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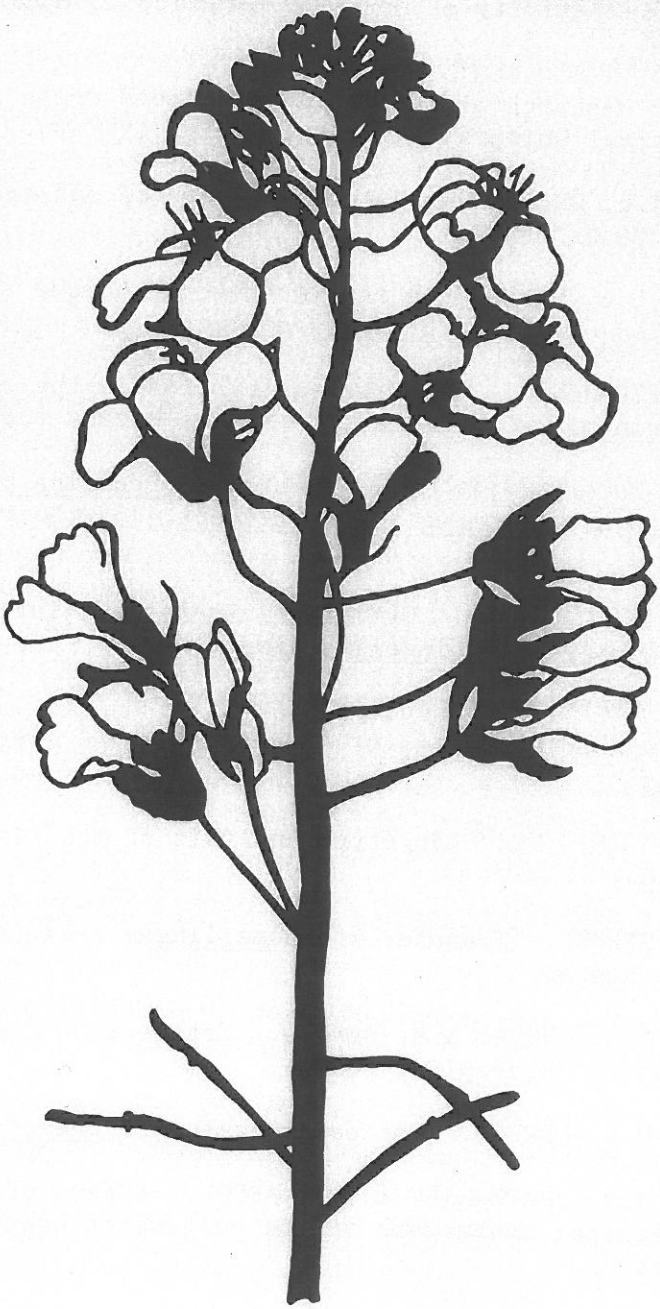

JUNE 1991

EUCARPIA

CONTENTS

EDITORIAL

Publication of Cruciferae Newsletter has been brought up-to-date by the production of this double issue. The editor apologises to authors whose contributions were affected by the hiatus in publication of the Newsletter and to all recipients, especially those who expressed concern that they had not received it.

The Newsletter has been produced since its inception by staff of the Scottish Crop Research Institute and the former Scottish Horticultural Research Institute and Scottish Plant Breeding Station, from which SCRI was formed. Regretfully, as research programmes have been changed, publication cannot be continued by this Institute. It is hoped that a new home for the Newsletter can be found shortly and that regular publication will be resumed under a new editor.

A.B. WILLS

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STATUS OF THE CRUCIFEROUS VEGETABLE COLLECTIONS OF THE NORTHEAST REGIONAL PLANT INTRODUCTION STATION

J.R. McFerson and B.E. Recchio-Demmin

The Northeast Regional Plant Introduction Station (NERPIS) in Geneva, New York maintains collections of the cruciferous vegetable species within the U.S. National Plant Germplasm System (NPGS). During the past two years, these collections have been considerably enlarged through acquisition of cabbage and radish collections held previously at Pullman, Washington and Ames, Iowa, respectively. NERPIS current holdings include:

Brassica oleracea	1105
B. rapa	115
B. spp.	55
Raphanus spp.	596
Arabidopsis spp.	6

REGENERATION AND EVALUATION

Several recent facilities additions and improvements have allowed for the expansion and modernization of seed regeneration and storage activities. During 1989, 10 portable screencages were purchased for controlled pollination. Construction was completed on an 18m x 60m field laboratory located on a new 13 ha farm. Cold room facilities were remodeled by installing moveable shelves and refrigeration units increasing seed storage space to approximately 200 m^3 at 5°C and 25% RH.

Twenty plant introduction (PI) accessions were regenerated at Geneva during the 1989 season; with improved facilities a targeted increase of 200 accessions is planned during 1990-91. NERPIS is presently establishing closer links with private U.S. seed companies and European genebanks in order to develop a cooperative, long-term regeneration strategy ensuring availability of a maximum number of accessions. We are also updating all crucifer documentation by identifying and filling gaps between the Geneva active collection and the base collection held at the National Seed Storage Laboratory in Fort Collins, Colorado. Information is also being updated in the Germplasm Resources Information Network (GRIN) in order to more accurately reflect the current baseline inventory.

C.E. Thomas and E.L. Jourdain, USDA-ARS Vegetable Research Laboratory, Charleston, South Carolina, have recently completed an evaluation of B. oleracea ssp. botrytis for resistance to downy mildew (incited by Peronospora parasitica Pers. ex. Fr.). This information is currently being entered into the GRIN system.

USDA/ARS Germplasm Res. Unit, NS Ag. Exp. Sta., Geneva, NY 14456-0462, U.S.A.

COOPERATION WITH EUROPEAN GENEBANKS

As part of our efforts to strengthen international cooperation among genebanks, J.R. McFerson, Geneticist/Curator, visited both the Plant Genetic Resources Center, Braunschweig, Federal Republic of Germany and the Center for Genetic Resources, Wageningen, The Netherlands from 28 August to 8 September 1989. The visit, sponsored jointly by the NPGS and the Office of International Cooperation and Development, hopefully is the first in a series of reciprocal visits and the genesis of several mutually beneficial cooperative projects focusing on Brassica spp. Areas identified for study include seed regeneration practices, documentation systems, seed-borne pathogens, and assessment of genetic diversity in a genebank setting.

COOPERATION WITH THE CRUCIFER CROP ADVISORY COMMITTEE

The NPGS relies heavily on technical advice of crop advisory committees to improve its operations. The Crucifer Crop Advisory Committee, chaired by P.H. Williams, comprises experts from genetics, breeding, pathology, biotechnology, and crop production. The committee meets at approximately 18-month intervals, usually in conjunction with the Crucifer Genetics Workshop.

RELATED RESEARCH

A considerable amount of diversity and complexity exists within the crucifer collections, making these taxa interesting model systems for studies of genetics and pathology as related to effective conservation and use. The research component of the Germplasm Resources Unit, directed by S. Kresovich, focuses investigations on the science of plant genetic resources management, including: (1) genetics, e.g., characterization and maintenance of genetic diversity, establishment of the relationships between molecular and cellular characters and whole-plant form and function; (2) plant pathology, e.g., disease detection, eradication, and screening; and (3) entomology, e.g., pollination biology and its effect on genetic structure and seed production in allogamous, heterogeneous accessions.

ACTIVITY OF CRUCIFER GENETICS COOPERATIVE (CrGC) 1989-1990

Paul H. Williams

In the last year, the Crucifer Genetics Cooperative has shown steady growth and activity. Most activity has been in the development and distribution of Brassica stocks that have unique phenotypes or genotypes that would make them useful for genetics, breeding, biological studies, research and education.. Since 1989, over 3000 packets of seed have been dispensed to researchers and teachers in 44 countries and 236 new members have increased the total membership to over 1500. Research activities with the stocks vary from conventional genetic and breeding applications to a variety of cellular and molecular biology applications including haploid plant regeneration, somatic embryogenesis, protoplast culture, molecular genetics of cytoplasmic organelles and gene vector relations.

Below is a list of the stocks currently available for distribution from the CrGC. Please note that we have significantly changed our assignment of CrGC stock numbers. Since most traits are placed in the background of a rapid cycling (RC) base population (BP) the seed stocks can be rapidly increased by the recipient. Along with the seed stocks, the CrGC supplies information on increasing the stocks and on evaluating specialized phenotypes such as host-pathogen interaction. Cultures of numerous crucifer pathogens are available upon request and with the provision of appropriate permits for transfer of the pathogen.

We are currently developing an increasing number of stocks with specific genotypes and phenotypes. When a particular trait is introduced into the rapid cycling background of a base population and when sufficient seed of a stock is produced, the stock is assigned a CrGC number and made generally available.

I would like to encourage anyone who has traits (qualitative, quantitative or cytoplasmic) that they would like to share with others to write the CrGC or send seed. We are also able to receive and are prepared to assist you in shipping pollen carrying useful genes. The CrGC will also receive genetic material on a confidential, proprietory or prepublication basis and to honor your wishes to not make the particular trait generally available through the CrGC until permission is granted in writing. All persons providing genes and stocks to the CrGC will be recognized as the source of those traits. In the case where the supplier of a trait is not the originator of the trait, I would also like you to provide information on the origins of the trait. A comprehensive system of stock origins, stock development (lineage), maintenance and distribution is being kept by the CrGC. This information may become increasingly valuable to users of these stocks. If you would like detailed information on any CrGC stock or have ideas that would help to improve the operation of the CrGC, we would be pleased to hear from you.

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RESEARCH ACHIEVEMENTS IN OILSEED CROPS AT HARYANA AGRICULTURAL UNIVERSITY, HISAR, DURING 1988-90.

J.S. YADAVA

A systematic and planned research work on improvement of different oilseed crops at Haryana Agricultural University was started in late sixties. Since then a good number of varieties in various oil crops have been evolved for commercial cultivation. The brief research highlights for the past two years during 1988-90 are given as under:

A. Rapeseed and Mustard:

1. Indian Mustard (Brassica juncea L.)

Three varieties namely, RH-8113, released in 1988 and RH-781 and RH-819 released in 1990 are developed. The variety RH-8113 is resistant to white rust and Alternaria diseases. The variety RH-781 has inbuilt tolerance to frost with high yield and the variety RH-819 is suitable for rainfed and drought cultivation. The variety RH-819 gave 30 per cent higher yield than a national standard variety, Varuna under rainfed conditions.

In advanced stage yield trials, the strain RH-8812 yielded significantly higher (2454 kg per ha.) over the best check variety RH-30 (2230 kg per ha.). This genotype was at par with RH-30 in maturity (134 days) and test weight (6.2 g per 1000 seed). A few other strains like RH-839, RH-8315, RH-848, RH-8602, RH-8805, RH-8865, RH-8904 and RH-8911 are developed for higher yield and are being tested in various yield trials. The strains, RH-8701 and RH-8814 are evolved for resistant to mustard diseases and high yield. The genotypes, RH-8816A, RH-8816B and RH-8825 are developed for long pod possessing average 20 seeds per pod against 14 seeds per pod of standard variety RH-30. The strains RH-8539, RH-8546, RH-8689, RH-8690 and RH-8693 are evolved for yellow seed coat colour and disease resistance with high yield.

In the interspecific crossing programme between B. juncea and B. carinata, the genotypes ISH-8805 and ISH-8807 which are significantly superior (1852 Kg and 1740 Kg per ha. respectively) to standard variety RH-30 (1607 Kg per ha.), are developed. They also possess resistance to white rust and Alternaria. In Brassica carinata (Ethiopian mustard), two genotypes namely, C_6YS_7B and Ethiopian Selection have been developed which are most promising in yield and possess resistance to white rust and yellow seed coat colour. In Brassica napus, the strains N-20-7-1 and Tower 5-2 have been developed for seed yield, earliness (125 days) and resistance to white rust.

2. Toria (Brassica Campestris var. Toria)

Recently a new variety, TH-68 has been released in 1990 which is high yielding (1400 Kg per ha.) and early maturing (89 days). Being early, it is most suitable for toria-wheat rotation. The other promising strains like TH-91, TH-94, TH-100, TH-101, TH-102, TH-120, TH-122 and TH-130 have been developed in early and late groups.

Breeder Oilseeds, HAU, Hisar-125004, India.

B. **Groundnut (Arachis hypogea L.):**

A new variety MH-4 has been released in 1988 for general cultivation. This variety gives at par yield (3500 Kg per ha. at 30x15cm spacing) with standard variety MH-2 (sown at 15x15 cm spacing). The other strains, MH-11 and MH-34 have been evolved for high yield (4180 Kg and 4006 Kg per ha). The strain MH-34 also possesses 54 per cent oil against 50 per cent in standard varieties. Another strain MH-42 possessing 60.5g 100 kernel weight against 53.8 g of boldest seeded standard variety M-13, have been developed. A few more genotypes viz. MH-46, MH-52, MH-53 and MH-56 are developed for high yield.

C. **Sesame (Sesamum indicum L.):**

The strains, namely, HT-15, HT-20, HT-21, HT-24, HT-35, HT-36, HT-45, HT-46 and HT-48 are developed possessing more than 1100 Kg per ha. yield against 973 Kg per ha of standard variety HT-1. Among these strains, HT-36 and HT-20 had oil content of 54 and 53 per cent respectively against 50 per cent of standard HT-1.

NOTE TO RECIPIENTS OF CABBAGE CVS. 'RICHELAIN' AND 'RICHESSE' SEEDS

M.S. Chiang and R. Crête

The newly released 'Richelain' and 'Richesse' cabbage cultivars, developed from the St-Jean Research Station, are resistant to races 2, 6 and 7 of the clubroot pathogen, *Plasmodiophora brassicae* Wor.

During the past two years we found that some of our breeding lines as well as the 'Richesse' cv. were susceptible to gray leaf spot and black leaf spot caused respectively by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.) Wiltshire, however, the 'Richelain' cv. seems to be tolerant or resistant to these leaf spot diseases.

Reference

Chiang, M.S. and R. Crête. 1987. Richesse: A new clubroot-resistant cabbage cultivar. Can. Hort. Council Rept. 1987:118-119.

Chiang, M.S. and R. Crête. 1989. Richelain: A clubroot-resistant cabbage cultivar. Can. J. Plant Sci. 69:337-340.

St. Jean Res. Stat., PO Box 457, St. Jean, Quebec, J3B 6Z8, CANADA.

A NEW IMPROVED TECHNIQUE FOR THE LARGE SCALE COLLECTION OF VIABLE *BRASSICA* POLLEN.

EK Ong and PE Taylor.

The use of pollen for genetic, physiological and biochemical studies has gained increasing importance. Pollen analysis can be a useful tool for the investigation of gene expression and regulation, pollen selection and its impact on evolution in higher plants and as a biological indicator to test the effect of biotic or abiotic stress. The study of these rely on the availability of a large amount of viable pollen. The problem with current methods is that the yield of pollen is very low. We have developed a new method that increased the yield of viable pollen by seven fold.

Plants of *Brassica napus* L and *B. campestris* L were grown at the Crops Research Institute, Horsham, Victoria and in the glasshouse at the University of Melbourne, Australia. The stem together with mature flowers were cut and kept indoors in vases at room temperature. After two days, mature pollen was collected by applying force with the forehand to the flowering tops of the cut stems, so that the anthers touched the glass plate (23 x 56 cm). The lipid-coated pollen stuck to the glass plate. Floral debris was easily removed by a gentle stream of air and by forceps and the pollen was scraped up with a razor blade. Pollen was collected from the same stems over a period of five days. Heavy thrip infestations are common in *Brassica* and contaminated the pollen on the glass plates. However, by leaving the plates undisturbed for about 20 min, these insects left the plate. This may be aided by insect attractants such as light or chemical or as we found, by placing freshly cut *Brassica* flowers on the perimeter of the glass plate. Using this method, at least 0.7 gram of pollen per hour was collected with a viability greater than 90% as tested with fluorochromatic reaction (Heslop-Harrison *et al.*, 1984). The amount of pollen collected was limited only by the size of the glass plate and the amount of plant material available. When vacuuming technique was used, powered either by battery (Evans *et al.*, 1987) or water pump, we collected on average 0.1 gram of pollen per hour. Thus the technique of collecting pollen onto glass plates is 7 times better than the vacuuming technique. We also found that by collecting pollen indoors, consistent yield were obtained in comparison to outdoor collecting which varied enormously depending on the weather and insects.

In conclusion, this new technique of collecting pollen onto glass plates has simplified the procedure while greatly increasing the amount of pollen collected when compared to earlier methods such as vacuuming or brushing dehisced anthers. The technique is also cost-effective as no equipment is needed.

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EXPERIMENTALS HYBRIDS IN THE COMPLEX HUTERA - RHUNCHOSINAPIS (CRUCIFERAE) OF SIERRA MORENA (SPAIN)

E. Sobrino Vesperinas

Experimental hybridizations have been made between three endemic species, Rhynchosinapis longirrostra (Boiss.) Heywood, Hutera leptocarpa Gonzalez-Albo and H.rupestris Porta, which are members of the tribe Brassiceae, they inhabit Sierra Morena (in the broad sense) on the Southern limit of the central table-land.

The principal differences between these three species, are in the size and form of the fruit, because the remainder of the characters, show a remarkable similitude (chromosomic number, seed, cotyledons, leaves and petals).

However the differences that we can find in the fruits are notable. In R.longirrostra they reach about 50 to 60 mm. in length, while in H.rupestris at the opposed extreme, they reach only 25 mm. maximum.

In the three species there are seeds in the valvar portion and in the beak, though the total number of seeds per fruit and their varies between the species. R.longirrostra has the most seeds/fruit and H.rupestris the least.

An additional difference is in the width of the rostrum and the valvar portion; in R.longirrostra both are similar, while the rostrum is broader and ventricose in H,leptocarpa, and the width is greater still in H.rupestris.

In this work have been utilized the taxonomic terminology proposed by TUTIN and col. (1964), where these taxa are considered to be in different genera. But, GOMEZ-CAMPO (1977) studied in detail the clinal variation of the species here treated and Rhyncosinapis hispida (Cav.) Heywood, concluded that both genera would best group under Hutera. Later, GREUTER and BURDET (in GREUTER and RAUS, 1983), considered that both genera must be nominated as Coincya by nomenclatural priority.

The facility of hybridization found (1 to 14 viable seeds for every 10 hybridized flowers) and the high fertility of the hybrids F_1 (pollen and seeds), confirmatted the grouping in only one genus.

The three species that have been studied support their morphological individuality as a consequence of their geographic isolation, because though adjacent elevated areas ; they are isolated by semiarid plains which are difficult to cross. This group of species morphological differentiation has not been accompanied by genetic barriers.

GOMEZ-CAMPO (1977) found populations of R.hispida, R.longirrostra and H.leptocarpa, that show intermediate and intergrading characters that he interpreted as different lines evolutionary; similary on this hypothesis the possibility of introgresive hybridizations could be considered on the basis of the crossing facility.

Another interesting aspect is the type of the fruit displayed by the F_1 hybrids, taking into account the important differences that exist between the parents.

The figure nº1 shows the fruit of H.leptocarpa X

Compania Espanola de Cultivos, Oleaginosos S.A., Cecosa, Genova 9-4, Madrid-4, SPAIN.

R.longirrostra and the correspondent parents. The hybrid fruit is similar in length to the male, whilst the index length rostrum/length fruit is closer to H.leptocarpa. The total number of seeds per fruit is intermediate, though
nearer to the female; however, the hybrid has the same number of seeds in the rostrum as R.longirrostra and in the valvar portion the number is identical to H.leptocarpa.Therefore is different to both parents, and appears that the inheritance of the number of seeds operates separately in every loculus.

In the figure nº2 is represented the fruit of the hybrid H.rupestris X H.leptocarpa and the parents. Hybrid fruit is intermediate between the parents. The main difference from H.rupestris being the considerable increase the number of seeds in the rostrum (from 2 to 6) in.

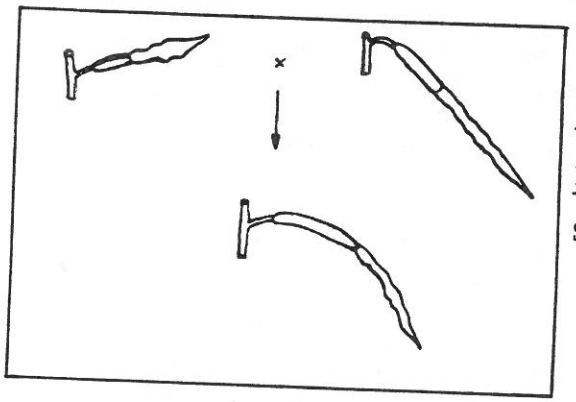

Fig.nº1 - Fruit morphology of the hybrid H.leptocarpa X R.longirrostra.

