENTIAL COOPERATION BETWEEN NETWORK COUNTRIES AND WITH CANADA,

1. White Rust:

   - Participants to send their diseased samples to Dr. Rimmer to identify races of white rust (caused by *Albugo candida*) at winnipeg, also willing to identify blackleg races.

   - Interested participants: to notify Rimmer, who will send invitation permit.
   - Collected stag heads should be stored in lab.

   Note: In this way Rimmer's collection can be augmented and other participants helped, not to be considered as general race identifying service lab.

2. - Dr. Qazi soliciting comments on his work in Pakistan, i.e. interspecific crosses for aphid resistance & pod size from *B. napus* to *B. juncea*, and for yellow seed coat color and pod angle from *B. juncea* to *B. napus*.

   - Dr. Downey commented that it depends on importance of characteristic to introgress (in particular situation); proof will be in the final result (need to relate to expected yield component compensation: thus need to test in field). Concerning pod angle for shattering resistances, need to try whether expected result occurs (note: Canadian *B. juncea* is not as appressed as that in Pakistan). He does not think that a close linkage exists between pod angle and shattering resistance.

   - Dr. B. Singh referred to the need to consider pod density (re: shattering resistance); also: observed shattering, narrow pod-angle *B. juncea*.

3. - G. Alemaw (Ethiopia) Sought information on species - resistance to black leg.

   - Dr. Rimmer indicated that those containing 'b' genome are generally more strongly resistant.

   - Dr. Downey indicated that some *B. junce* lines are susceptible.

4. - Kumar (India) expressed concern about possibly different races of white rust affecting pods and leaves in India.

- Dr. Rimmer indicated that same isolates can induce stag head and white rust on leaves; thus variability relating to part of plant affected may not be applicable.

5. - Dr. Yan Zhang (China) indicated that 'free' (low) glucosinolate *B. napus* and *B. juncea* are still in early stages of development; however, *B. campestris* line BC86-18, with segregation averaging half normal glucosinolate content, is available for distribution. Possibility of selecting approximately 0 glucosinolate should exist.

B. BRASSICA SUB-NETWORK: RECOMMENDATIONS/ACTIONS

6. - Dr. Omran expressed his concern that due to the death of the Sub-network Chairman, up-to-date information on implementation not yet available in all cases. His files have not yet been accessed. From 1989 recommendations:

6.1. Seed Registry:

- Requested to send descriptions of released varieties to Dr. Omran, for Publication in Oilcrops Newsletter.

6.2. Germplasm Exchange:

- Over 60 lines were received and dispatched to participants. Request to send more relevant lines to Dr. Omran: eg. via Dr. Downey.

6.3. Training:

- Agronomy/breeding course held in Pantnagar, India, Dec. 1989; Quality course held in two days preceding this sub-network meeting (April 21-22, 1990) in Shanghai, China.
- For future trainings: Dr. Mundel expressed plea for attempt to ensure greater homogeneity of candidates in training, education and experience
- Dr. Omran suggested that for the proposed plant protection training, governments will be requested to nominate candidates working as pathologists or entomologists with B.Sc. or above.
- Dr. B. Singh offered to hold this training course in Pantnagar. This offer was accepted by the participants.

6.4. Information exchange:

- Dr. Omran stated that the Network contracted with Indian scientists for the following reviews/abstracts:

a) Screening and Breeding Techniques for Alternaria Blight Resistance in Oilseed Brassicas : A Review. by Drs. Basudeo Singh and S.J.Kolte.

b) Screening and Breeding Techniques for Drought Resistance in Oleiferous Brassicae : A Review. by Drs. Arvind Kumar and J.N. Sachan.

c) Technical Bulletin on Identification of Diseases and Insect Pests of Brassica Oilcrops (In Colour). by Drs. S. J. Kolte, G. C. Sachan and D. R. C. Bakhetia.

The completed copies are expected in next few months. These will be sent to all participants by Dr. Omran.

- A Review on White Rust, compiled by Drs. Verma and Saharan, is being published with CAB and IDRC cooperation.

- Dr.Rimmer informed that the American Phytopathological Society is preparing a Compendium of Brassica Diseases.

- For Cruciferae Newsletter, 1 - 2 page summeries may be forwarded to Dr.Omran for provision of Camera-ready copy for publication.

6.5. Equipment :

To a querry from Dr. Mundel about equipment, Mr. Ker suggested that details of manual/bullock draion/tractor driven seeding and/or harvesting equipment be provided to omran for announcing in newsletter. Details to include as much information as possible, including photos,transport cost, exportability, etc.

6.6. Steering committee information:

- Network 'draft constitution' to be approved for the next Steering Committee Meeting.

- Dr. B. Singh assumes responsibility as chairman of Sub-Network. China nominated Dr. Zhang Yan to act as Co-Chairman. Thus, she will also be asked to represent the Brassica Sub-Network on the Steering Committee of the Oilcrops Network.

6.7. Next Sub-Network Meeting:

- Dr. Downey invited the participants to hold the next Brassica Sub-Network meeting in Saskatoon, Canada, July 1991, in conjunction with the 8th Rapeseed Congress. Accommodation at University would be possible and relatively inexpensive.

- Dr. Yan Zhang suggested to add 1 or 2 days in Dr. Mc Gregor's lab for quality training.

- As an alternate site invitation from Pakistan (from Dr. Rana, presented by Dr. Qazi) to hold Sub-Network meeting, and whole Network Steering Committee meeting in Islamabad, early in 1991).

- Dr. Omran requested participating countries to seek other donors (including own governments), and to communicate commitments by July-August 1990.

- Note from Dr. Mc Gregor: In conjunction with the International Rapeseed Congress, the International Standards Organization (Subcommittee 2, Technical Committee 34 will meet in Saskatoon, July 1991. Sub-network representatives may wish to pursue possibility of their country sending Brassica representatives.

6.8. Suggestions/Queries for Next Meeting:

Dr. Mateo

- Is past emphasis on information exchange desired direction? or is more collaborative work sought?
- Is it worthwhile to share experiences on processing/marketing?

- What possibilities exist for private sector and national government support to Network? as well as other donors: IDR, WB, CIDA, etc. (these could be approached for integrating with Network activities).
- To cut costs, may need to consider spacing meetings further apart.

Dr. Downey

- I am happy to see the value of personal/professional contacts established by Sub-Network meetings.

- As information from breeders' perspective has tended to dominate, I may wish to bring in non-breeders for a day. I refer also to BARD-project in Pakistan: the integrated approach from research to extension to processing and marketing to consumer.

Dr. B. Singh

- I suggest that next meeting emphasizes papers on areas of potential collaboration and potential need. eg. *Orobanche*, seed production, etc. Thus: country papers only to present new developments.

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