Size: x 0'5

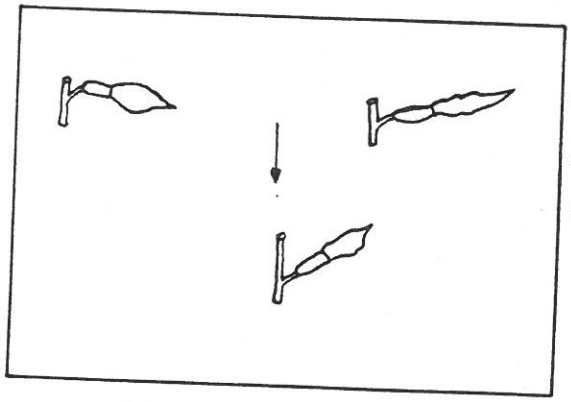

Fig.nº2 - Fruit morphology of the hybrid H.rupestris X H.leptocarpa.

Size: x 0'5

It should be interesting to study the hybrid F_2 generation, that could provide profound knowledge of the inheritance of the line of the fruit reduction in Brassiceae, which would be entranced, where could be found species, that associate important differences in the fruit and interfertility.

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INTERGENERIC HYBRIDIZATION IN BRASSICA JUNCEA x SINAPIS PUBESCENS AND B. NAPUS x S. PUBESCENS, AND THEIR CYTOLOGICAL STUDIES

Nobumichi INOMATA

The subtribe Brassicinae in tribe Brassiceae consisted of five genus, Brassica, Diplotaxis, Eruca, Erucastrum and Sinapis (Mizushima 1952). Intergeneric hybridization between Brassica and Sinapis arvensis was reported by Mizushima (1950) and Inomata (1988). The present paper intends to obtain the intergeneric hybrids and the fundamental data on introducing a gene or genes from Sinapis pubescens to Brassica.

The materials used in the experiment were B. juncea ((2n=36) FW 1-2 which have white petal, B. napus (2n=38) subsp. oleifera cv. Aomori No. 1, B. napus subsp. oleifera cv. Karat and S. pubescens (2n=18). Reciprocal pollination was made between Brassica and S. pubescens, and the ovary culture was carried out according to the previous papers (Inomata 1978, 1985). Chromosome pairing at the first meiotic division and the pollen fertility were examined by acetic carmine.

Table 1 shows the results on ovary culture. The ovaries explanted were almost no infected by mold and bacteria. Developing embryo was not obtained in all cross combinations except one late torpedo-shaped embryo in the cross of S. pubescens x B. napus. Many seeds were obtained in the cross of B. juncea x S. pubescens and B. napus x S. pubescens, but no seed was obtained in the reciprocal crosses. Artificial pollination was made as a control of ovary culture in four crosses. In one of them, B. juncea x S. pubescens, two seeds were obtained in 15 flowers were pollinated. The morphological characteristic of leaf and petal color of the F_1 hybrids was intermediate between the parents. The F_1 hybrids in the cross of B. juncea x S. pubescens and B. napus x S. pubescens showed 27 and 28 chromosomes, respectively. Table 2 shows the results on the first meiotic division at the PMCs. One of three F_1 hybrids in the cross of B. juncea x S. pubescens was examined. The mode of chromosome configuration was $1_{II}+25_{I}$. The range of chromosome association showed $(0\text{-}6)_{II}+(15\text{-}27)_{I}$. Table 3 shows the results on the first meiotic division at the PMCs. One of five F_1 hybrids in the cross of B. napus x S. pubescens was examined. The mode of chromosome configuration was $10_{II}+8_{I}$. One trivalent chromosome was observed. The range of chromosome association showed $(0\text{-}1)_{III}+(2\text{-}11)_{II}+(6\text{-}24)_{I}$.

No pollen fertility was obtained in both F_1 hybrids, B. juncea x S. pubescens and B. napus x S. pubescens. Crossability of the both F_1 hybrids was examined in self-pollination, open pollination and backcrossing to the maternal plant. No seed was obtained in self-pollination and the backcrossing. Four and six seeds were obtained in open pollination of the F_1 hybrids in B. juncea x S. pubescens and B. napus x S. pubescens, respectively. In another F_1 hybrids cytological studies and crossability were not examined until now. The F_1 hybrids in B. napus x S. pubescens showed a perennial plant like the habit of S. pubescens. But the F_1 hybrid, B. juncea x S. pubescens, died by the summer.

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Acknowledgment

I would like to thank Dr. Banga, S.S. at the Department of Plant Breeding of Punjab Agricultural University, India for providing the seed of Brassica juncea FW 1-2, and also like to thank Dr. Gómez-Campo, C. at Escuela T.S. Ing. Agronomos Universidad Politecnica, Madrid for providing the seed of Sinapis pubescens 3823-75. Finally, I would like to thank the Plantech Institute, Japan for providing the seeds of Brassica napus cv. Aomori No. 1 and cv. Karat.

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Table 1. Intergeneric hybridization between Brassica juncea and Sinapis pubescens, and between B. napus and S. pubescens through ovary culture

Cross combination	No. of capsules examined (A)	No. of embryos further cultured Late torpedo	No. of seeds obtained	No. of hybrids obtained (B)	B/A x 100
B. juncea x[1)] S. pubescens	54	0	8	3	5.6
reciprocal cross[1)]	65	0	0	0	-
B. napus x[2)] S. pubescens	103	0	9	5	4.9
reciprocal cross[3)]	57	1	0	0	-

1: B. juncea used in the experiment was B. juncea WF 1-2. 2: B. napus used in the experiment was B. napus cv. Aomori No. 1. 3: B. napus used in the experiment was B. napus cv. Karat.

Table 2. Chromosome configuration at the first meiotic division of the F_1 hybrid between Brassica juncea and Sinapis pubescens

Cross combination	First meiotic division					
	No. of PMCs observed (%)	$1_{II}+25_I$	$2_{II}+23_I$	$5_{II}+17_I$	$3_{II}+21_I$	Other types
B. juncea x S. pubescens	50	16 (32.0)	8 (16.0)	8 (16.0)	7 (14.0)	11 (22.0)

Table 3. Chromosome configuration at the first meiotic division of the F_1 hybrid between Brassica napus and Sinapis pubescens

Cross combination	First meiotic division					
	No. of PMCs observed (%)	$8_{II}+12_I$	$9_{II}+10_I$	$10_{II}+8_I$	$11_{II}+6_I$	Other types
B. napus x S. pubescens	50	2 (4.0)	5 (10.0)	22 (44.0)	17 (34.0)	4 (8.0)

CYTOPLASMIC-GENETIC RELATIONSHIP BETWEEN *BRASSICA NIGRA* AND *SINAPIS ALLIONI*

S.S.Banga and K.S.Labana

Genetic relatedness between *Brassica nigra* (BB; 2n = 16) and *Sinapis allioni* (SS; 2n = 18) has not been experimentally explored so far. However, the meiotic analysis of the hybrid between *B.nigra* and *S.arvensis* (SS; 2n = 18), a species closely related to *S.allioni*, revealed as many as eight bivalents including seven allopairs and one autopair (Mizushima,1968). Very high frequency of bivalency indicated that either B and S chromosomes of two genomes are structurally less undifferentiated or they have sufficiently long genetically homeologous regions to allow frequent chiasma formation (Mizushima,1950; Mizushima,1968). In this communication we report chromosomal as well as cytoplasmic relationship between these two species.

MATERIALS AND METHODS

B. nigra was pollinated as female with pollen from *S. allioni*. Fifteen seeds obtained from about 1900 pollinations resulted in six adult plants. Meiotic studies were carried out on young buds fixed in Carnoy's solution containing ferric acetate as mordant and squashed in 2 per cent acetocarmine.

RESULTS AND DISCUSSION

Only one plant out of six turned out to be a true hybrid. The remaining five plants resembled the pollen parent and were suspected to be of patromorphic origin. The solitary F1 hybrid plant was tall, solid stemmed, and late like *B. nigra* but had intermediate leaf shape whereas patromorphic plants were dwarf and hollow stemmed like *S. allioni* plants. The cytological analysis confirmed the visual observations. The hybrid plant had 2n = 17 with varying number of bivalents (Table 1) the maximum being 8II. The mean bivalent frequency was 4.25. One trivalent was frequently present. The univalent related defects such as irregular and unequal division were encountered during anaphase -1 but 9-8 distribution was most frequent. There was unequal distribution of chromosomes at anaphase-II, and microspores released were of variable shape and size. Only about 30 per cent of pollen was deeply stained. No seed setting was observed on selfing or backcrossing. The cytological analysis of the five

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Table 1: Bivalent frequency* in the PMC's of B. nigra x S. allioni F_1 hybrid

2II	3II	4II	5II	6II	7II	8II
59 (17.4)	68 (20.1)	32 (18.3)	77 (22.8)	36 (10.7)	21 (6.2)	15 (4.4)

Mean bivalent frequency : 4.25

* Each configuration had varying number of univalents and sometimes one trivalent

Figures in parentheses indicate per cent of the total cells.

suspected patromorphic plants confirmed their somatic chromosome number to be 2n = 18 like the male parent S.allioni. No meiotic irregularities were observed. However, all the five plants were completely male sterile with about five per cent pollen fertility.

Occurrence of very high bivalent frequency in the hybrid, suggests a chromosomal homology between BB and SS genomes. Mizushima (1980) also observed close homology between B. nigra and Sinapis arvensis. The species S. arvensis is very closely related to S.allioni as their F_1 hybrid is normal and fully fertile (Harberd, 1972). However, the developmental imbalance brought about by the interaction of nigra cytoplasm with allioni nucleus in patromorphic plant results in male and female sterility hence it is inferred that the cytoplasms of nigra and allioni are different.

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KARYOTYPIC STUDY AS A AID IN ANALYSING THE EVOLUTION AND RELATIONS OF THE SIX OILSEED SPECIES IN GENUS BRASSICA

Du Xin and Tang Ze-jing

From the karyotypic analysis in the six species of genus Brassica (Table 1), we discuss their evolutionary level and path, the factors which affect evolution and the relations among the six species.

Table 1. The results of karyotypic analysis of the six species

Species	Karyotype formula	Various range of the arm tatio
B. campestris	2n=20=12m+2sm(sat)+6sm	1.29-2.53
B. oleracea	2n=18=12m+2sm(sat)+4sm	1.06-2.19
B. nigra	2n=16=8m+4sm(sat)+4sm	1.32-2.27
B. juncea	2n=36=16m+6sm(sat)+10sm+2st+2t	1.08-8.30
B. carinata	2n=34=14m+6sm(sat)+14sm	1.08-2.73
B. napus (E)*	2n=38=20m+4sm(sat)+10sm+4st	1.19-3.76
B. napus (J)*	2n=38=24m+4sm(sat)+8sm+2st	1.03-3.33

(E)*: material of B. napus originated from Europe
(J)*: material of B. napus originated from Japan

Species evolution should have an affect on the chromosomes, so, by means of karyotypic analysis, we can comprehend the evolutionary level and path of the species and the factors which affected their evolution. First of all, Levitzky (1930) pointed out a concept about the karyotypic symmetry and asymmetry, he discovered that the main current of karyotypic evolution in flowering plants was that the karyotype changed gradually from symmetry to asymmetry. Stebbins (1958) classified the karyotype using karyotypic symmetry as a standard.

Based on Stebbins' classification and our results, B. juncea, B. napus and B. campestris belonged to class 2B, whose karyotype was more asymmetric; while B. nigra, B. oleracea and B. caritana belonged to 2A, whose karyotype was more symmetric. According to Levitzky's review, the more asymmetric a karyotype a species has, the more evolved it is. Therefore, B. campestris is furthest evolved among the elementary species, and B. juncea and B. napus among the amphidiploid species.

B. juncea is one of the main cultivated species, it originated from cold, high mountains, where the ecologic conditions were very complicated. B. napus originated from the Mediterranean coast, it is cultivated throughout the world. Under the action of both natural and cultivated conditions, they have a higher level of evolution, and so does B. campestris, because it has a long cultivated history and wide cultivated range. B. nigra and B. caritana are less cultivated, most

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of their characters are primitive, they have a lower level of evolution.

The main factors leading to karyotypic asymmetry are the pericentric inversion, the inequivalent reciprocal translocation and fragment loss in chromosomes. The pericentric inversion only changes the arm ratio of the chromosome, it does not change the size and length of the chromosome; the inequivalent reciprocal translocation only changes the arm ratio and length between the chromosomes, it does not change the nuclear DNA amount, the fragment loss in chromosome changes not only the arm ratio but the nuclear DNA amount. From S.C. Verma's study about the nuclear DNA amount in Brassica and our experiment about the comparison of the number of various chromosomes, we believe that the fragment loss in chromosome, the pericentric inversion and the inequivalent reciprocal translocation have very important roles in the evolution of Brassica.

Based on our results, the karyotype of the three elementary species are more similar, they have closer relationships. Most researchers thought that the elementary species originated from an ancestral parent (n=6) by means of chromosome proliferation, the amphidiploid species originated by hybridisation between two elementary species. After their origin, they underwent mutations, genetic reconstitution, pericentric inversion, inequivalent reciprocal translocation and fragment loss in chromosomes during their evolution, and became the extant species.

In our experiment, we also analysed the karyotypes of B. napus and B. napella (the material of B. napus originated from Japan), the result indicated that there were greater difference between them, giving support to the view that B. napus and B. napella must be divided into two species or two varieties of one species.

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KARYOTYPE ANALYSIS OF THE SIX SPECIES OF GENUS BRASSICA

Du Xin and Tang Ze-Jing

The six species of genus Brassica are very important in agriculture, they are cultured extensively as oilseed crops. As cytogenetic study of these crops could explain their origin, evolution and relations there have been many such studies of the genus Brassica over the world, but the small size of their chromosome makes accurate analysis difficult. Furthermore, owing to the distinct methods and materials used by the researchers, their results were usually different from each other. Otherwise, a karyotype analysis of Brassica carinata, which is one of the amphidiploid species has not been reported.

MATERIALS AND METHODS

Using the wall degradation hypotonic methods, the somatic karyotypes of the six species of genus Brassica: Brassica nigra, B. oleracea, B. campestris, B. napus, B. juncea and B. carinata were studied, we employed the idiogram and karyotypic formula to show the structure and morphology of the genus.

RESULTS AND DISCUSSION

The idiograms of the six species are omitted. Based on the results, the following questions are discussed:

1. The similarities and variances of the karyotypes of the six species. There are many similarities among the six species, especially the three elementary ones in chromosome size, morphology and composition, those of the latter species being composed of M and SM types. But during the long-term evolutionary process, the karyotype have changed under various natural and cultivated conditions. The variances are displayed mainly in the number, size of satellite chromosome, the ratio of large/small chromosomes, and in their subtle structures.

In the six species, the karyotypes of amphidiploid species are not composed simply of the karyotypes of their two elementary species, there were greater differences between the elementary species and the amphidiploids.

2. The satellite chromosome in the six species. On the basis of our results, the number of satellite chromosomes in the species were: B. campestris and B. oleracea, one pair; B. napus and B. nigra, two pairs; B. juncea and B. carinata, three pairs. It was shown that the number of satellite chromosomes of the amphidiploid species equalled to the sum of satellite chromosomes of their two elementary species.

The other characteristic of the satellite chromosome in the six species was their diversification and heterozygosity. The morphology and number of satellite chromosomes differed between species, which showed the diversification and the morphologies of satellite chromosome varied in the same species, which showed the heterozygosity.

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J. Zhu and D. Struss

The genetic base of resistance to blackleg (*Phoma lingam*) used in present rapeseed varieties is narrow and almost exclusively derived from the French variety "Jet Neuf" (Wittern 1984). The B genome of the genus *Brassica* is assumed to be another source of resistance to *Phoma lingam* (Roy 1978). Sacristan and Gerdemann (1986) using interspecific crosses between *B. carinata*, *B. juncea* and *B. napus* attempted to transfer the *Phoma*-resistance of the B genome into *B. napus*. We have been successful this year, 1989, in transferring the resistance from *B. nigra* into oilseed rape (AACC) by generating *B. napus /B.nigra* addition lines.

Our addition lines were developed by crossing *B. nigra* with *B. napus*, reciprocally . The F_1 s were subsequently backcrossed to the oilseed rape variety "Andor". 10-12 plants of each line were inoculated at the stem base by an aqueous pycnospore suspension of *Phoma lingam* at a concentration of 10^6 spores/ ml. Plants were then kept in a plastic foil chamber. Disease symptoms were scored 5 to 7 weeks after inoculation using a scale from 1 (no symptom) to 9 (collapse). Monosomic addition lines, euploid sister plants, the parents and the variety "Jet Neuf" were tested side by side. The average infection rate of the tested material is shown in Fig. 1. *B. nigra* and "Jet Neuf" showed high resistance and "Andor " was highly susceptible. From the tested addition lines six also showed resistance. Of a particular interest were six other lines, which were derived from addition lines but possessed 2n= 38 chromosomes, and nevertheless expressed resistance (Fig. 1; columns without i dot). Obviously this may result either from recombinational events or, less likely, from chromosome substitutions. These genotypes appear to be cytological stable and can be used to improve today´s breeding materials.

More details are now under investigation in backcross progenies. It is known from other experience (e.g. fatty acid content) that the resistant addition lines contain different B genome chromosomes. Thus the *B. nigra* resistance to *Phoma lingam* is obviously controlled by several genes each of which contributes to blackleg resistance quite considerably.

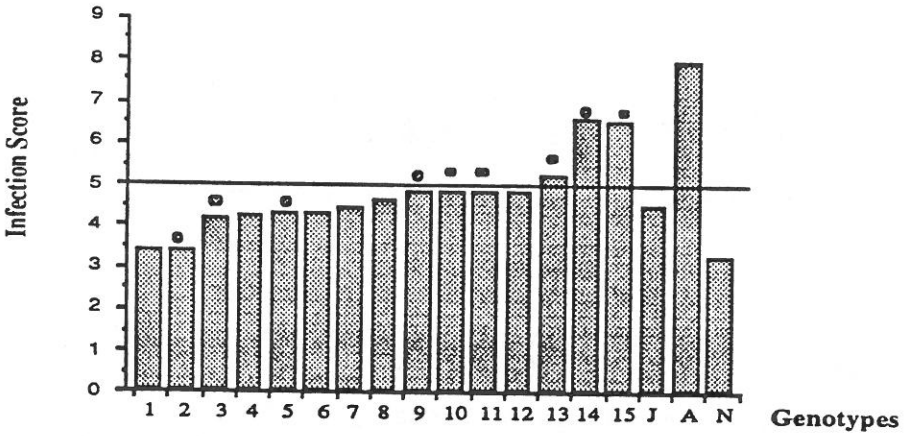

Fig. 1 : *Phoma* resistance of 15 *B. napus* lines derived from crosses with *B. nigra*: Columns with i dot represent monosomic addition lines. Score 5 marks the borderline of resistance. J=Jet Neuf, A=Andor, N= *B.nigra*

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ESTIMATION OF PLOIDY LEVELS IN B. NAPUS BY CHLOROPLAST COUNT

M.O. LUCAS, A.M. CHEVRE and M. RENARD

INTRODUCTION

Varying ploidy levels can be observed among B. napus plants regenerated from embryos obtained by anther culture (RENARD and DOSBA, 1980). A rapid and reliable technique for evaluating ploidy levels is necessary in order to distinguish the haploids, which are then colchicine doubled, from the diploids.

Cytological techniques of chromosome counting are reliable, but impractical for use with large numbers of plants. Morphological observation of inflorescences is generally too late, and cannot be used to assess ploidy levels in male sterile plants.

These reasons led to a search for a technique more rapid than those previously used, but equally reliable. The method selected was a chloroplast count in stomata guard cells, already used for sugarbeet (ESSAD and TOUVIN, 1959). This technique is based on the correlation existing between chloroplast numbers and levels of ploidy. This has been confirmed by studying diploid and tetraploid kale (CHEVRE et al., 1989). The method was applied to B. napus.

MATERIALS AND METHODS

Vegetal material

A preliminary experiment was carried out on hybrid plants obtained by anther culture following a B. napus x B. nigra cross.

A second study used the technique on male sterile rapeseed plants.

Techniques used

- Chromosome counts on mitoses of root meristems taken from plants in full growth, after vernalization (JAHIER et al., 1989).
- Morphological observation of inflorescences just before flowering : haploids show smaller buds, smaller petal surface and atrophied stamens.
- Chloroplasts counts in stomata guard cell have been described by CHEVRE et al., 1989.

RESULTS

The preliminary experiment was carried out after vernalization on plants produced by anther culture after a B. napus x B. nigra cross. 24 plants were studied : these were classified into two groups according to average numbers of chloroplasts in the stomata.

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A comparison between these results and those obtained by chromosome counts shows that the two groups correspond to two types of plant : haploid plants show average chloroplast numbers of 0.1 ± 1.1, whereas for diploid plants the averages are 16.2 ± 2.2. Results of the two techniques show a level of convergence of 100 % for diploids and 90 % for haploids.

The technique was then applied to male sterile rapeseed plants. 108 plants were analysed at the end of vernalization. These results also indicate the existence of two groups : the larger of these concerns chloroplast numbers between 7 and 10 and can be assimilated to the haploid type ; a second group, wider in range, shows chloroplast numbers varying between 11 and 20.

Morphological observation of inflorescences (bud size) was carried out on 28 plants, and confirmed the results of chloroplast counts, except in one case.

CONCLUSION

The technique of chloroplast count in stomata cells is easily carried out and interesting from several points of view. Early classification of plants before vernalization would allow colchicine doubling of haploids well before flower formation, thus increasing efficiency.

Moreover, this type of classification would make it possible to keep only the haploid plants plants, eliminating all diploids and thus avoiding the risk of keeping diploids originating from unreduced gametes.

The technique, however, is still lacking in precision. In particular, it cannot identify aneuploid or mixoploid plants. Results obtained suggest that the wider-range group could include both aneuploids and mixoploids as well as diploids. Further study is necessary in order to define the different levels of ploidy to be encountered in the descendence of *B. napus* anther culture.

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The Basic Number of *Brassica* Genomes: X=3 ?

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It has been advocated that the *Brassica* genomes, A=10, B=8 and C=9, have been ascended through aneuploidy from a common archetype. A basic genome of X=6 is believed to be the common archetype. Still a lower basic number, X=3, was suggested on the basis of observations made on secondary association at meiosis of *B. oleracea* (Hussein and Abobakr, 1976).

We favour the hypothesis that the basic number of *Brassica* may be X=3. However, this needs to be confirmed by more cytogenetical, molecular and other types of studies. Nevertheless, the present report is meant to stimulate interests on this topic amongst *Brassica* researchers.

We list the following points in support of the hypothesis that the basic number for *Brassica* is X=3:

I. Based on our results of isozyme studies, we suppose that the nuclear structural genes for phosphoglucomutase enzyme occur as a triplicate in *B.campestris* and *B. oleracea*. There is evidence that the three copies of this locus may be located on three separate chromosomes in *B. oleracea*.

II. In haploids of *B. campestris* and *B. oleracea*, the occurrence of one trivalent was sometimes observed (Armstrong and Keller 1981, 1982). The AC amphihaploid showed the formation of pentavalents (Attia and Röbbelen, 1986). The occurrence of hexavalents was also demonstrated in *B. napus* (Newell et al., 1984). These facts support the basic number of X=3 in *Brassica* if chromosome pairing is interpreted as an expression of homology or residual homoeology.

III. According to Röbbelen (1960), the three *Brassica* genomes were all constituted of six basic types of chromosomes which were designated as A, B, C, D, E and F. Each genome has two nucleoli. The two nucleoli are attached to chromosomes 1 and 2 (both of type A) in the *B. campestris* genome, to chromosomes 1 and 3 (types A and C) in the *B. nigra*

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genome and to chromosomes 1 and 4 (types A and C) in the *B. oleracea* genome. If chromosome types A and C are considered as potential nucleolar organizers, then the potential maximum number of nucleoli can be as many as three in the *B. campestris* genome (with chromosomes A, A and C) and in the *B. oleracea* genome (with chromosomes A, C and C).

IV. The trigenomic hybrid (2n=29, AAC) derived from the cross between *B. napus* (AACC) and *B. campestris* (AA) produced gametes with a non-random distribution of chomosome numbers between 10 to 19. There was a preponderance of gametes with 13 and 16 chromosomes (Nwankiti, 1970), which possibly reflected the preferential survival of 'balanced' gametes carrying certain groups of 3 or 6 chromosomes of the C-genome.

V. *B. oleracea* (CC, n=9) is a species with great morphological diversity. The n=9 may be a triplicate of the basic genome with X=3 or a combination of two ancient archetypes with X=3 and X=6.

VI. Of the twenty *Brassica* species with n=8, 9 or 10, four species have n=8 (20%); ten species have n=9 (50%) and six species have n=10 (30%) (Gomez-Campo and Hinata, 1980). Species of n=8 and 10 may be derived from species of n=9 by loss or gain of one chromosome.

As a conclusion, it is more likely that *Brassica* species with n=8, 9, and 10 have evolved through the pathways as indicated above under V and VI than through aneuploidy from a common archetype of X=6.

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EFFECT OF PLOIDY ON SOME PHYSIOLOGICAL CHARACTERS IN THE CULTIVATED SPECIES OF BRASSICA

P A Kumar, S R Chatterjee and Y P Abrol

Polyploidization has played an important role in plant evolution. A better understanding of the physiological consequences of polyploidization could facilitate the development of agronomically useful genotypes. Increases in nuclear ploidy have been associated with changes in both the quantitative and qualitative expression of enzymes (Gottlieb, 1982), net photosynthesis (Joseph *et al* 1981), photorespiration (Garrett, 1978), stomatal conductance (Randall *et al*, 1977), photosynthetic electron transport (Timco and Vasconcelos, 1981), anatomical characteristics (Byrne *et al*, 1981) etc. The purpose of this investigation was to examine the effects of nuclear allotetraploidy on some physiological characters viz., net photosynthetic rate, stomatal conductance and activities of two key enzymes involved in nitrogen assimilation, nitrate reductase (NR) and glutamine synthetase (GS) in the leaves of six cultivated species of *Brassica*.

Six cultivated species of *Brassica* (*B.nigra* (L.) Koch cv. IC 257; *B.oleracea* L.var *alboglabra* Bailey; *B.campestris* L.ssp.*oleifera* var. brown sarson cv. Pusa Kalyani; *B.carinata* Braun Strain 1; *B.juncea* Czern cv. Pusa Bold and *B.napus* Strain 706) were grown in the field. Sampling was done at the time of flowering. Net photosynthetic rate and leaf stomatal conductance were measured at different canopy levels using a portable photosynthesis system. (LI-COR 6000). NR activity *in vivo* and GS activity were assayed in the leaves according to Klepper *et al* (1971) and Mohanty and Fletcher (1980), respectively.

Table 1 shows that B.juncea exhibit high rates of net photosynthesis followed by *B.oleracea* and *B.nigra*. It is clear from the data that there is no positive correlation between photosynthetic rate and chromosome number, nor is there any gene dosage effect. Similar conclusion can be drawn from the values of stomatal conductance and the activities of NR and GS. The tetraploids of *Brassica* employed in our study were evolved from different parental species and the gene action may not be strictly additive. In allopolyploid series of tall fescue (4X,6X,8X and 10X) net photosynthesis increased significantly with ploidy so also leaf stomatal conductance and Rubisco activity. However, such effects were not discernible on any of the parameters studied by us.

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Table 1: Effect of ploidy on photosynthetic rate, leaf stomatal conductance and activities of NR and GS in the cultivated species of Brassica

Species	Chromosome No. (2X)	Net photosynthesis ($mgCO_2$ dm^{-2} s^{-1})	Stomatal conductance ($cm\ s^{-1}$)	NR activity ($\mu mol\ g^{-1}$ dry wt hr^{-1})	GS activity ($\mu mol\ mg^{-1}$ prot min^{-1}
B.nigra	16	0.81±0.05	0.65±0.02	53.3±1.50	0.13±0.004
B.oleracea	18	0.84±0.05	1.36±0.12	27.3±0.96	0.12±0.008
B.campestris	20	0.78±0.04	1.21±0.12	32.8±4.92	0.18±0.01
B.carinata	34	0.81±0.07	1.44±0.11	32.0±3.16	0.13±0.006
B.juncea	36	0.86±0.02	1.18±0.09	55.5±2.38	0.17±0.01
B.napus	38	0.66±0.03	1.63±0.07	26.0±6.98	0.14±0.006

NATURAL CROSS-POLLINATION IN INDIAN MUSTARD

Ram Bhajan, Y.S. Chauhan and K. Kumar

Indian mustard (*Brassica juncia* L. Czern & Coss.) is a naturally self-pollinated crop (11). However, reports indicate the occurrence of variable amounts of natural outcrossing as 14% (4), 7.6 - 18.1% (5) and 11.94 - 24.0% (2). The discordence in the results of various studies stems partly from genotypic differences and rests on environmental conditions that differ in time and space (9,10). The inconsistencies in the results of earlier studies stress the need for more studies on this aspect because the knowledge of natural breeding system is important for determining the breeding methods to be used and for maintaining the genetic purity of the stocks.

Different genetic markers such as flower colour (6), seed colour (1,2) and erucic acid (8) have been used in such studies. The use of flower colour has proved unsatisfactory due to colour discrimination by pollinators (3), and the use of erucic acid as a genetic marker requires extensive laboratory facilities. Yellow seed coat colour, on the other hand, makes it necessary to grow the crop to maturity. In the present study, a purple leaf mutant was used as a genetic marker. This mutant had deep anthocyanin pigment on the entire upper surface of the leaves and is digenically inherited with complementary genes (1). This marker enabled to have the scoring of outcrossed plants in the test population within 20 days of sowing.

To facilitate the natural outcrossing, 13 small concentric plots, each consisting of one plant of the cultivar Laha-101 (normal green leaves, recessive marker) in the centre and surrounded all around by purple leaf mutant, were laid out during winter season of 1986-87. The percentage of cross-pollination was determined by scoring purple pigmented plants in the progenies of open-pollinated test plants grown during 1987-88.

A perusal of Table 1 indicates that the average natural cross-pollination was 16.60%, although values for individual plants ranged from 7.38 to 29.31%.

Table 1. Percentage of cross-pollination in Indian mustard

Test progeny	No. of plants		Total plants	Percent cross-pollination
	Normal green leaf	Purple pigmented leaf		
1	96	16	112	14.29
2	305	34	339	10.03
3	119	18	137	13.13
4	352	80	432	18.52
5	199	82	281	29.18
6	128	12	140	8.57
7	226	18	244	7.38
8	164	68	232	29.31
9	222	18	240	7.50
10	180	30	210	14.29
11	140	28	168	16.67
12	393	36	429	8.39
13	315	125	440	28.41
Total/mean	2839	565	3404	16.60

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The substantial amounts of natural outcrossing as observed in the present study and corroborated by earlier findings point to the need for reorientation of current breeding procedures and the methods employed to maintain the genetic purity of stocks/varieties.

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General Statement on the Studies and Utilization of Rapeseed Heterosis in China

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According to the pretiminary data, the sown area in the last autumn for rapeseed hybrids in China is more than 0.4 million hectares, about 18 hybrids (including nine cytoplasmic male sterile hybrids, one genic male sterile hybrid, two self-incompatible hybrids and six chemical hybrids) are taking part in the regional test of provinces in China. Four hybrids (one cytoplasmic male sterile hybrid, one genic male sterile hybrid, two chemical hybrids) have passed the examination in provinces.

The main ways on the utilization of rapeseed heterosis in China may be briefly sttated as follkows:

A Spontaneous hybrids (or hybrids of self-bred slow-witted lines).

The Agricultural Science of Menyuian Institute in Qinghai province planted the two high combility varieties of Brasssica campestris row by row, e.g., one row one variety, another row other variety nearby. After ripening, the seeds were harvested mixly, and yield of this hybrid is 28.9-31.8 percent higher than that of two parents. Tian, Z.K.(1986) utilizing the property of the slow reaction of self fertilization and poor seed set of selfpollination of B. campestris, used the homozygous line (e.g. self-bred slow-witted line) as female parent to plant one by one with male parent and got the hybrid whose hybrid ration is more than 80 percent.

B. Self-incompatible hybrids

Liu H.L., Fu T.D. (1975, 1977) developed self-incompatible lines 211, 271 and their hybrids of B. napus. Li, S.Q. (1978), Zhang, Q.H. (1979, 1982), Gao Yong-tong (1980), Wang, J.X. (1981) got the self-incompatible lines and their hybrids respectively. In order to overcome the self-incompatibility, Fu, T.D. (1981) developed the self-incompatible line and its maintainer and restorer, and realized using these three lines to produce hybrid. Hu, D.Z., An, C.T.(1983) reported using ten percent salt water (Nacl) to spray flowers during flower period to overcome incompatibility in Gansu province firstly. Fu, T.D. (1984, 1985), Si Ping (1985) demonstrated that five percent of salt water is the suitable concentration to overcome incompatibility in Wuhan. Yin J.Z. (1990) suggested that eight percent is more suitable in Xingjiang province. Si Ping (1985) completed a series of research works on the time and the mechanism of overcoming incompatibility by salt water. the results show that, during the flowering period spraying salt water every three-five days is effective. This method is economical and easy, and its effect is equal to that of bud pollination by overcome incompatibility.

C. Chemical induced male sterile hybrids (e.g., Chemical hybrids)

The rapeseed hybrid research group of Hunan Agricultural college (1979), Guan C.Y. etc (1981, 1987) reported the results by using male gametocides to kill pollen grains and produce hybrid seeds. They selected out "Male Gametocide No.1", "MG4" etc chemical substances which can kill pollen grains more than 80 percent, and thought that microspore was the most sensitive

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period to male gametocides. Pan T.etc (1980) has done some studies on the chemical hybrid. The "Shuza 2", a double high chemical hybrid, has passed the regional test of Sichuan province; its mean yield was 24.15 percent higher than the check and the planting area was about five thousand hectares.

D. genic male sterile hybrids

Since Liu, G.H. et al (1973) and Yibing Institute of Sichuan province discovered genic male sterile line 87A of B. campestris in 1965 and developed genic male sterile line Yi-3A of B. napus L. in 1972 respectively, many Institutes began the research and development of genic male sterile hybrid. Li, S. L. etc (1983, 1984) reported that the yield of the double high genic male sterile hybrid, "23A×4190" of B. napus L., when taking part in the regional test of Shanghai during 1981-1983, was 34.9, 34.1 and 33.7 percent higher than the check respectively. Oil Crops Institute bred the double high hybrid, "hybrid 03", its yield was 11.4 percent higher than the check in the two running years' regional test of Hubei during 1988-1989; Du. H. P., Li, S. L. (1986) demonstrated that Shanghai genic male sterility was controlled by two pairs of dominant interaction genes, and suggested a new hypothesis of using genic male sterile "three lines" to produce hybrids. Hou, G. Z. etc (1990) discovered the genic male sterile line 117A, which was controlled by two pairs of recessive genes. The low erucic acid hybrid, "Shuza 1", of B. napus, which was developed by Pan, T. etc, passed the regional test and examination of Sichuan province in 1989, its mean yield was 20.3 percent higher than that of the double high check variety, its planting area for show and experiment was about nine thousand hectares.

E. Cytoplasmic male sterile hybrids.

Liu, G.H. et al (1973) discovered cytoplasmic male sterility line "Shantian A" of B. campestris in 1965. Since Fu T. D. et al (1981) found Polima cytoplasmic male sterility (pol CMS) of B. napus in 1972 (Discovery and studies of pol CMS in B. napus passed the scientist appraisal held by the Agricultural Ministry in January, 1990), the Crop Institute of the Agricultural Science of Hunan Academy, utilizing pol CMS, developed the pol CMS sterile line "Xiangai A", and set up the "three lines" in 1976. (Cui D. X., 1979; Liu H. L. etc, 1985, 1987). Li, D. R. (1984) found the Shan 2A CMS of B. napus in 1976, and set up the "three lines" in 1983. The mean yield of its double high hybrid, "Qinyou 2", was 27.4 percent higher than the check during the three years regional test of Shanxi during 1984-1986. This hybrid was registered in 1986, its planting area is about ten thousand hectares, and this result have passed the appraisal of the Agricultural Ministry. Till this year, the planting area has reached 0.4 million hectares. Now the single and double low "three lines" of CMS were set up in China. 8 low erucic acid CMS hybrids are taking part in the regional test in some provinces, and their planting area is about ten thousand hectares; the double low hybrids are being made and their yield are being tested.

Shi, H. Q. etc (1986) found the cytoplasmic male sterility

of B. juncea in 1973 and developed sterile line "Ou Xin-A" of this CMS. The CMS "three lines" of B. juncea have been made, the yield of the hybrid is being tested. The ogu CMS was imported from France, Canada and west Germany during 1980-1982. Many studies were done in China.

Besides the development of rapeseed hybrids, many institute conducted some basic research works.

Now, the main subjects of our works are:

(1) Closely combining heterosis breeding and quality breeding, and emphasising the development of double low CMS "three lines" and thier hybrids.

(2) The cytoplasmic male sterility is the main object of producing rapeseed hybrids and also give some considerations to other ways.

(3) Enriching restoring gene pool and look for new cytoplasmic male sterility, and also searching for strains with the highest combining ability and rapeseed hybrids with higher productivity.

(4) Stressing studies on the hybrid cultivation technique for high yield and hybrid production technique.

(5) Further studies on the inheritance of different kinds of male sterility.

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A NEW MODEL OF SEX EXPRESSION IN RAPESEED

S. K. Gupta and S. V. Bali

Generally the sex expression of an individual plant may be of the following types:

Hermaphrodite - plant with only hermaphrodite flowers
Monoecious - plant with both staminate and pistillite flowers
Androecious - plant with only staminate flowers
Gynoecious - plant with only pistillate flowers
Andromonoecious - plant with both hermaphrodite and staminate flowers
Gynomonoecious - plant with both hermaphrodite and pistilite flowers

Rapeseed(*Brassica napus*)is a self pollinated plant and the sex expression is hermaphrodite one with six stamens four with long and two with short filament. A new model of sex expression which is being reported here was found in some collections of *Brassica napus*. About 100 collections comprised of different Brassica species were grown during rabi 1989. Two plants with new sex expression were found in the HPN and HNS series of *Brassica napus*. The observed plant showed some pods at the upper part and male sterile (Pistillate) flowers at the lower part of the plant. The buds were without anthers with extended stigma The sex expression of the reported plant is gynomonoecious type.

The implication of above information for the practice of breeding of rapeseed are manifold and quite obvious. We shall mention here one explicitly: the possible development of functional female (pistillate) flowers at the lower pod of the plant may lead to the attainment of F_1 hybrids only when pollinated with foreign pollens. Although this study is a preliminary one yet it is of great interest to the Brassica breeders.

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A NEW GENE FOR MALE-STERILITY IN RAPESEED, BRASSICA NAPUS L.

R.Theis

Male sterility has been useful in many crop plants for the production of high yielding hybrid cultivars (for review see Kaul 1988). But up to now there is no effective system available for the genetical emasculation of B.napus, although various CMS systems are under investi - gation in many laboratories. Encouraging results have been obtained with the Polima CMS first described by Fu (1981). The source of its S-cytoplasm, however, is still unknown.

Recently male-sterility of a similar phenotype has been discovered in our nursery which might be revealing with regard to the origin of the Polima system. The male sterile plants occurred for the first time in 1981 in a backcrossing programme for the improvement of resynthesized rapeseed (Röbbelen, pers.comm.). The seed parent used had been a normal B.napus and the pollinator the resynthesized rapeseed (B.oleracea x B.chinensis). Several male-sterile plants were noticed by their flowers with narrow petals, short filaments and rudimental whitish anthers containing no pollen.

Histological studies showed an early premeiotic inhibition of anther ontogeny. In anthers of male-sterile plants no differentiation of sporogenous tissue became microscopically visible in 2 µm thick cross-sections of toluidine blue O stained slides. The common resin embedding method was used. The same developmental blockade was also observed in the Polima CMS as well as in the nap CMS system derived from cv. 'Isuzu natane' (Shiga et al. 1983).

For a study of inheritance male-sterile plants of this resyn origin were pollinated by the winterrape cv.'Jet Neuf'. All F_1 plants were male-fertile. These plants were selfed and also used to again pollinate resyn male-sterile plants for generating a backcross progeny. In the F_2, the segregation conformed with a 3 : 1 ratio (265 mf and 69 ms) with a Chi square of less than 0,05, while the BC_1 fitted well to an 1:1 segregation (192 mf and 181 ms). The results indicated that our resyn male-sterity is inherited as a monogenic recessive gene.

Under conditions of higher temperatures partial restoration to male-fertility was observed in the field and greenhouse as well.

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SELF-INCOMPATIBILITY TESTING IN *BRASSICA NAPUS*

Stuart Gowers.

Pollen tube screening is usually considered better than seed set for studying self-incompatibility (SI)(Zuberi *et al*, 1981). However, unusual results have sometimes been observed with some *B. napus* material (Gowers, 1979; Ayotte & Harney, 1985) and, because of this, I usually use seed set for testing my lines for SI. However, it is a week to ten days before a reasonable assessment of SI can be made from pistil size. In some cases it is necessary to have a compatibility result within a day or two, to enable the necessary pollinations to be carried out, or to allow repeat scores to be made, and pollen scores are therefore necessary.

With some such cases, I wanted immediate scores but also wanted the back up of getting seed set scores. Instead of doing seperate pollinations for each score, I only removed the stigma and 2-3 mm of style and left the rest of the pistil in place, 24 hr after pollination. I was able to stain and squash the stigma (aceto-carmine or as Lewis and Crowe, 1958) to get an immediate score on pollen germination and penetration, and seed development appeared to continue as normal so that a seed set score was obtained as well.

From a test with 30 plants, 13 were scored as compatible on pollen germination and penetration, 13 were scored as SI and 4 were scored as partially SI. Of the latter, three were SI on seed set (<1 seed per pollination) and one was partially SI (2.5 spp). From the other two groups, one plant scored as compatible appeared SI on seed set, and two plants scored as SI were only partially SI on seed set (2.5 and 5 spp). Seeds produced from pollination of these two plants were therefore able to be discarded.

Although only a small trial, mis-scoring was shown to be possible by either method. Using this technique to get seed set results as a back-up to pollen germination seemed to work well, and allowed plants to be selected on both sets of results.

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GENETICS OF SELF-INCOMPATIBILITY IN A JAPANESE VARIETY OF RADISH, RAPHANUS SATIVUS cv TOKINASHI

S. C. Verma, T. S. Sareen and Jasveer Kaur

Introduction: Recently, Lewis et al. (1988) have established a novel gametophytic - sporophytic system of self incompatibility in Raphanus sativus (var. radicula) from Poland. Their hypothesis is contrary to the general belief of an entirely sporophytic incompatibility in the family Cruciferae, controlled by multiallelic single S locus (Richards 1986). It is, therefore, necessary to examine other material of cultivated radish for a full reassessment of the incompatibility system in R. sativus. It may be recalled that Verma et al. (1986) analysed two mating matrices in diallel of families of selfing origin, one in each of Japanese cultivars, Miyashige and Shogoin. The data obtained by them were complex, but it was possible for them to accommodate these data on a one sporophytic S gene by making an important assumption that the mutual relations between a pair of alleles is affected by the background genotype. The present studies are initiated in another Japanese cultivar, named Tokinashi, and its seeds were obtained from Prof. I. Fukuda of Tokyo Women Christian University, Tokyo (Japan).

Methods: The seeds were sown in beds in the university botanic garden at Chandigarh during winter. The procedure of making controlled pollinations, the processing of pollinated stigmas for staining, and scoring of compatible and incompatible pollen on the stigma are given elsewhere (Lewis et al. 1988, Verma et al. 1989).

Observations: The corolla are usually white with purplish veins. At anthesis, the four inner stamens lie above the level of stigma, and introrse dehiscence makes self - pollination inevitable. Since anthers begin to dehisce after anthesis, emasculation of the just-about to open flower-buds is not necessary unless it is required for confirmation of suspected results. Stainability test showed pollen fertility to vary from 78.3 to 98.2 %, and this information is useful when scoring of emptied or compatible pollen grains is done. Between plants the average number of ovules per ovary varied from 3.2 to 5.8, whereas the average number of seeds per siliqua in open pollinations varied from 2.6 to 4.6. These data impress upon the unsuitability of seed-set as a parameter to assess the status of pollinations. The nature of compatibilities ought to be based on cytological examination of carefully pollinated stigmas, particularly when the incompatible pollen are rejected at the stigma papillar surface (Lewis et al. 1988, Verma et al. 1989). The

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ovule number can serve as an index to rate the compatibility levels of the pollinations (see Verma et al. 1989).

Nine plants of the parental sample were mated in a diallel, and the data on pollen - stigma interactions are summarised in Fig. 1. The entries in each square are a mean of three replicates, and they represent from top downwards: total number of pollen grains adhered firmly to the stigma papillae, the number of grains amongst them which showed germination, and the number of germinated pollen whose pollen tubes had penetrated the stigma papillae (& hence emptied). The germinated pollen which are unsuccessful in penetration of the stigma papillae constitute the incompatible grains, and are dark stained.

♀ \ ♂	1	2	3	4	5	6	7	8	9
1	41 18 0	113 40 14	69 40 •22	41 20 5	83 38 •20	79 26 8	54 28 9	94 40 •20	96 59 10
2	24 10 2	40 0 0	63 41 •22	53 39 •20	21 12 6	49 22 10	54 42 •21	47 36 •20	87 45 •27
3	70 26 5	97 72 52	55 14 0	94 61 •31	49 28 •15	34 23 16	50 15 8	29 10 0	51 35 •16
4	70 45 •27	62 22 13	65 22 2	45 5 0	59 22 8	36 2 0	37 2 0	38 1 0	140 23 0
5	70 28 0	178 62 20	112 37 14	34 7 0	66 7 0	49 14 0	22 10 0	108 30 10	95 40 10
6	114 51 •30	88 27 5	66 19 3	79 45 20	82 25 7	31 0 0	70 8 0	88 16 0	45 19 9
7	73 21 0	33 0 0	48 28 •16	32 2 0	53 16 4	32 4 0	28 5 0	60 30 4	45 26 •14
8	75 9 0	82 19 0	53 18 0	43 5 0	72 36 •19	103 31 3	56 17 3	35 13 0	22 0 0
9	105 37 10	33 9 0	82 51 •27	67 34 8	26 8 2	16 0 0	53 12 8	135 26 0	23 3 0

Fig. 1 Mating matrix in diallel

All the nine plants are fully self-incompatible, and the self pollen are rejected at the stigma surface, as is characteristic of homomorphic SSI in angiosperms. The most important and striking observation of the occurrence of both incompatible and compatible types of pollen grains from the same anther, when tested on a given stigma, require explanation. At least 17 of the crosses show a near 1:1 ratio of compatible and incompatible pollen grains (marked •). It demonstrates semi-compatibility, which is not expected in SSI.

Discussion and Conclusion: The demonstration of semi-compatibility in this sample of Japanese radish suggests that the gametophytic - sporophytic system as postulated by Lewis et al. (1988) will be common in radish.

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GENETICS OF SELF-INCOMPATIBILITY IN THE WILD RADISH, RAPHANUS RAPHANISTRUM (CRUCIFERAE) - III. INTER - SIB POLLINATIONS IN A BUD-SELFED FAMILY

S. C. Verma, T. S. Sareen and Usha Devi

Introduction: The controversy over the multigenic control of self-incompatibility in members of Cruciferae continues, despite the general belief that SI in this family is mediated by a single sporophytic multi-allelic S locus (Nasrallah & Nasrallah 1986). Richards (1986) stated explicitly that in multi-allelic sporophytic systems, "all the pollen grains from a single male will show the same behaviour on a given female parent (i.e. semi-compatibility does not occur)". That semi-compatibility occurred in several controlled crosses within SI crucifer species was pointed out by Verma (1987a,b). Elaborate studies on Raphanus sativus and Brassica campestris have established a complex system of gametophytic - sporophytic control in these taxa (Lewis, Verma & Zuberi 1988, Zuberi & Lewis 1988). More recently, studies on the genetics of SI in wild radish, Raphanus raphanistrum, have revealed a complex SI system that did include a gametophytically operating factor within the overall sporophytic control (Verma et al. 1989a,b). We present here data on inter-sib compatibilities in diallel of another family of bud-selfing origin in wild radish to further impress upon the involvement of a gametophytic gene G in the SI system of radish.

Material and Methods: The original seed material came from a wild population at Davis (California, USA) through the courtesy of Dr M. Stanton. The methods adopted are the same as given by Verma et al. (1989a,b). Compatibilities are judged from cytological examination of restrictedly pollinated stigmas (Lewis, Verma & Zuberi 1988) and the analysis of mating matrix may be done as proposed by Verma et al. (1989a,b).

Results: Out of 17 sib-seeds only 8 plants survived and matured to bear flowers. Stainability test revealed 92.6 to 97.8 % pollen fertility. The number of ovules per ovary varied from 4 to 7, and in open pollination the number of seeds produced per siliqua was 2 to 5. Obviously, little reliance can be placed on seed set data for a full comprehension of the SI system in this species.

The fate of pollen grains upon pollen-stigma interaction in sib-diallel pollinations is summarised in Fig.1 The entries in each square are the means of 4 replicates and represent from top downwards: total number of pollen adhered firmly to the stigma papillae, the number amongst these which germinated, and the number of germinated

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whose pollen tubes had successfully penetrated the stigma papillae (emptied grains, unstained). The germinated ones whose pollen tubes were denied penetration comprise the incompatible pollen (dark stained). Three of the observations are relatively more important: i) all of the 8 plants are fully self-incompatible with zero penetration, ii) the sib-diallel matrix cannot be satisfactorily fitted into the three genotypic groups expected on a single sporophytic S locus model (Fig. 1) in spite of the low number of sibs in the selfed family, and iii) some of the crosses manifest noticeable semi-compatibility (see squares marked • in Fig. 1). From amongst the germinated pollen grains, about 50 % were successful in penetrating the stigma papillae, and the remaining were frustrated in their effort (see Verma et al. 1989a,b for more details on this aspect).

Discussion: The most striking observation of semi-compatibility between sibs is contrary to the expectations based on an exclsively SSI system (cf. Richards 1986). The occurrence of semi - compatibility within the sib-diallel of a selfed family is in itself a strong evidence in favour of the involvement of a gametophytic factor (the G gene) complexing the overall sporophytic system of incompatibility in the wild radish, like the one reported for Raphanus sativus by Lewis, Verma and Zuberi (1988).

♀ \ ♂	1	8	2	3	5	6	4	7
1	8 3 0	121 40 0	94 54 •32	189 153 •68	204 90 23	73 24 11	104 50 35	203 118 •60
8	104 50 0	28 2 0	124 72 •30	115 95 •34	154 89 •30	41 2 0	54 2 0	178 82 •35
2	109 5 0	128 72 59	14 0 0	53 12 0	107 27 0	124 0 8	123 94 •59	84 71 53
3	40 8 0	52 21 7	32 0 0	13 0 0	12 0 0	23 0 0	142 103 9	84 31 7
5	148 80 0	88 41 15	78 0 0	46 0 0	20 0 0	63 0 0	139 52 15	63 11 7
6	120 70 •48	76 5 0	59 8 0	42 2 0	45 5 0	45 0 0	112 73 54	129 97 84
4	59 3 0	78 0 0	113 64 •23	103 66 •28	108 18 0	82 44 10	15 0 0	74 22 22
7	108 90 24	142 94 •55	108 71 64	109 63 •35	59 24 13	62 9 0	64 5 0	6 0 0

Fig.1 Sib-diallel mating matrix

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THE ESTIMATION OF SELF-INCOMPATIBILITY IN WINTER OILSEED-RAPE (Brassica napus L.)

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Introduction

Self-incompatibility (s.i.) is a well suitable tool for the production of F1-hybrid seed in the Brassica family because of the prevention of selfing in a S-homozygous line and the unlimited crossing between two lines homozygous for different S-alleles.
For the S-allele is not related with any visible trait the selection of s.i. individuals and the following selection of the wanted S-homozygous genotypes is very difficult and needs very extensive and labourious test-pollinations (selfing and crossing). The most necessary prerequisite is therefore a screening method for the sure and reproducible distinction between a compatible and an incompatible pollination. In this paper we will report on our method for investigation in s.i.

Methods

There are two different possibilities to study the incompatibility reaction:
1.microscopical investigation of the pollen tube growth and counting the number of pollen tubes per pistil (pt/p)
2. estimation of the seed set by counting the number of seeds per flower (s/fl) resulting from a certain pollination.
In order to get sure and reproducilble informations on incompatibility we study both traits.

1. Microscopy

The staining of pollen tubes with fluorescence dyes as for example anilin blue provides a good means for making them visible in the UV - light. They are well to distinguish from other kinds of tissue in the pistil. We use a so called "combined solution" containing anilin blue, sodium hydroxide, tertiary potassium phosphate and the commercial rinsing medium "Fit" as wetting agent (NAETHER,1971). This solution serves as a fixative for the pollinated pistil. But when heating up to 100 Celsius degrees for some minutes the pistil will be stained, macerated, and bleached in one step. After carefully squeezing this pistil in a drop of glycerine it is ready for observation by a fluorescence microscope. It is also possible to store the pollinated pistils in the "combined solution" without cooking up to 12 months and perhaps more, but in darkness.

2. Seed set

Therefore the pollinations are made by hand, preventing uncontrolled pollinations by bagging the inflorescence. Seed set may be counted already in the green siliqua when the seeds are well developed.

Screening procedure

As already mentioned we study the pollen tube growth as well as the seed set. Pollen tube growth provides an immediately information on incompatibility and seed set is a means for its confirmation. All pollinations are conducted on the plant, whether in greenhouse or in field.

The inflorescences are bagged in the bud stage removing single open flowers and thus preventing any contamination with foreign pollen. When crosses are intended the buds are emasculated.

Some two days later when the buds have opened they are pollinated (self or cross) by hand. They are bagged again. In common we pollinate 5 to 10 flowers per inflorescence.Twenty four hours later the pistils are removed taking care that the seed vessels remain intact on the plant.

The pistils of one inflorescence are put into a small test tube, and after adding the "combined solution" it stays for 11 minutes in a cooking water bath. Then it may be prepared and observed as described before. To be classified as incompatible the average from all pollinated pistils of the inflorescence must not exeed 5,0 pt/p .

The remained seed vessels grow to siliquas. When the seeds in the siliquas are well to see, the green siliquas can be opened to count the seeds. The average from the pollinated flowers of the inflorescence must not exeed 1,0 seeds per flower to be classified as incompatible.

By this procedure it is possible to compare the pollen tube growth and the seed set in a direct manner for the values of both traits originate from the same inflorescence. With some additional expense it is possible to get the values of both traits from the same single flower.

The results of the both investigations do not always correspond. Particularely in the first generations the incompatibility shows often an instable behaviour and then it is possible to observe even negative correlation coefficients. But the more stable the incompatibility is the more both methods correspond. In 1988 we found in advanced generations (S-homozygous lines and other frequently selected incompatibles) that the correlation between pollen tube growth and seed set was r= 0,80 in the greenhouse and r=0,85 in the field, respectively.

For that reason we classify a test pollination only then as incompatible when the number of pollen tubes per pistil is below 5.0 as well as the number of seeds per pollinated flower is below 1.0 .

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RELATIONSHIP BETWEEN THE ORIGIN AND EVOLUTION OF RAPESEED AND THE DEVELOPMENT OF CYTOPLASMIC MALE STERILE "THREE LINES"

Fu Tingdong and Yang Guangsheng

Analysing the data collected about the cytoplasmic male sterile three lines in B.napus, B.campestris and B.juncea in the world, we can conclude: (1).It's easy to find CMS and its restorers in the variety populations with lower degree of evolution and primary species in the original center and nearby. (2).It's easy to find maintainers in the area far from the original center and the higher evolved variety populations. These view are based on the follow facts.

I: The basic species of B.napus, the allopolyploid (n=19, AACC), are B.campestris (n=10, AA) and B.oleracea (n=9, CC) (U, 1935). B.napus originated in the regions along the shore of Mediterranean Sea in Europe, was spread to Asia and other regions in the world. What is the relationship between the origin and the cytoplasmic male sterility in B.napus:

First: The valubale male sterile cytoplasm, polCMS, was found in the variety "polima" in 1972, which was introduced to China from Poland in 1960s, Poland closes to the original center in Europe(Fu, T.D.1981,Liü,H.L. Fu, T.D.1987). The restoring fertility genes of polCMS exist among the European varieties of B.napus (Agricultural Academy of Hunan province, 1979,Liu, H.L.,Fu, T.D.1987,Fang, G,1987) and the ancestral varieties of B.campestris (Chuei,D.X.1976, Yang,G.S.1987). Varieties (or strains) of B.napus were used as the pollinators to hybridize Xianai A,the polCMS sterile line, the percentage of Chinese and Japanese varieties, which can restore fertility of Xianai A is 35.0-50.0%(Chuei,D.X. 1979). Fu,T.D. and Yang,X.L.(1985) used 28 European varieties and 31 Asian varieties as the pollinators to testcross with polCMS sterile lines, five European varieties and restore their fertility, eg,17.9percent, only one Asian variety has retoring fertile ability,e.g, 3.2 %. The maintainers can easily be found in no-European varieties and Asian varieties, these varieties belong to the variety population far from the original center.

Second: "Qinyou No.2" is a hybrid variety (double high) of cytoplasmic male sterility which is used firstly to production greatly in China. The male sterile cytoplasm of "Shan 2A", which is the female parent of "Qinyou No.2",and its restoring fertility genes are come from European variety of B.napus, and also the maintainer from Asian variety of B. napus.

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Third: Fu,S.Z.(1986) used European variety Mutsu as female parent, whose cytoplasm is male sterile,e.g, napCMS, and backcross with Isuzu,and developed CMS sterile line "MI". Although the cytoplasm of most European and Asian varieties are napCMS (Rousselle, 1978, Shiga,T.1973), e.g, TCMS or SCMS. In total 131 Japanese varieties, 23 can restore the fertility of napCMS, 79 partialy, and in total 68 European varieties, 62 can restore the fertility, 4 partialy restore, 2 have no restoring genes. These results suggest it is easy to find restorers of napCMS of B.napus in European and maintainers in Asian varieties.

II: It is known that B.juncea (n=18, BBCC)came from the spontaneous cross between two basic species B.campestris (n=10,AA) and B.nigra (n=8,BB) (U,1935). The original center of B.juncea are in Middle Asia, India, the west of China(Vavilov,N.I.1926, Hemingway,T.S.1976, Liu,H.L.1985). Anand etc.(1978)and Shi,H.Q. (1986)respectively find the cytoplasmic male sterility in Indian Mushtard (B.juncea) and in Chinese variety of B.juncea. The two CMS of B.juncea are found in the original centers of B.juncea. The MS-4 cytoplasmic male sterile line of B.juncea is found in the F_2 of the cross between RLM-198 and EJ-33, the both are varieties ofB.juncea. The male sterile cytoplasm of RLM-198 originated from India, the maintainer EJ-33 came from Europe (Banga,S.S.1985). The restoring genes of Indian Mustard CMS exist among the varieties of the two basic species, e.g, B.campestris and B.nigra (Anand,1987); and the restoring genes of Chinese CMS of B.juncea exist among Chinese varieties of B.juncea; the maintainers among the European varieties of B.juncea (Shi, H.Q.1986). These facts also demonstrate the view: It's easy to find male sterile cytoplasms and their restorers among the varieties in the original center and find maintainers among the varieties far from the original center.

III: B.campestris (n=10,AA) originated in the north-west of China (Hu, X.S.1955). The Chinese southern varieties of B.campestris is evolued from the B.campestris of the north-west of China in the process of cultivation (Liu,H.L.1985). It's easy to find the male sterile plants in the progenies of the crosses, in which the north-west varieties of B.campestris as the female parents and the southern varieties of B.campestris as the male parents (Fu,S.Z. 1980). And also when the varieties of B.campestris are used as the pollinators to testcross with polCMS sterile line of B.napus, the restorer can be found in the north-west varieties of B.campestris and most southern varieties are maintainers (Chuei,D.X.1979).

Another male sterile cytoplasm studied internationally is the oguCMS discovered by ogura (1968), no restorer is found among the Asian varieties and a few restorers found in European varieties of Raphanus sativus (Bennet,1975). This agrees with the view: It's easy to find the restorers in the original center and the nearby regions, because Raphanus sativus originated in Europe.

DISCUSSIONS ON EXPLOITATION AND BREEDING OF CYTOPLASMIC MALE STERILE LINES OF CRUCIFEROUS VEGETABLE PLANTS

Wei Yu-tang Wei Bao-qin

Abstract

In crucifer family, in genus Raphanus stable cytoplasmic male sterile (CMS) lines have been developed and are used in seed production in large area, while genus Brassica, in addition to CMS lines, male sterile (MS) lines have also been bred. the percentage of MS plants was about 50%. these could be used as dual-purpose male sterile lines for producing F1 hybrids. since plants of the CMS type are mostly sensitive to the environment, when used for seed production, adaptability to the region and the season for seed harvesting should be taken into account.

Because there exists to a certain extent a correlationship between male sterility and late self-fertilization, when the two are combined developing male sterile late self-fertilizing lines for seed production, the percentage of the hybrids could be as high as 100%.

currently, in Brassica plants, it is still impossible to substitute male sterile lines for self-incompatible lines in seed production. therefore, in the future, more attention should be given to the study of the hereditary mechanism of male sterility and the rules governing genetic variation, and while exploring the sources of male sterile cytoplasm in genus Brassica, efforts should be made to create new sources of cytoplasm through remote crossing, in order to develop superior CMS lines to replace the self-incompatibla lines.

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STUDY ON LEAF YELLOWING OF RADISH-CMS NON-HEADING CHINESE CABBAGE (B.CAMPESTRIS SSP.CHINENSIS L.)

Ren Chengwei and Cao Shouchun

In order to improve the abnormality of leaf yellowing of Radish-cytoplasm male strile non-heading Chinese cabbage ,2 Radish-CMS non-heading Chinese cabbage lines,as maternal parents, were mated with 99 non-heading Chinese cabbage cultivars , as paternal parents, in spring of 1988. 198 hybrids produced were cultivated in a randomized complete block experiment with 2 replications in autumn-winter of 1988.

Through regular observations on index of leaf yellowing (ILY) in the whole experiment, it was found that the value of ILY of a hybrid changed along with the growth, like (S) CURVE with 2 peaks that one was in cotyledon period and the other was in cold winter. The ranges of the changes in different hybrids were depending on their nuclear genotypes and atmospheric temperature.

The analysis of variance for ILY of the experiment on December 10, 1988, just before harvest, showed that the effects of the hybrids, the paternal parents and the maternal parents were all highly significant. 26 hybrids with slight yellowing were screened out although non-yellowing ones were not found. 7 paternal cultivars with obtuse sensitivity to Radish-CMS in leaf yellowing were selected for further evaluations and combining ability test, of which 6 cultivars were with green leafstalks.

With the hypothesis that the maternal and the paternal materials used in the experiment were selected at random, NC2 quantitative genetic design method was applied to analyze ILY The results showed that under the Radish-CMS genetic backgrounds the additive variances were 108.34 for paternal materials and 91.34 for maternal materials respectively and the dorminate variance was 29.28.

From the results above three possible ways were suggested for further improving the leaf yellowing abnormality. These were to change the nuclear genotypes of the sterile materials lines, to select corresponding paternal cultivars with obtuse sensitivity to Radish-CMS in leaf yellowing, and to cultivate this kind of hybrids away from low-temperature season.

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PRELIMINARY STUDY ON RESTORATION OF FERTILITY AND CYTOPLASMIC EFFECTS OF CMS ON SEED NUMBER AND SEED YIELD IN INDIAN MUSTARD(*BRASSICA JUNCEA*)

.N.S.VARMA, S.C.GULATI and RAJNI RAMAN

Though the cytoplasmic male sterility was first reported by Rawat and Anand in 1979 the pollen fertility restoration through genes incorporated from *B.campestris* and *B.nigra* appears to be incomplete and inconsistant. These cms plants have petaloid or rudimentary anthers that lack pollen grains. The sterile cytoplasm also affects the floral parts and the development of siliquae. In most of the cms plants, there is reduction in flower size the pods are deformed, thickened and twisted with a fewer seeds(3-5 seeds) per pod. The seed size and number in cms plants also show a drastic reduction under open pollination.

The cms source is being maintained at Division of Genetics, I.A.R.I., by recurrent back-crossing to five elite backgrounds viz., Pusa Bold, Pusa Barani, Varuna, RLM-198 and Prakash. Inspite of 6-8 generations of back-crossings with mf parents these cms lines tend to be late in flowering and maturity, poor in performance with reduced flower size and deformed and twisted siliquae. During 1988-89, cms lines with three genetic backgrounds(Pusa Bold, Varuna and Pusa Barani) were crossed to a number of germplasm entries, agronomically superior cultivars and with some synthesized *B. juncea* lines. The *B.juncea* lines were earlier synthesized by crossing different subspecies of *B.campestris* with *B.nigra* at Division of Genetics(Raut and Shyam Prakash,1985).

The F_1 hybrids with sterile cytoplasm were studied for the restoration of fertility and seed yield, in a replicated trial in 1989-90. It is interesting to note that at flowering stage, fertility restoration could be ascertained merely by looking at the floral parts, particularly the size of corolla. In the fertile hybrids, the size of the flowers was normal, whereas, in the sterile hybrids flowers were smaller in size. The pollen fertility in hybrids having normal sized flowers was later found to be more than 85 %, in most of the cases. At the podding stage also, the pod development in the fertile hybrids was normal, without any deformity, with larger well developed seeds as compared to sterile hybrids. Data pertaining to seed yield and seeds per pod are presented in the table.

Among a number of male parents used as pollinators for making the F_1 hybrids with cms lines, five lines could restore the pollen fertility in the hybrids. Of these two lines were from synthesized *B.juncea* and remaining were from germplasm collection. It was observed that the degree of restoration was different for different mf lines. The synthesized *B.juncea* lines 1563 and 1564 could restore the fertility in all the three cms lines. Whereas, GP 174 and GP 247 could only partially restore the fertility in Pusa Barani(ms)

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and Varuna(ms) respectively. GP 4, though, could restore fertility in Pusa Bold(ms) completely, had only partial restoration in other two cms lines. Similar differential restoration by mf parent in different genetical backgrounds of cms is also observed by Anand(personal communication) . It seems that restoration of fertility in sterile cytoplasm is not only dependent upon the gene/s in the restorer but also upon the genotypes of the cms. Data on seed yield also indicates some promise of heterosis as some of the fertile hybrids either outyielded the checks or yielded at par with the checks. Studies are in progress to confirm the results and to workout the genetics of restoration.

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table 1. Mean seed/pod (SP) and seed yield(g)/ plant (SYP) in some hybrids, restorers and checks.

Restorers	Pusa Bold(ms)		Varuna(ms)		PusaBarani(ms)		Restorer Mean	
	SP	SYP	SP	SYP	SP	SYP	SP	SYP
Germplasm lines:								
1. GP 4	12.33	15.05	5.54	11.12	7.47	9.56	10.86	9.41
2. GP 174	11.61	13.47	7.07	8.32	7.60	4.07	11.27	6.89
3. GP 247	13.78	11.96	6.82	6.13	7.70	6.78	12.20	8.19
Synthesized lines:								
4. 1563	12.00	19.92	12.07	17.33	11.87	18.43	11.13	7.32
5. 1564	-	21.14	-	27.79	-	7.76	11.33	9.53
Check mean*	13.07	13.47	10.67	15.02	10.76	15.75		

* Mean of mf checks-Pusa Bold,Varuna and Pusa Barani.

INDUCED POLYGENIC VARIATION IN MUSTARD (Brassica juncea L. Czern and Coss)

S.V.S. Mahla, B.R. Mor and J.S. Yadava

The present investigation was envisaged with a view to induce variability for several agronomic traits in two mustard genotypes, RCU101 from India and Domo-4 from Canada, and their hybrid by using ethylmethane sulphonate (EMS) and gamma rays. Dry seeds of the parents and F_1 were treated with 0.5,0.75 and 1 per cent EMS concentrations and 80,100 and 120 kR doses of gamma rays. The seed from M_1 generation was taken to raise M_2 generation . No M_1 plants produced seeds from the 0.75 and 1 per cent EMS treatment in parents and 1 per cent EMS treatment in hybrid.

The M_2 generation along with the untreated genotypes and F_2 population was field evaluated in randomized block design with three replications. The M_2 plants were scored for chlorophyll mutations at seedling stage and other mutants at maturity. Different agronomic traits like primary and secondary branches, number of silique on main stem, siliqua length, seeds per siliqua, seed yield, 1000-seed weight, and oil content were studied in M_2 generation.

The spectrum of chlorophyll mutations in the M_2 generation spread to four type: albina, chlorina, striata and albo-viridis. EMS induced higher frequency chlorophyll mutations than gamma rays. The data clearly indicated the specificity of different mutagens to produce different types of chlorophyll mutations. The differential response of physical and chemical mutagens with regard to production of chlorophyll mutants has also been reported by Ehrenberg et al (1961).

Frequent shifts in mean values of mutagen treated populations (M_2) were observed in comparison to controls. There was clear evidence for enhanced mean values for fruiting zone, primary and secondary branches, seed yield, and 1000 seed weight in most of the mutagen treated populations of parents and hybrid. The increase in mean values in irradiated and or chemical mutagen treatment populations for primary and secondary branches, yield and oil content has been reported in Brassica species (Kumar and Yadava, 1988). However, the mutagenesis in this study tended to decrease the mean values for siliqua length, seeds per siliqua and oil content. The enhancement of mean values for these characters was more conspicuous in EMS treated populations. The direction of shift in mean values was found to vary with genotype, mutagen, concentration/does and character.

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The change in mean values in mutagen treated populations was usually followed by changes in magnitude of variability. Substantial variability has been induced by EMS and gamma rays for all traits in the parents and hybrid, but the extent of variation depended on genotype, concentration or dose of mutagen and the character. The magnitude of variability induced by EMS was greater than that of gamma rays. Among the three doses of gamma rays, generally lower doses (80 and 100 kR) were more effective than the higher dose (120 kR) for induction of variability.

The mutagenesis of the F_1 hybrid resulted in greater variability in the M_2 generation than the F_2 population. But the induced variability in the mutagen treated populations of the parents was greater than in the mutagen treated populations of hybird. Similar findings were also reported by Emery and Wynne (1976). Obviously, mutagenesis of F_1 hybrid has no greater advantage than the mutagenesis of parents. The estimates of genetic variance, heritability and genetic advance revealed that selection in mutagen treated popuations would bring out considerable improvement for most of the traits.

The study revealed that high yielding mutants appeared in the M_2 generation of parents and hybrid. These mutants will be assessed for adaptability and yield responses in subsequent generation in multilocation trails.

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MUTAGENIC EFFECTIVENESS AND EFFICIENCY IN MUSTARD (*Brassica juncea* L. Czern and Coss)

S.V.S. Mahla, B.R. Mor and J.S. Yadava

The present investigation was carried out to find effectiveness and efficiency of ethylmethane sulphonate and gamma rays in mustard. Dry seeds of two varieties RCU 101 and Domo-4 and their F_1 hybrid were treated with 0.5, 0.75 and 1 per cent of EMS concentration for 12 hours and 80, 100, and 120 kR of gamma rays. The plants of 0.5 and 0.75 per cent EMS treatment in parents and 1 per cent EMS treatment did not reach maturity. The seeds from M_1 progeny were taken to raise M_2 population. Mutagenic effectiveness and efficiency of mutagens were computorized according to method suggested by Konzak *et al*. (1965).

The study clearly indicated that EMS was more effective and efficient than gamma rays. The earlier studies also indicated that alkylating agents were efficient and effective than gamma rays (Augustine *et al* 1975, Mohan, 1984). The comparison of EMS concentrations revealed that EMS 0.5 per cent was more effective and efficient than the higher concentration (0.75%). In the EMS 0.75 and 1 per cent treatments of parents and EMS 1 per cent treatment of hybrid, the survived M_1 plants were completely sterile, suggesting that lower concentrations of chemical mutagenic treatments for longer period always result in a higher frequency of mutation and hence the efficiency was higher at lower concentrations. The undesirable effects of sterility and lethality increase with

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increase of concentration of mutagen. Several studies suggested that good results of chemical mutagens depend on careful attention to concentration, duration of treatment, temperature, and pH of mutagenic solution and water content in the seeds (Sigurbjornsson, 1983).

Among the doses of gamma rays 100 kR was more effective and efficient than 80 and 120 kR doses. The effectiveness of mutagen in this study appeared to genotype specific. The EMS was more potent in RCU 101 than in Domo-4 and hybrid. On the other hand gamma rays were more effective in Domo-4 than RCU 101 and hybrid.

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EXPERIMENTS WITH EXOGENOUS DNA UPTAKE IN *BRASSICA JUNCEA* (L) COSS

Shashi K. Banga, S.S. Banga and K.S. Labana

Possibility of genetic transformation through the application of exogenous DNA to the germinating seeds or pollen grains has been a subject of considerable debate. Preliminary experiments to evaluate this technique were conducted in Indian mustard. Strain WF-2 recessive for white flower colour and green leaves was used as the recipient whereas strain PJ-1 dominant for yellow flower colour and purple leaves was used as the donor parent. DNA was extracted from very young leaves of the field grown donor parent using a slightly modified protocol of Marmur (1961). For seed treatment the DNA precipitate was dissolved on 0.01% NaCl while for the pollen treatment, the DNA was suspended in .3 M sucrose solution. The treatment was effected immediately after DNA extraction.

Seed Treatment:

After presoaking in water for 8 hours, the germinating seeds were immersed in DNA solution for 48 hours and then transferred to field. Screening of more than 2000 seedlings/plants did not reveal any transformant. One hundred random plants were selfed to raise F_2 generation. F_2 generation also did not show any evidence for transformation.

Pollen Treatment:

Fresh pollen from recipient plants was immersed in DNA solution of the donor parent. The resultant paste was applied to the stigma of the recipient parent. More than 250 buds of the recipient parent were pollinated this way. Seed setting and seed fertility was very low as only 96 seeds were obtained out of which 16 plants could be raised. All these plants had normal green leaves of the recipient. Out of these sixteen plants, two were yellow flowering instead of expected white flowering ones. Interestingly, both of these plants had green leaves. A distinct possibility could be that in spite of care taken, these yellow flowering ones resulted from pollination with stray pollen grain. If this was so then the F_2 progeny resulting from these plants should show normal Mendelion segregation of digenic recessive epistasis i.e. 12 yellow : 3 pale yellow : 1 white. However, screening of more than 1000 plants in each of the two families raised from selfing of the two suspected transformants did not show any fit with expected ratios. More than 97 percent plants had either completely yellow flowers or various hues of yellow flower colour and about 2 percent plants showed variegation for flower colour i.e. yellow streaks on white flower. Significance of this requires some explanation. Our previous results (Banga *et al.*, 1989) have shown that white flower colour in WF-2 results from the loss of gene activity due to integration of a transposable element at Y locus. This element responds to the signals of a regulator when located in cis position. However, normally the regulator is closely linked to the dominant allele for yellow flower colour and both of them are inherited together. Genomal stress caused by irradiation or

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chromosome breakage during wide hybridization releases this regulator and when this is brought in cis position with the receptor, excision followed by transposition restores gene activity leading to yellow streaks in white background. DNA isolation could have resulted in the similar genomal stress in the donor parent. The variegated plants bred true on selfing with expected rate of germinal revertants (.01%).

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THE VERNALIZATION REQUIREMENT OF SYNTHESIZED B. NAPUS AND THEIR ANCESTRAL PARENT LINES AND THE INHERITANCE OF THIS TRAIT IN THE INTERSPECIFIC CROSS B. OLERACEA X B. CAMPESTRIS

Dr M.R. Ahmadi

The vernalization requirements of 70 synthesized amphidiploid rapeseed lines and their ancestral parents were evaluated in the greenhouse. The mode of inheritance of this trait in the interspecific cross of B. oleracea x B. campestris was also determined. Synthesized winter rapeseed genotypes with high vernalization requirements and those with spring-form genotypes were examined.

A two year experiment was carried on in three locations. The data gathered from this experiment about cold resistance were compared with vernalization requirements.

Many of the rapeseed lines with high vernalization requirement were completely susceptible to cold.

The varieties of B. campestris oleifera in cross with B. oleracea had more bolting inducing effect on offspring than did B. campestris pekinensis varieties.

The varieties of B. oleracea ssp. alboglabra bolted without vernalization. B. alboglabra, B. botrytis and B. gengylodes mothers, had also a bolting inducing effecton offspring in this interspecific cross.

(Author's abstract of a longer paper submitted to Cruciferae Newsletter - Ed.)

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ON THE INHERITANCE OF SEED COAT COLOUR IN WINTER OILSEED RAPE (Brassica napus L.)

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Introduction

To improve the quality of rapeseed (Brassica napus L.) the breeder is interested in reducing the fibre content of the seed which causes an increased energency concentration of the meal for animal feeding. The main proportion of the fibre is localized in the seed coat and thus a thinner seed coat causes a lower fibre content of the seed. There exists a narrow relationship between colour and proportion of seed coat. Yellow seeded types have a thinner coat than normal black ones. In contrast to other Brassica species like B. campestris or B. juncea in B. napus stable and uniform yellow seeded types are not available. With recurrent selection on seed coat colour we didn't have the expected success. Therefore we begun to study the inheritance of that trait in B. napus. In this paper we report on our first results.

Material and method

Five genotypes with different seed coat colour were crossed by hand and their F1- and F2-progenies were scored for seed coat colour. All seed samples were classified into one of the following four classes: (1) black seeds, (2) black seeds with some dark brown or pale spotted seeds, (3) brown seeds and (4) yellow-brown seeds. The seed colour of the parents was black (line A), brown (line B) and yellow-brown (lines C,D and E),respectively. Following crosses were made: black x brown and brown x black, black x yellow-brown and yellow-brown x black.

Results

The main results of the investigation are:

1.) The classification of the seed coat colour is difficult because the samples from one line and also from one plant had no uniform colour. The distinction between two classes was sometimes difficult, too. The character is obviously influenced by environment.

2.) The seed coat colour is fully determined by the mother plant but not by the pollinator. Between reciprocal crosses are no differences.

3.) The F1-progenies show that black is completely dominant over brown and incompletely dominant over yellow-brown.

4.) The genetic difference between the black seeded parent (1) and the yellow-brown seeded ones (4) is determined by two or three factors (see table 1).Yellow-brown seed coat represents the fully recessive genotype. If at least one locus is homozygous dominant the seeds are black.Unequal gene action may be supposed because there are only some brown segregants.

5.) The genetic difference between the black (1) and the brown parent (3) is determined by 4 factors (see table 1). Black colour is expressed if in the homozygous or the heterozygous type at least two genes show a dominant allele. The fully recessive genotype has brown seeds.

6.) Further experiments are necessary to investigate the relationship between the yellow-brown and the brown parents. It may be supposed that the brown parent and the brown segregants in the F2-progeny of the the cross yellow-brown x black have not the same genotype

7.) In this investigations we didn't find yellow seeded types, too.

Table 1: Segregation of seed coat colour in the F2-progenies of crosses between parents with different seed coat colour

cross		nb. of plants with seed coat colour (1)	(2)	(3)	(4)	assumed nb. of genes	Chi-square
A x B	obs.	569	24	3			
	exp.	566	28	2		4	1,1
B x A	obs.	570	23	1			
	exp.	564	28	2		4	1,5
A x C	obs.	344	220	20	15		
	exp.	346	243		9	3	4,0
D x A	obs.	352	224	13	8		
	exp.	345	243		9	3	0,4
A x E	obs.	307	224	38	26		
	exp.	344	242		9	3	37,3 *
	exp.	335	223		37	2	14,3 *

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explanations: obs. ; exp. :observed and expected number of plants, respectively
Chi-square value for alpha 5% and 2 d.f.: 5.99
for colour classification see text

GENETICS OF SEED TRAITS IN INDIAN MUSTARD (*Brassica juncea* L. Czern & Coss)

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Traitslike germination percent, seedling dry weight and electrical conductance of seed leachate are important components for determining the planting value of seed. Information regarding the genetic system controlling seed traits in Indian mustard is meagre for above mentioned seed traits. Genetics of seed traits in mustard is reported here under.

MATERIALS AND METHODS

Fifteen lines were crossed with DIR-313, TM-22(referred to as testers L1 and L2) and their F1 hybrid(designated as L3) during *rabi* 1989-90. Seeds of these(30 Single and 15 three way) crosses along with 15 lines and 3 testers were evaluated for germination percent, seedling dry weight(mg/seedling) and electrical conductance of seed leachate(μmhos/cm/g) following ISTA (1976) rules. Transformed data(arcsin) were used for statistical/genetical analysis(Ketata *et al.* 1976).

RESULT AND DISCUSSION

Both additive and dominance components were important with predominance of additive effects for all the traits(Table 1). Based on the estimates of average degree of dominance, incomplete dominance was noticed for all the traits studied. Which is in agreement with the findings of Yadav *et al.*(1988). Indication of dominance of decreasing alleles for electrical conductance of seed leachate and ambidirectional dominance was recorded for germination percent and seedling dry weight.

Partitioning of total epistasis into additive X additive (i) type and additive X dominance plus dominance X dominance(j+l) type revealed the importance of both 'i' and 'j+l'types epistasis for all the traits(Table 2). However 'i' type epistasis was much larger in magnitude for germination percent and seedling dry weight,where as,'j+l' type epistasis was higher in magnitude for electrical conductance of seed leachate. Sagwal *et al.* (1990) also reported the importance of higher order epistatic effect ('i' type epistasis) in the inheritance of seed vigour traits.

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Table 1. Estimates of additive (D), dominance (H), degree of dominance (H/D) 1/2 and correlation coefficients (r) between sums and differences

Components	Germination (%)	Seedling dry weight (mg)	Electrical conductance of seed leachate (μ mhos/cm/g)
D	200.34**	36.09**	182.38**
H	67.57**	5.38**	65.65**
(H/D) 1/2	0.58	0.39	0.60
r	0.36	0.25	0.50*

Table 2. Analysis of variance to detect the presence of epistasis.

Source	a.f.	Germination (%)	Seedling dry weight (mg)	Electrical conductance of seed leachate (μ mhos/cm/g)
'i' type epistasis	1	649.12**	42.51**	303.99**
'j+l'type epistasis	14	334.81**	3.85**	475.29**
Total	15	355.77**	6.43**	463.87**
'i' type epistasis X blocks	2	42.92	0.001	3.07
'j+l'type epistasis X blocks	28	66.39	0.030	11.87
Total epistasis X blocks	30	64.82	0.02	11.29

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GENETICS OF QUALITATIVE TRAITS IN INDIAN MUSTARD (*Brassica juncea*)

B.K. Brar, S.S. Dhillon, Kaur Singh and K.S. Labana

Inheritance of qualitative traits like flower colour and open verses appressed pods is of paramount importance as they could be used as markers for varietal identification. The knowledge of nature of gene action would help in the choice of appropriate method for the transfer of such characters. Keeping this in view an attempt was made to study the nature of inheritance of these marker traits.

Five yellow flowered varieties i.e. RH-30, pusa bold, Varuna, DIR-202 and PR-18 were crossed with white flowered variety Rai B-85. F_1 plants had yellow flowers indicating the recessiveness of white flower colour. The proportions of yellow, intermediate and white flowered plants in F_2 (Table 1) fitted well to 12:3:1 ratio (P0.05 and 0.01) suggesting flower colour is governed by two epistatic genes Symbols Y_1 and Y_2 designate two genes where Y_1 is epistatic over Y_2. Singh *et al* (1964), Bhuiyan (1986) and Rawat and Anand (1986) also reported similarnature of gene action whereas Safiul and Alam (1986) have reported duplicate gene action (15:1) for flower colour.

Table 1 :

Sr. No.	Pedigree	Segration in F_2		
		Yellow	Inter-mediate	White
1.	Varunaxx white flower (W.F.)	371	89	40
2.	PR-18 x W.F.	302	80	27
3.	DIR-202 x W.F.	398	98	26
4.	RH-30 x W.F.	255	68	24
5.	Pusa Bold x W.F.	351	97	32

Inheritance ofopen VS appressed pods was studied in the F_2 progeny of cross RC-781 x UVR-4. The proportion ofopen VS appressed pods was 254:247, which fitted well to 9:7 ratio indicating that two supplementary genes control the positioning of pods, whereas Nayar and George (1970) have

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reported duplicate gene action (15:1) for flower colour.

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BREEDING OF SLOW BOLTING *BRASSICA CAMPESTRIS* VARIETY WITH NO LOW TEMPERATURE SENSITIVITY

S.YUI, H.YOSHIKAWA and Y.KUGINUKI

As reported before (Yui and Yoshikawa 1988), slow bolting variety 'Osaka Shirona Bansei (OSB)' bolts and flowers without any chilling (vernalization) treatment under long day (16 hours) and comparatively high temperature (20-25°C) condition. The purpose of this study is to clarify the response of OSB to low temperature treatment.

Experiment 1. Selection line FNC, which is a progeny line of flowering OSB with no chilling treatment, was sown on April 16, 1988. Seven days after sowing, the plants were transfered and chilled for 31 days at 2-7°C cold chamber. After the treatment, they were transfered to a phytotron (20-25°C, 16 hours). As a control of chilling treatment (0 days), seeds of FNC were sown in a glasshouse. Nine days after sowing, they were transfered to the same phytotron. Twenty two and 20 plants were cultivated for 31 days and 0 days treatment, respectively. Fig.1 shows leaf numbers of FNC at flowering. In 0 days treatment, 14 out of 20 plants flowered. It means that FNC has almost no chilling requirement for its flowering. Leaf numbers at flowering ranged continuously between 17-29. In 31 days treatment, 19 out of 22 plants flowered. Among the 19 plants, 4 plants flowered very early with leaf numbers of 12-16. The rest 15 plants flowered with 21-29 leaf numbers, which was the same leaf numbers of 0 days treatment. After the experiment, seeds of flowering plants in 0 days treatment (with no chilling requirement) and seeds of slowly flowering plants in 31 days treatment (with low or no chilling sensitivity) were obtained. These two lines were named as FNC0 and FNC31, respectively.

Experiment 2. Similar experiment was carried out using FNC0 and FNC31 with 0 days and 63 days chilling treatments. Fig.2 shows leaf numbers of FNC0 at flowering. Seventeen out of 21 plants flowered in 0 days treatment with leaf numbers of 25-67. In 63 days treatment, all plants flowered with leaf numbers of 11-22. It means that all the plants of FNC0 were vernalized with the chilling treatment. Fig.3 shows the result of FNC31. Eighteen out of 21 plants flowered in 0 days treatment with leaf numbers of 31-76. In 63 days treatment, 11 plants out of 22 flowered very early with leaf numbers of 11-16, however, 50% of the plants flowered slowly with leaf numbers of 29-63. It seemed that there was no effect of chilling treatment for the latter half of FNC31.

In FNC31, which has no low temperature requirement for its reproductive growth, there was much diversity for low temperature sensitivity. It might be possible to breed a slow bolting OSB with no low temperature sensitivity and no low temperature requirement. As OSB belongs to *B.campestris* var.*chinensis*, it may be also possible to introduce the unique slow bolting character to Chinese cabbage (*B.campestris* var.*pekinensis*).

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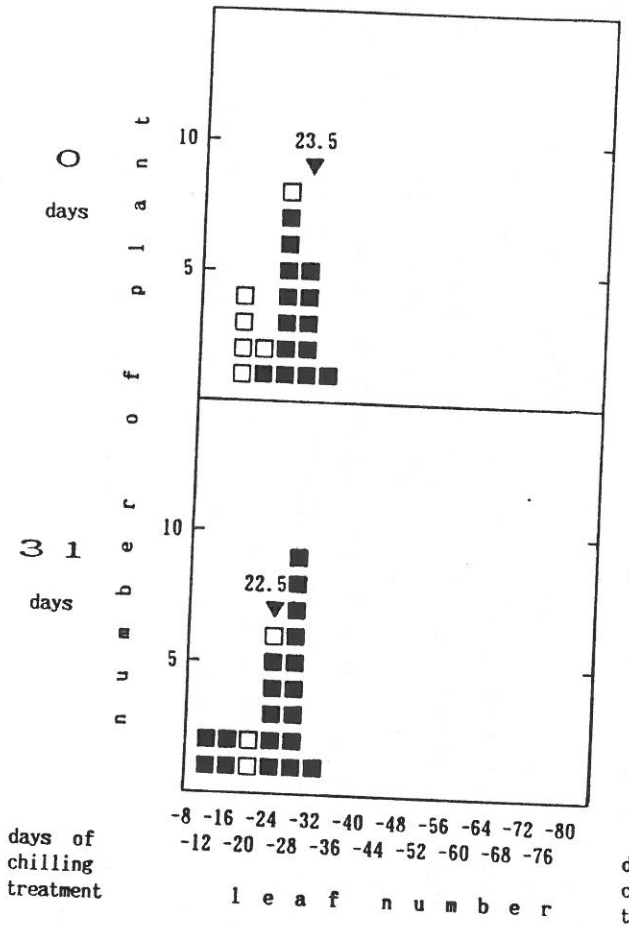

Fig.1. Effect of chilling treatment on leaf numbers of FNC at flowering
(■ : flowering plant
□ : non-flowering plant
▼ : mean leaf number of flowering plants

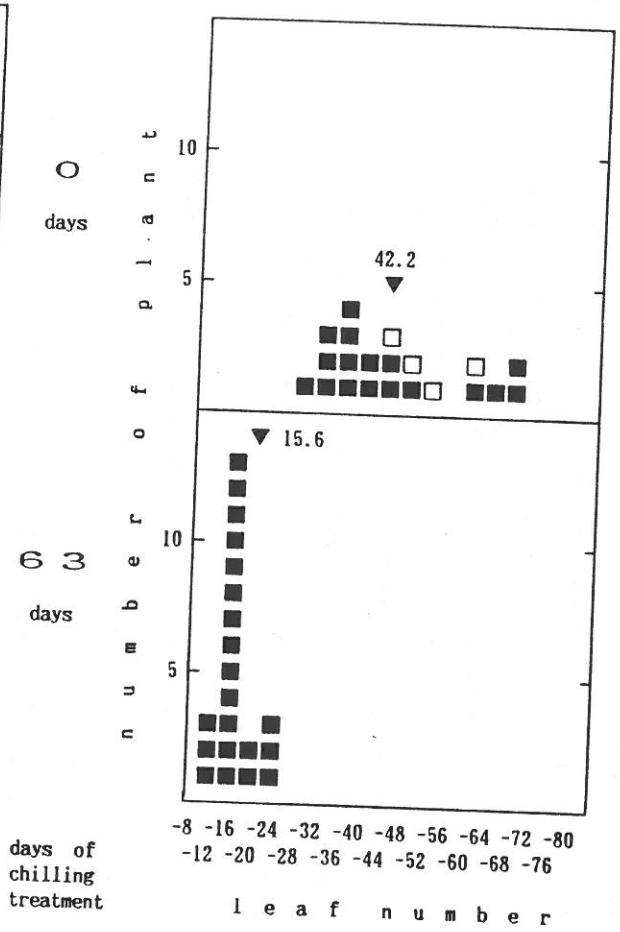

Fig.2. Effect of chilling treatment on leaf numbers of FNC0 at flowering
(■ : flowering plant
□ : non-flowering plant
▼ : mean leaf number of flowering plants

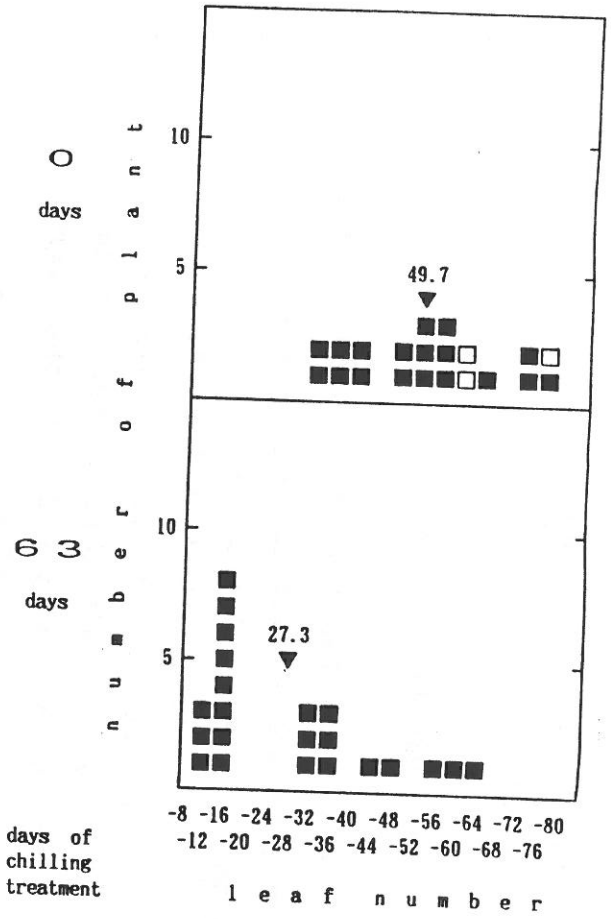

Fig.3. Effect of chilling treatment on leaf numbers of FNC31 at flowering
(■ : flowering plant
□ : non-flowering plant
▼ : mean leaf number of flowering plants

SELECTIVE STRATEGIES ON THE BREEDING FOR QUALITY IN BRASSICA NAPUS

Yongming Zhou and Houli Liu

It has been revealed that there was close relationship between quality characters and agronomic characters in Brassica napus (Zhou and Liu, 1987). Further studies have been conducted to understand whether selection for quality traits, such as seed oil content, fatty acid composition in seed oil, and glucosinolate content in meal, could bring negative effects on seed yield and other agronomic traits in the crop. Comparison of selection for quality traits in various generations has been made to decide adequate period in which selection for both quality traits and seed yield is efficient. The materials used for this investigation included segregating hybrid populations orinated from two crosses from three varieties with differnt contents of erucic acid and glucosinolates, and lines selected from different generations of these two crosses. The main results are summarized as follows.

Seed yield and seed oil content of the plants with below 2% erucic acid decreased strikingly than the plants with above 10% erucic acid in reciprocal F_2 populations (F_2 and RF_2), and the same trend was found among the other characters, such as total number of siliquae per plant and number of seed per siliqua. The differences of agronomic traits between groups with various glucosinolate content were not very significant in the same populations. There was no real difference in seed yield between lines with varied erucic acid content and glucosinolate content which were selected from F_3 (BCF_2) lines, but lower seed oil content was often accompanied with lower erucic acid among the lines. The lines with different contents of erucic

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acid and glucosinolates have been isolated from $F_4(BCF_3)$ lines in which the alleles for erucic acid and glucosinolates have been kept heterozygous for two generations. The comparisons of agronomic traits among the lines showed that the lines with below 2% erucic acid were higher than the lines with above 20% erucic acid in seed yield per plant, but unfortunately, lower seed oil content still stayed with the lines with below 20% erucic acid. The lines low in glucosinolate content had nearly equal seed yield to the highest seed yield lines with above 40 μmol/g of glucosinolate content. Two lines with doube-low traits and higher seed yield than the control with high content of erucic acid and glucosinolates have been identified from F_5 (BCF_4) lines. The results above implicated that the genotypes with high seed productivity could be lost due to strict selection for quality in earlier segregating generations. In contrast, it may be a more efficient selective method to keep the populations heterozygous in quality characters and select plants with expected quality characters in advanced generations.

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PROSPECTS OF PRODUCING F_1 HYBRID CAULIFLOWER FOR SPRING & SUMMER GROWING IN HILLS.

S.R. Sharma, H.S. Gill & K.S. Kapoor

Experiments were conducted during the last few years at IARI Regional Station, Katrain (Kullu Valley), H.P. to explore the possibility of growing cauliflower all the year round in the hills. The period from October to April is coverd by the snowball types while for the remaining period, a line from a group of Indian cauliflower has been identified and released under the name of Pusa Himjyoti (Gill et. al., 1987). It performed most sucessfully during summer & rainy season (May to Oct.) in the hills of northern India at an altitude ranging from 1050-1650m above m.s.l. However its curd weight varied from 400-700 gm with leaf number of 14-24 depending upon period of its availability. Another line (KK 104) was also found promising but had cream coloured curd and self blanching habit which was lacking in Pusa Himjyoti. In order to improve upon the curd weight and plant type of Pusa Himjyoti and curd quality of KK 104, these lines were crossed with eight promising Snowball lines/cultivars namely Pusa Snowball K-I, Pusa Snowball-I, Sel-12, Erfurter Supermax, EC 12013, King, Dominant & Igloo.

The F_1 hybrids were assessed during summer months. It was observed that all the F_1 hybrids involving Snowball x Snowball or Indian x Snowball groups either did not form the curds at all or formed unmarketable curds with pink, green and yellowish green pigmentation and small leaves inside the curds. The hybrids were also late by 10-15 days than the respective female parent. Similar results have been reported by Watts (1964). However F_1 hybrid of Pusa Himjyoti & KK 104 gave the best performance. Non curd/unmarketable curd forming habit of Snowball types was found to be dominant over the Indian group as its expressibility is dependent on high tempetrature (Dickson and Lee, 1980, Crisp & Innes, 1983).

The production & release of F_1 hybrids involving two groups of cauliflower for this season is ruled out. However, there is a possibility of improving the yield, curd weight & plant type by developing F_1 hybrids between two cauliflower lines i.e. Pusa Himjyoti and KK 104. Work on the isolation self-incompatible lines from Pusa Himjyoti is in progress as high level of self-incompatibility has been reported in this group of cauliflower by Chatterjee & Swarup, 1985. This will open the way for producing F_1 hybrids with better curd quality and plant type for growing in hills during summer and rainy months thereby improving the economy of hill farmers and help in meeting the increased tourist demand during these months.

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EVALUATION OF BROWN MUSTARD GERMPLASM IN SRI LANKA

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Abstract

An islandwide germplasm collection of mustard was followed by the evaluation of the germplasm at RARS, Angunukolapelessa, Sri Lanka. Study reveals that the differences in characters plant height, dates from sowing to flowering, number of pods per primary branch and weight of 1000 seeds are significant.

Brown mustard Brassica juncea (L.) Czern & Coss (2n=36), is widely grown in the southern dry zonal part and in some other regions in Sri Lanka. Cultivation is carried out by subsidiary level farmer whose inputs are low. In Sri Lanka mustard is mainly used as a condiment to local dishes rather than an oil crop. It has an attractive market price and a demand. Interest for mustard cultivation is increasing due to it's export potential. Farmers of different regions grow a variety of indigenous cultivars. This research was undertaken to collect, multiply conserve and evaluate mustard germplasm in Sri Lanka.

Materials and Methods

Seeds were collected from seven mustard growing regions in Sri Lanka. Collections were multiplied three seasons and conserved at Plant Genetic Resources Centre (PGRC), Sri Lanka prior to evaluation. One exotic line from one locality was evaluated at RARS, Angunukolapelessa (Lat-6°N and Long-81°E) in 1987/88 Maha rainy season (Sept.-March). Lines were raised in a RCBD with four replicates. Data on seven agronomic characters and yield were recorded.

Results and Discussion

Biometrical analysis of data on quantitative measurements of characters reveals distinct variation in germplasm collections (Table 1B). Analysis of variance indicates significant differences in plant height, dates from sowing to flowering, number of pods per primary branch and weight of 1000 seeds. It implies the use of indigenous variability in discriminating mustard population in these characters. Table 1A shows character measurements of germplasm from different locations. Wallawaya entry has shown the shortest plant height, least number of primary branches per plant, highest 1000 seed weight and a satisfactory yield. Hambegamuwa collection has given the highest mean plant yield. Kuda Oya, Wellawaya and Angunukolapelessa sources have resulted in satisfactory yield levels compared to other sources.

In general it can be infered that Sri Lanka inherits a wealth of variation in some characters in B.juncea. Locality differences were also observed to be important in some characters like 1000 seed weight, and number of pods per primary branch and dates from sowing to flowering. An appropriate approach towards the genetic improvement of this crop would be both to use existing variability and the creation of genetic variability through technical means wherever necessary. This step has already been initiated in the Regional Agricultural Research Station at Angunukolapelessa, Sri Lanka.

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Table 1A. Means and standard errors of characters in different germplasm collections.
Table 1B. Mean, coefficient of variation (CV%), variance and range of characters studied.

Entry/parameter	Plant ht. (cm)	Dates from sowing to flowering	Dates from sowing to 50% flower.	Number of primary branches per plant	Number of pods per primary branch	Dates from sowing to maturity	Weight of 1000 seeds (g)	Seed yield (kg/ha)
Table 1A.								
Angunukolapelessa	76.71±3.7	39.00±0.3	43.00±0.3	9.85±0.9	48.00±2.8	89.00±0.6	1.47±0.1	1115
Kuda Oya	64.71±2.3	37.00±0.6	41.00±0.4	10.14±0.5	45.30±3.5	88.00±0.3	1.64±0.1	1710
Mahailluppallama	75.00±4.3	37.00±0.5	41.00±0.5	10.14±0.5	44.30±5.5	89.00±0.7	1.48±0.1	945
Rahangala	60.57±2.6	39.00±0.3	44.00±1.2	9.14±0.4	32.70±1.8	86.00±0.7	1.18±0.1	352
Buttala	63.71±2.3	41.00±0.9	46.00±1.2	9.57±0.8	30.70±7.4	86.00±0.4	1.52±0.1	424
Wellawaya	60.00±2.4	38.00±0.9	44.00±1.9	9.14±1.0	41.90±4.4	92.00±0.3	2.14±0.1	1500
Hambegamuwa	65.28±1.2	39.00±0.5	45.00±1.4	11.42±0.6	44.10±1.6	89.00±0.3	1.65±0.1	1940
Table 1B.								
Mean	67.00	38.73	43.24	9.92	58.61	88.00	1.60	-
CV%	13.85	5.13	7.60	18.44	23.44	1.71	2.40	-
Varience	5.53**	3.67**	2.23	1.30	4.88**	2.50	5.14**	-
Range-Max	36.00	44.00	51.00	14.00	77.00	92.00	3.20	-
-Min	46.00	35.00	39.00	7.00	26.00	86.00	1.00	-

** = significant at P=0.01.

EFFECT OF MIXTALOL ON YIELD COMPONENTS OIL CONTENT FATTY ACID COMPOSITION IN RAYA (*Brassica juncea* L. Czern & Coss)

Rajinder Sharma, Lakhvir Singh and Gurbaksh Singh

Foliar spray of aqueous solution (1 ppm) of Mixtalol, a mixture of biologically active naturally occurring long chain fatty alcohols was applied at anthesis stage to study its effect on yield and its components and oil content and its quality in raya (*Brassica juncea* L. Czern & Coss) cv. RLM-198. The experiment was conducted in the field keeping three replications and the yield contributing parameters viz. number of siliqua per plant, length of siliqua, number of seeds per siliqua and 1000-seed weight were studied in a sample of five plants selected at random from each plot measuring 18' x 5'. Total yield per plot was also recorded after harvesting and the data was analysed statistically. Oil content was determined by the method of Kartha and Sethi (1957) and verified by NMR whereas fatty acid composition was measured by Luddy *et al*. (1968).

Mixtalol significantly increased the number of siliqua per plant leading to an improvement in yield over control (Table 1). The length of siliqua, number of seeds per siliqua and 1000-seed weight, however, remained unchanged. Data presented in Table 2 show that mixtalol increased the oil content and percentage of palmitic acid (16:0), Stearic acid (18:0) and Oleic acid (18:1) as compared with control. Linoleic acid (18:2) and Linolenic acid (18:3), however, decreased with this treatment. A higher level of oleic acid and low linoleic acid are known to be important for better nutritional and keeping quality of oil. The improvement in yield can be attributed to enhanced pollen germination and tube growth leading to an increase in the number of fertilized flowers (Sharma and Malik, 1977) and increased mobilization of photosynthates towards developing siliqua as proposed earlier by Sharma and Singh (1988). Mixtalol has been reported to increase root length, shoot length and dry matter accumulation in paddy, wheat, sorghum and maize (Menon and Srivastava, 1984). An increase in the oleic acid and decrease in linoleic acid has also been obtained in groundnut by the use of some phenolic compounds (Sharma *et al*., 1988). From the present studies it can be concluded that with the use of Mixtalol, we can obtain higher yield as well as oil of better nutritional and commercial value with higher levels of oleic acid and erucic acid.

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Table 1. Effect of Mixtalol on yield contributing parameters in Raya cv. RLM-198

Treatments (ppm)	Number of siliqua/ plant	Length of siliqua (cm)	Number of seed/ siliqua	1000-seed weight (g)	Total yield/ ha (kg)
Control	385	3.7	12.3	1.602	849.171
Mixtalol(1)	416	3.9	12.8	1.804	914.952
C.D. at 5%	26	N.S.	N.S.	N.S.	23.220

N.S. = Non significant

Table 2. Effect of Mixtalol on oil content and its quality in Raya cv. RLM-198

Treatments (ppm)	Oil content (%)	Fatty acid (per cent)					
		*16:0	18:0	18:1	18:2	18:3	22:1
Control	38.0	3.05	1.01	10.17	15.94	10.25	47.42
Mixtalol(1)	38.9	3.15	1.04	10.67	15.02	9.95	47.68

* 16:0 = Palmitic acid, 18:0 = Stearic acid, 18:1 = Oleic acid, 18:2 = Linoleic acid, 18:3 = Linolenic acid, 22:1 = Erucic acid

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EFFECT OF DEFOLIATION ON SEED YIELD AND YIELD COMPONENTS IN INDIAN MUSTARD (*BRASSICA JUNCEA* (L) COSS)

Gulzafar; M.L.Gupta and K.S.Labana

The role of leaves as the main site of photosynthesis is an established fact and needs no justification. In addition to leaves, other plant parts also play some role in photosynthesis particularly after anthesis. In rapeseed and mustard crops during fruiting stage numerous green siliquae with an apparently large photosynthetic area are produced and must have a role to play in the source and sink relationship of the said plants. Pandaya (1975) reported that in Indian mustard during fruiting period, leaves had a limited role to play towards seed production and pod photosynthesis assumed greater importance. In the late fruiting stage the lower half of the leaves although forming a greater bulk of the photosynthetic leaf material had no assimilatory contribution towards seed formation.

The objective of the study was to investigate the effect of leaf removal on oil content, seed yield and its components in *Brassica juncea*.

The experiment was conducted at oilseeds Experimental Area, Department of Plant Breeding, Punjab Agricultural University, Ludhiana, during 1985-1986 in a randomized complete block design with three replications.

Ten competitive random plants were selected from each entry in all the replications. At flower initiation five plants were mechanically defoliated completely and the rest of the five were kept as such. Observations were recorded in both populations on seed yield/plant, oil content, 1000-seed weight, seed-chaff ratio, siliqua length, main shoot length, siliquae number, plant height, primary branch number and secondary branch number. Mean performance of the normal Vs defoliated plants of the geographically diverse entries was compared by "t" test.

Normal and defoliated plants differed significantly among themselves for all traits, except for 1,000-seed weight, primary and secondary branch number.

The significant differences between normal and defoliated plants clearly indicated that defoliation adversely affect the performance of the genotypes. Defoliation reduced the photosynthates resulting in low seed

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yield and oil content. Freyman et al (1973) in Brassica campestris and Clark (1978) in Brassica napus reported reduction in seed yield on leaf defoliation but Pandaya (1975) reported reduction in seed yield upto only 37.5% on complete defoliation while in the present case, the range of reduction in seed yield as compared to normal plants was from 49.54% (in PR2001) to 88.09% (in RH8130).

The lack of effect on branch number could be attributed to the fact that branch primordia are developed and determined before anthesis. Once the flowering shoot is established the developing sink is dependent on green pod walls for assimilate supply. This can be the justification of lack of defoliation effect on seed size.

Though the branch number remained relatively unaffected, their development in terms of length and number of effective flowers borne was severely restricted due to absence of assimilate supply from leaves and competition with developing seeds and shoots share a common metabolic pool. This hypothesis also explains the drastic reduction in length of main shoot, siliquae number and even plant height, the multiplicative effect of restricted plant canopy was manifest in very low yield of defoliated plants.

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Novel variations in the X=9 *Brassica* species

Shahryar F. Kianian and Carlos F. Quiros

The *Brassica oleracea* cytodeme (X=9) represents a group of highly polymorphic species. However, the evolutionary relationship is still hypothetical. In an effort to understand this relationship, we are surveying the variability of these species with molecular markers.

High level of polymorphism in the zymograms of the isoenzymes GOT, LAP, MDH, PGM, 6-PGDH, and TPI was observed for the species classified under this cytodeme. Most interesting was the variation for 6-PGDH which is determined by a series of highly conserved duplicated loci (1). As far as we know no variation for the three banded pattern of the plastid isozymes is observed within cultivated *B. oleracea* (1). However, while surveying other species of this cytodeme, variations including loss and gain of isozymes were observed for the species *B. montana*, *B. cretica*, *B. insularis*, *B. incana*, and for some wild *B. oleracea* accessions (figure 1). This type of polymorphism can be explained by the presence of a third duplicated loci in all the wild species and of a null allele for locus 6-PGDH 1 in the species *B. cretica*, *B. incana* and the wild *B. oleracea* accessions. Thus, in general the determination of the 6-PGDH isozymes is more complex for most of the wild species in this cytodeme than for the cultivated *B. oleracea*.

A similar situation was observed for ribosomal genes on EcoRI genomic digests for the wild species representing perhaps additional duplications. *B. alboglabra*, *B. cretica*, *B. drepanensis*, *B. incana*, *B. insularis*, *B. montana*, and *B. rupestris* displayed higher level of polymorphism for the intergenic rDNA spacer than *B. oleracea* (figure 2). Upon further analysis, we believe that this variation represents at least four loci, two of which might be linked.

We are now in the process of analysing F1 and F2 progenies hoping to learn more about the inheritance of these traits and their evolutionary significance. Since none of these variant phenotypes are observed in the *B. oleracea* horticultural types, our preliminary data supports the monophyletic origin of the different *B. oleracea* morphotypes.

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Figure 1

Isozyme 6-PGDH

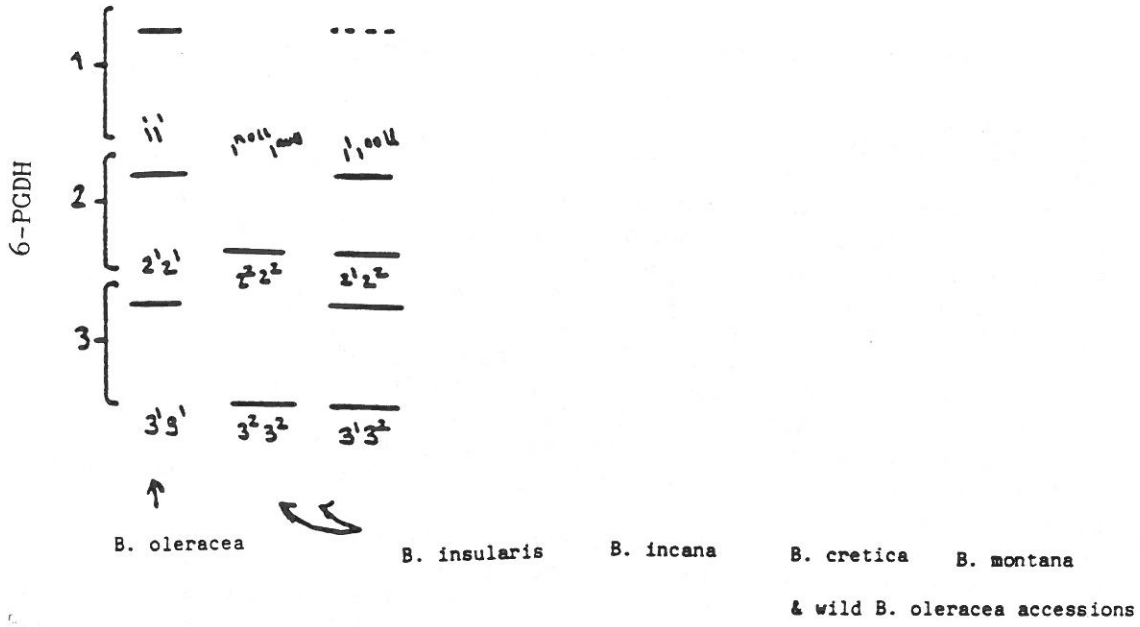
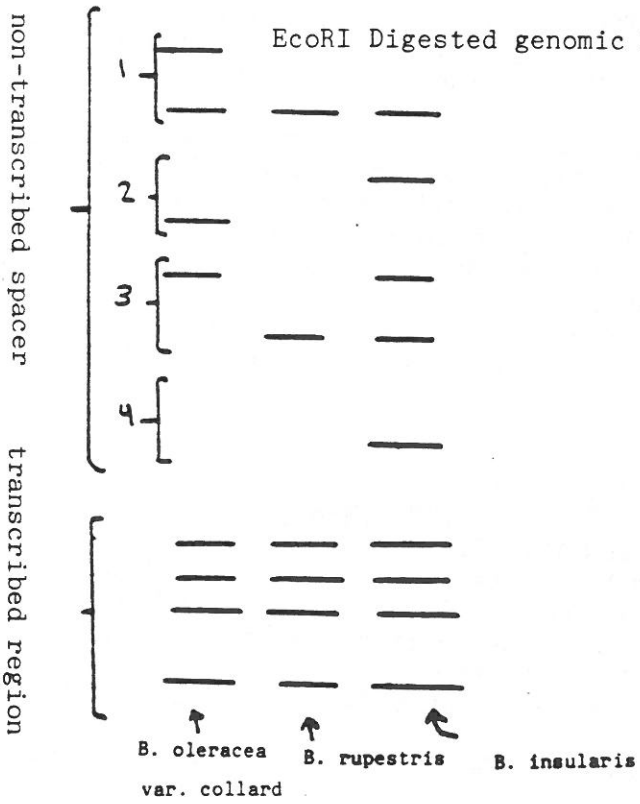


Figure 2



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FIRST RESULTS OF RAPESEED VARIETY IDENTIFICATION BY ISOZYME ELECTROPHORESIS

A.M.CHEVRE, R.DELOURME, F.EBER, P.ARUS

Genetic analysis of several isozymes has been made for diploid parental species of rapeseed: Brassica oleracea (Arus and Orton,1983) and B.campestris (Truco,1986). These systems have been used for studies of phylogenetic relationships (Vaughan et al,1970; Truco and Arus,1987; Chen et al,1989), identification of interspecific hybrids (Sjödin and Glimelius,1989) and characterization of addition lines (Quiros et al,1987). The first attempt of isozyme detection in different rapeseed varieties was reported by Thorpe et al (1987).

We have tried to characterize five french rapeseed lines used in our breeding and interspecific programs: Brutor, RP1 (spring lines), Darmor, Bienvenu, Samouraï (winter lines). Ten isozyme systems were studied: malate dehydrogenase (MDH), isocitric dehydrogenase (IDH), leucine aminopeptidase (LAP),6-phosphogluconate dehydrogenase (6PGD), aconitase (ACO), phosphoglucoisomerase (PGI), triose phosphate isomerase (TPI), glutamate oxaloacetate transaminase (GOT),phosphoglucomutase (PGM) and acid phosphatase (APS).

Young leaves were crushed in a Tris-HCl 0.1M buffer containing 1% glutathione. Starch method used was reported by Quiros and McHale (1985) and buffer systems by Shields et al (1983). MDH,IDH,LAP,ACO,6PGD were separated on G buffer system (pH 6.1), GOT,TPI on C buffer system (pH 8.3) and PGM on E buffer system (pH 7.0). PGI was studied on G,C and B (pH 5.7) buffer systems. Acrylamide separation seemed to be more efficient for GOT (Tris-HCl gel buffer pH 7.0) and APS (Tris-HCl gel buffer pH 8.9) (Truco,1986).All the staining procedures were reported by Vallejos (1983). Twenty and ten plants per line were studied on starch and acrylamide respectively.

The patterns are shown in figure 1. PGM was the only monomorphic system. Only GOT allowed the discrimination of all the lines. The winter lines were more similar. Four and five systems differentiated Darmor from Bienvenu and Samouraï respectively. Winter and spring lines differed for six to nine systems. Some variability was observed in few plants for 6PGD-2 in Brutor and for APS-1L in Samouraï.

The variability within B.napus will now be considered with a much higher number of varieties of different origins and the genetic of each system will be studied more precisely.

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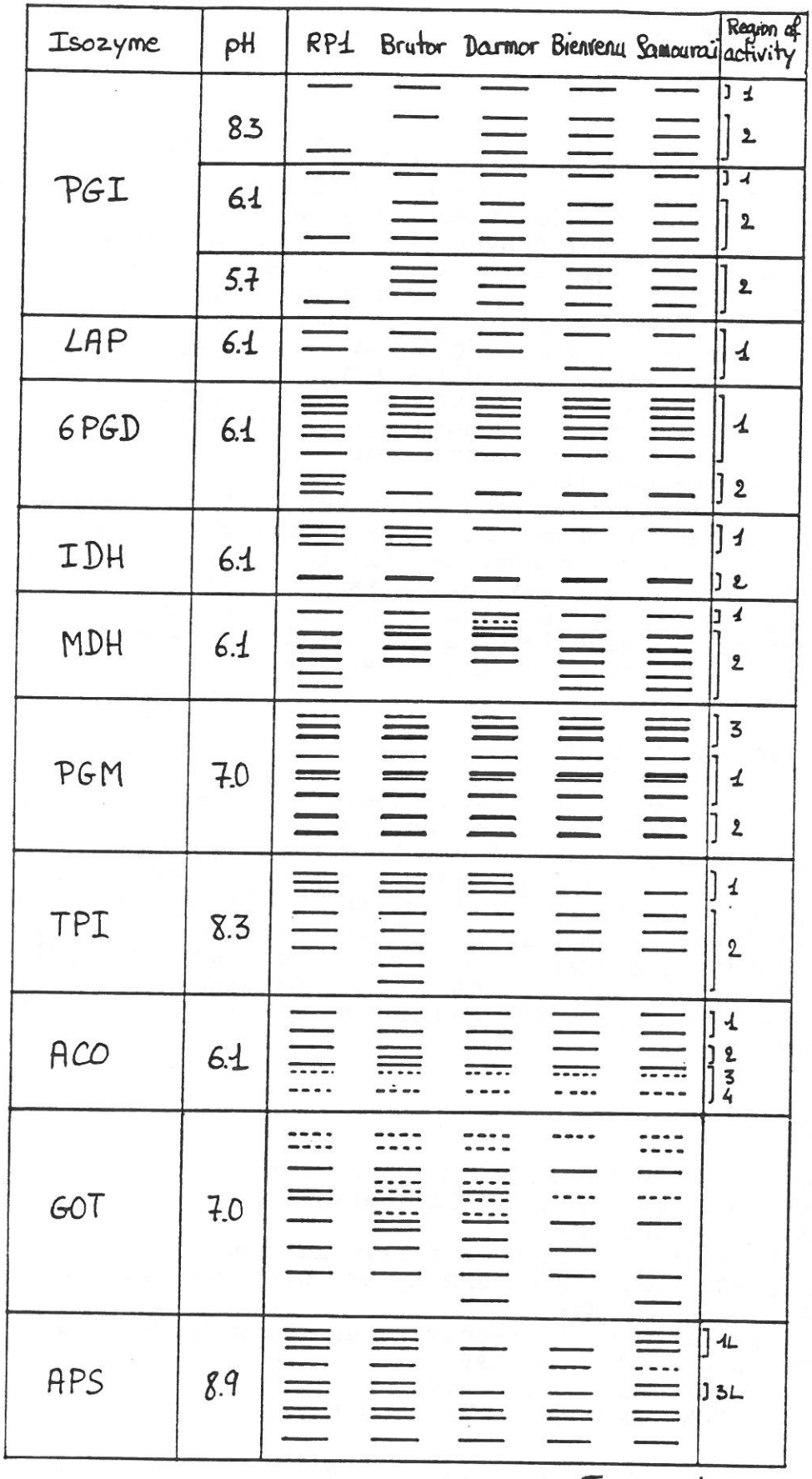
Isozyme
pH
RP1
Brutor
Darmor
Bienvenu
Samourai
Region of activity
PGI
8.3
6.1
5.7
LAP
6.1
6PGD
6.1
IDH
6.1
MDH
6.1
PGM
7.0
TPI
8.3
ACO
6.1
GOT
7.0
APS
8.9
1
2
3
4
1L
3L

Figure 1

OPTIMIZATION OF STARCH GEL ELECTROPHORETIC TECHNIQUES FOR USE IN GENETIC CONSERVATION OF Brassica oleracea L.

Z.H. Guo, M.R. Nolan, B.E. Recchio-Demmin, J.R. McFerson, and S. Kresovich

With the cole crops, B. oleracea, 12 enzymes have been resolved via starch gel electrophoresis. Of those, six enzymes involving 11 polymorphic loci have been genetically defined, including acid phosphatase, aspartate amino transaminase, alcohol dehydrogenase, leucine amino peptidase, phosphoglucoisomerase, and phosphoglucomutase. The remaining six undefined systems consist of beta-glucosidase, beta-galactosidase, esterase, triose phosphoisomerase, 6-phosphogluconate dehydrogenase, and malic dehydrogenase. For different plant species the same enzyme usually can be resolved by similar protocols. However, some critical methodological details require optimization for a given enzyme of a particular species (Wendel and Weeden 1989).

The objectives of this study were (1) to compare the effects of different procedures and factors on the resolution of various enzymes, (2) to develop a standard protocol for the utilization of isozyme assays for B. oleracea, and (3) to resolve more enzyme systems for future applications in genetic studies and effective conservation and utilization of cole crop genetic resources.

MATERIALS AND METHODS

Plant material for study included 82 entries of B. oleracea L. representing a spectrum of plant introduction (PI) accessions, commercial cultivars, and genetic stocks of six morphotypes: cabbage (subsp. capitata), cauliflower (subsp. botrytis), broccoli (subsp. italica), Brussels sprouts (subsp. gemmifera), kohlrabi (subsp. gongylodes) and kale (subsp. acephala). Cotyledon, meristem, and pollen were used as tissue sources for the assays. Systems of Wendel and Weeden (1989) and May et al. (1988) were utilized for the assay.

RESULTS

* Characterization of enzyme systems:
  - Well resolved, genetically defined (3)
    AAT, GPI, and PGM
  - Well resolved, not genetically defined (13)
    MDH, ME, IDH, 6-PGD, GAPDH, GR, DIA, PER, Alpha-EST, FDP, TPI, MPI, and MUP
  - Not satisfactorily resolved, genetically defined (3)
    ADH, Alpha-ACP, and LAP
  - Well resolved, no variation found (6)
    SKDH, GDH, CAT, SOD, ALD, and PEP-GL

USDA/ARS Germplasm Res. Unit, NS Ag. Exp. Sta., Geneva, NY 14456-0462, U.S.A.

* Thirteen additional enzyme systems were resolved via starch gel electrophoresis for the first time in B. oleracea.

* In total, 25 enzyme systems involving 52 loci (among which 11 have been defined genetically) were resolved in this study.

* Of the 25 enzyme systems, 19 showed variability and yielded 33 polymorphic loci or zones of activity.

* No differences were found in banding patterns and staining intensities of cotyledon and meristem tissues. Pollen yielded enzymatic activity strong enough to be useful for elucidation of the quaternary structure of selected enzymes.

* Gel system pH had major effects on the resolution, staining intensities, and mobilities of all the enzymes. In most cases, an optimum gel system could be detected for each enzyme system.

* No extraction buffer proved to be more efficacious across the enzyme systems tested.

* No consistent pattern was observed in resolution across the range of starch concentrations and enzyme systems evaluated.

* The presence or absence of sucrose in the gel had no consistent effect across the enzyme systems evaluated.

For additional information on this or subsequent activities, please contact the **USDA-ARS Germplasm Resources Unit.**

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EVALUATION OF HEAT-TOLERANCE AND ITS CORRELATED CHARACTERS IN NON-HEADING CHINESE CABBAGE

Liu Weixin and Cao Shouchun

Heat-tolerance of non-heading Chinese cabbage was related to ecological type and developing rate. Southern ecological type and F_1 hybrids were more heat-tolerant than the others. In the process of heat-tolerance evaluation, morphological, physiological characters and field performance should be considered.

Non-heading Chinese cabbage [Brassica campestris L. ssp. chinensis (L.) Makino] is an important vegetable in southern area of China but the low yields, heavy diseases and bad quality were the main problem in summer production. Heat-tolerance of non-heading Chinese cabbage had been investigated in 1960 (Cao Shouchun). Recently, reports on heat-tolerance of non-heading Chinese cabbage have not been found. The objective of this study is to determine the heat-tolerance of non-heading Chinese cabbage and to select the characters related to heat-tolerance.

The experimental materials were 23 cultivars and F_1 hybrids. The sowing date of the materials was 2 July 1988. The design was a randomised complete block with 2 replications. The experimental unit consisted of 40 plants. Plant and row spacing were both 18 cm. The management was as the same as the field management. Morphological characters and disease were investigated according to "the investigated method of characters in non-heading Chinese cabbage". The heat-killing temperature was analysed as described by Zhu Yuelin et al. The other characters were analysed by standard method.

There was significant difference between cultivar heat-tolerance (Table 1). According to field performance, Pu Tung Pe Tsai variety and Tsai Tai variety were more heat-tolerant than that of the other types. Ai-Khan-No.3, Fu-Shan-Wu-Ye, Ai-Za-No.1, Si-Jiou-Cai-Xin, AB-Ai and Duan-Bai-Geng were heat-tolerant cultivars and could be used in summer production. Because of their bad quality, Ru-Gao-Mao-Cai and Wu-Chang-Huong-Cai-Tai could not be used in summer production. Early-bolting cultivars were more heat-tolerant than late-bolting ones.

Compared with heat-sensitive cultivars, heat-tolerant cultivars generally have a greater number of leaves, and their leaves are thicker and longer, their plants are higher and the plant width is smaller, the palisade tissue is thicker also. In physiological characters, net photosynthesis and Chl a content/Chl b content were positively correlated with heat-tolerance respectively.

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Table 1. Heat-tolerance evaluation of non-heading Chinese cabbage

Cultivar	Plot yield (kg)	Index of heat injury	Anthracnose index
Pu Tung Pe Tsai Variety			
Hei-Ye-Po-Tou	4.72 cd	14.17 c	25.83 ab
Fu-Shan-Wu-Ye	7.68 a	11.25 c	3.16 c
Ai-Kang-No.3	6.64 b	13.54 c	8.96 c
Xue-Ke-Qing	1.72 ef	5.63 c	3.13 c
Nan-Nong-Ai-Jiao-Huang	3.78 d	12.92 c	29.59 ab
Mu-Yan	3.09 de	10.58 c	13.75 bc
Ai-Za-No.1	5.53 c	11.67 c	13.26 bc
AB.-Ai	3.19 de	19.17 bc	29.38 ab
Duan- Bai-Geng	3.85 d	11.71 bc	21.85 b
Ai-Jiao-Huang	4.54 cd	14.79 c	24.99 ab
Liang-Bai-Ye	2.59 e	14.58 c	25.22 ab
Ai-Za-No.2	3.40 de	15.63 bc	26.88 ab
Wu-Bai-Ye	1.93 ef	28.54 ab	24.98 ab
Nanjing-Si-Yue-Bai	3.37 de	14.79 c	27.64 ab
Tsai Tai variety			
Si-Jiou-Cai-Xin	-	8.13 c	6.14 c
Wu-Chang-Huong-Cai-Tai	4.75 cd	9.83 c	21.47 b
Tai Tsai variety			
Yuan-Ye-Tai-Cai	2.92 de	25.42 ab	33.44 a
Hua-Ye-Tai-Cai	1.05 fg	17.19 bc	25.04 ab
Xu-Zhou-Tai-Cai	1.47 f	35.83 a	34.71 a
Ta Tsai variety			
Wu-Ta-Cai	0.35 g	30.63 ab	13.38 bc
Shanghai-Xiao-Ba-Ye	0.97 fg	10.21 c	13.53 bc
Duo Tou Tsai variety			
Ma-Er-Tou	1.28 fg	19.38 bc	11.18 c
Ru-Gao-Mao-Cai	5.50 c	6.25 c	11.50 bc

Table 2. Correlation between different characters and heat-tolerance

Characters	Anthracnose index	Index of heat injury	Heat-killing temperature	Plot yield
Height of plant	0.259	- 0.036	0.325	0.491*
Width of plant	0.011	- 0.428	0.129	0.590**
Length of leaf	0.198	- 0.083	0.423*	0.652**
Width of leaf	0.259	- 0.216	0.347	0.664**
Number of leaf	- 0.601**	- 0.333	- 0.130	0.085
Thickness of leaf	- 0.14	- 0.513*	0.015	0.709**
" palisade tissue	- 0.218	- 0.526*	- 0.040	0.555**
" spongy tissue	0.051	- 0.399	0.128	0.512*
Chl a/Chl b content	- 0.340	- 0.471*	0.140	0.092
Cell extract content	- 0.031	- 0.094	- 0.161	- 0.415
Net phytosynthesis	0.188	- 0.209	0.318	0.439
Protein content	- 0.004	0.08	0.084	0.035
Sol. sugar-content	- 0.205	- 0.222	0.308	0.220

Degree of freedom = 20, PO.05 = 0.423, PO. 01 = 0.537.

The responses of *Brassica camperstris* L. to aluminium toxicity

J.Meng* and N.Thurling**

It has been widely proved that aluminium toxicity is a major cause of poor crop growth in acid soils and 40% of arable land in the world is potentially concerned with such a problem (Foy,1987). The effect of Al concentration varied with and within plant species. The roughly range would be 1 μM to 560 μM depending on the pH and composition of culture solution and the tolerant creterio been used. This study developed a Al-solution culture system which could distinguish tolerant and sensitive genotypes of *Brassica* and tested the response of *Brassica* genotypes to Al toxicity for the purpose of breeding Al-tolerant rapeseed cultivars.

The experiments were carried out at a phytotron with the temperature of 25°C day/20°C night and 24 hours a day aeration for solution. The composition (μM) of nutrient solution was $Ca(NO_3)_2$,424;KNO_3,390;NH_4NO_3,256;$MgSO_4$,101.5;KH_2PO_4,22;NaCl,8.5; H_3BO_3,5;$MnSO_4$,5;FeEDTA,5;$CuSO_4$,0.12;$ZnSO_4$,0.10;$CoSO_4$,0.036; Amonium molybdate,0.015. $Al_2(SO_4)_3$ was added to the nutrient solution and the pH of Al solution was adjusted to 4.2 or 4.8 using 1N HCl and 1N KOH at the beginning of Al treatment and no further pH adjusting through the experiment. Seeds were germinated on stainless steel screens covered with cotton qauze above an aerated solution for 4 days until roots were about 20 mm long. The seedlings or were clipped by 5 folded foam strips inserting into 5 slipts of a plastic lid above a pot containing nutrient solution and Al solution or retained on the germinating qauze rowedly and then transfered the whole screen-qauze-seedling to 5-l pots. Root lenth could be measured regularly without any injury by the later way.

Roots from seeds of a composite population of *B.campestris* L.developed well in the solution of low Al concentrations and were dramatically inhibited when Al concentration increased to 12 μM at pH4.2 and 60 μM at pH4.8 or above. The lateral root just appeared in the nutrient solution stopped growing shown a "stubby root" symptom in the inhibiting Al solution,and no matter how fast one root of seedling grew in the nutrient solution,its growth stopped in Al-toxicity solutions as other slow-growing roots. The development of top parts of plants slowed down a few days later after root growth stopped in Al solutions. The higher of Al concention and the lower of the solution pH,the severer of toxic symptom. With initial pH4.8,small plants growing in 100 μM Al solution just bloomed a few flowers and that of in 300 μM could not flower at all.

10 μM Al solution with initial pH4.2 was chosen as a critical criterion for distinguishing tolerant and sensitive genotypes of *Brassica camperstris*. 28 half-sib families derived from the

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composite population were tested. Table 1 shown a distinquish genetic difference among some of families. Tens of tolerant and sensitive individual plants and several tolerant half-sib families had been selected from our experiments.

Table 1. Root elongation and final pH of solution as response to aluminium*

	SF4	SF12	SF17	SF55	SF61	SF95
Root elongation mm	2.30 ±2.91	1.97 ±1.45	0.63 ±1.03	51.88** ±14.46	1.63 ±1.45	1.37 ±2.14
Final solution pH	4.35	4.10	4.17	4.70	4.40	4.23

* Data came from measuring of 4 days after roots exposed to aluminium solution.
** Significant at p=0.01

It was obvious and interesting that some tolerant families such as family 55 had an ability to increase the pH of solution while other sensitive families decrease the pH of solution when growing each family separatly. Since increasing pH of solution resulted in precipitation of Al, increasing pH of solution might be one of machanisms of tolerant to Al toxicity for some *Brassica* genotypes.

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Field- and greenhouse- evaluation of *in vitro* selected salt tolerant plants of *Brassica juncea* L.

Sunita Jain, R.K.Jain, H.S.Nainawatee and J.B.Chowdhury

Plant tissue culture techniques provide a promising and feasible approach to develop salt tolerant plants. *In vitro* selection of salt tolerant cell lines have been reported for several species (for review see Dix 1986). Efficacy of *in vitro* selection system, however, has been limited particularly due to failure of selected tolerant cells to regenerate plants. Secondly, in a few cases, where plants have been regenerated, the progeny were not analysed for salt resistance, with some notable exceptions (Bhaskaran *et al.* 1986, Nabors *et al.* 1980, McCoy 1987, McHugen 1987).

We have described earlier an *in vitro* selection system to screen directly for NaCl-tolerant plants of Indian mustard using cotyledon cultures, which possessed a very high morphogenetic potential (Jain *et al.*, 1986; 1989). In this communication we summarise some of our recent findings on agronomic performance of the progeny of selected NaCl-tolerant and non-selected somaclones and parent variety under normal field conditions and salt-stressed sand pots in the greenhouse.

The three (SR-1, -2 & -3) plants recovered after screening on high salt media (Jain *et al.* 1986) were multiplied by axillary bud culture on NaCl free medium. The salt-tolerant shoots were maintained for a total period of 3 months on a NaCl free medium before bringing it back onto stress media to check the stability of the selected salt-tolerance trait. All the three selected salt-tolerant shoots grew at 0.5 and 1.0% NaCl, wheras the control shoots from non-selected somaclones as well as seedlings turned brown and died.

While two of these somaclones flowered and set seeds, third one (SR-1) grew slowly, had abnormal leaf morphology and was sterile. The seed of the plants were sown in the field to raise R_1 segregating generation. Data were recorded for yield, other agronomic components and oil content. The somaclonal lines, both selected salt-tolerant and non-selected, showed tremendous amount of variation for all the characters studied. One of the two tolerant somaclones (SR-2) invariably showed reduced height, longer reproductive phase and higher 1000 seed weight. Based on the agronomic performance of R_1 plants of these somaclones, some plants were selected and their progeny were evaluated for agronomic performance under field conditions and salt-tolerance in the greenhouse using sand pot culture method. Most of the lines bred true for their specific characteristics.

In greenhouse using sand pots, irrigation with various NaCl (30, 60 & 90 meq/l) concentrations did delay the seed germination but all the genotypes showed invariably more than 95% germination in all the treatments by day 7. Data taken on plant biomass after 6 weeks of seeding indicated vigorous plant growth in selected tolerant as well as non-selected somaclonal lines as compared to their progenitor. By comparing flowering in each salinity level and across diferent genotypes, SR-2 line flowered significantly (15 days) earlier than the parent progeny. It was interesting to note that while increase in salinity does delay flowering in parent progeny, it make it flower earlier by 2-5 days in tissue culture raised plants irrespective of the *in vitro* selection factor. At harvest, data was recorded for a number of agronomic parameters. In general, plant height, yield, other yield components and oil-content decreased with an increase in salinity in parent and control and salt-tolerant somaclones and decrease being significantly high in 'Prakash', however, with some exceptions. Notably, SR-3 line showed a marginal increase in seed yield

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and number of siliqua at low salinity levels. Amongst parent 'Prakash', selected tolerant and non-selected (CP-5) somaclones, percent reduction in seed yield per plant at 90 m eq/l was maximum in 'Prakash' (57%) and minimum in SR-3 line (31%) and others ranged in between. Percent reduction in oil-content was significant and nearly equal in the different genotypes with increasing salinity, with an exception to SR-2 line. SR-2 line displayed similar characteristics as it showed under field conditions. It had significantly low number of siliqua and higher 1,000 seed weight as compared to control. The percent reduction in all the parameters studied in this genotype was markedly less as compared to that in parent 'Prakash'. Under sand pot culture, this genotype showed the maximum oil content, and it declined by only 8.2% as compared to 23% in parent at 90 m eq NaCl. At 90 m eq NaCl, 1,000 seed weight decreased to 1.9 g or below except for that in SR-2 line, which maintained a fairly bold seed size and 1,000 seed weight of 2.43g, a weight comparable to that of other genotypes under non-saline conditions. This variant also seemed to be temperature sensitive as higher day temperature (>28 °C) adversely affected siliqua formation in this genotype much more than others. Another marked difference between stressed and non-stressed plants was early maturing in stressed plants of all the genotypes. SR-2 line, while behaved similarly under salt stressed conditions, matured invariably about a week later than the parent plants.

Selection for salt tolerance accompanied extensive somaclonal variation, both undesirable and the one which could be desirable too. For e.g. while it was deterrent for the successful recovery of 'SR-1' salt tolerant line characteristics like low height, longer reproductive phase and bold seed of SR-2 and high yielding capacity of SR-3 could also be desirable for *Brassica* breeding programmes. It is essential to mention here that accompanying somaclonal variation may not be a result of selecting for salt-tolerance, as extensive somaclonal variation have also been observed in non-selected plants reported earlier in this species (Jain *et al.*, 1989).

In the present study, mechanism of salt tolerance is not clear. It is interesting to note that even non-selected somaclone, showed increased salt-tolerance as compared to parent progenitor plants. CP-5 and other selected tolerant somaclones undoubtedly showed increased vigor in one way or other under normal field conditions and it could be one reason for enhanced salt-tolerance (McHugen 1987). Salt-tolerant somaclones, SR-2 and SR-3, differed in their salt tolerance during vegetative and reproductive phases as indicated by their mean salt-tolerance indices. SR-2 had better tolerance indices during vegetative phase and later for 1,000 seed weight and oil-content. SR-3 somaclones, while performed just better than the parent progenitor during vegetative phase, proved to be most tolerant later as indicated by seed-yield based indices. These observations suggest that different somaclones may have different salt-tolerance mechanisms. Further detailed cytological, physiological and biochemical characerization of these somaclones is in progress and will through more light on the possible salt-tolerance mechanisms operating there on.

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SELECTION OF RAPE (BRASSICA NAPUS L.) CALLUS CULTURES RESISTANT TO OXALIC ACID

Wu Chunren Liu Houli

Infection of rape by Sclerotinia sclerotiorum (Lib) de Bary causes serious damage to crops in China.While a high degree of resistance is unknow,only few less susceptible cultivars and breeding lines have been found (Wu,1989).

Oxalic acid,a phytotoxin produced by S.sclerotiorum , appears to figure prominently in the pathogenicity of the pathogen (Rai and Dhawan,1976;Wu and Liu,1989). The toxin and culture filtrates have been widely used to select resistance/ tolerance from excised leaves (Tu,1985),cell suspensions(Noyes and Hancock,1981) and seedlings (Huang and Dorrell,1978) of sunflower and bean.

The studies reported here were to determine if a response to oxalic acid could be observed in rape callus cultures and, if so,to determine the possibility of using cell culture techniques to select for callus resistant to the toxin.

Materials and Methods

Callus cultures of winter rape cultivars(lines) hua No8 and hua No13 (high susceptible); 083 and 821 (low susceptible) were initiated from hypocotyls as described by Song(1987). Callus was divided into small uniform pieces(approx.50±5mg F.wt) weighted,and three or four pieces were placed in flasks containing the appropriate medium with different concentrations of oxalic acid(for control was pure tissue culture medium).Fold increase in fresh weight was calculated by dividing the increase in weight by the initial weight.

Result and Discussion

The response of rape callus to oxalic acid is shown in Table 1. The data generally indicated that fold increase of callus decreased with concentration of oxalic acid or with duration of feeding. At a given concentration of oxalic acid and at a given time,the calli from cultivars(lines) less susceptible to the pathogen survive a higher dose of oxalic acid than the more susceptible cultivars(lines).

Callus from culture medium containing 1.0mM oxalic acid that have been cultured continuously in the presence of toxin for 120 days,was weighted and transferred to medium with 0 and 2.5 mm oxalic acid. The growth rates from 6 clones in the presence of the high toxin concentration are compared to the growth on medium with 0 mM oxalic acid in Table 2. Significant differences were observed among both low and high susceptible cultivars (lines). Further comparison of relative growth rates on oxalic acid and control media indicated that resistance remained stable and perhaps increased slightly in some clones.

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Table 1. Effect of oxalic acid on growth of rape callus*

Cultivar (line)	Concentration of oxalic acid (mM)						
	0.0	0.25	0.5	1.0	2.5	5.0	10.0
821	4.5a	4.3a	3.6a	3.1a	1.7a	0.8a	0.0
083	4.4a	4.5a	3.9a	2.9a	1.9a	0.4a	0.0
Hua No8	4.3a	3.8b	2.9b	1.4b	0.6b	0.0	0.0
Hua No13	4.6a	3.8b	2.7b	1.1b	0.4b	0.0	0.0

*Means of fold increase in fresh wt were obtained from weights of 10 inocula.Means in a column followed by the same letter are not significantly different(Dancan's multiple range test, p=0.05).

Table 2. Tolerance of variant rape callus to oxalic acid*

Cultivar (line)	Clone number	Fold increase in F.wt	
		Control	oxalic acid (2.5 mM)
821	W821-6-3-1	14.8	17.2
	W821-4-3-1	19.3	12.4
	W821-1-5-3	9.3	6.3
Hua No8	W08-4-1-2	21.2	14.2
	W08-5-3-3	13.7	15.4
	W08-5-4-3	11.3	8.9

*Figures are fold increase in fresh wt.from weights of 10 inocula/treatment after 90 days.The control for each clone was grown on pure culture medium.

Our results with the callus screening test were consistent with the field epiphytotic data on cultivar (line) susceptibility to S.sclerotiorum.The selection of toxin-resistant variants of rape callus can be made possible by the inhibitory effect of the oxalic acid on growth of rape callus.This inhibition could provide a positive selection system in which susceptible cells presumably die or grow very slowly while more resistant cells grow faster and can be subcultured for further screening. Induction and selection for novel resistance /tolerance to S.sclerotiorum from haploid embryogenic cultures of rape after a mutagen treatment using oxalic acid as selecting agent are in progress.

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A RAPID PRELIMINARY TECHNIQUE TO SCREEN FROST TOLERANT GENOTYPES OF BRASSICA

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Brassica is prone to frost damage in several parts of Asia, Europe, America and Austrelia. Seed damage amounting to as high as 90% has been reported in India (Yadava and Bhola, 1977). Therefore, it is essential to develop some frost tolerant genotypes. Screening of genotypes need some laboratory/field techniques. Most of the laboratory techniques (Fowler, Simnovitch and Pomeroy, 1973; Bringham and Jenkins, 1975) have a drawback that these involve leaves or exposed seeds. The freezing damage to the leaves may not necessarily be correlated to its seed damage whereas, the exposed seeds get damaged during separation from siliqua. The field test using Movable Freezing Chamber (Ohlsson, 1986) is most suitable test, as the freezing treatment is given directly to field grown plants, but test speed is too low. Therefore, it is essential to develop some technique which is rapid and involves freezing to unexposed seeds. The comprehensive account of the present technique is described.

Record the date of flowering initiation of the genotypes to be screened. Thirty-five days after flowering initiation, 10 main shoots bearing siliquae from each genotypes are given oblique cut during early morning hours before 4.00 A.M. of each genotype. Insert 10 twigst of each genotype in three replicated 6" pots containing sandy soil which is already maintained at field capacity. The different genotypes (the number depending upon the size of the deep freezer) are placed randomly in deep freezer at about 4.00 A.M. already fixed at 2°C. Allow this temperature upto 30 minutes. Bring down the temperature to -3.0°C for 60 minutes, followed by -4.0°C for another 30 minutes. Switch off cooling unit and open the deep freezer after another 30 minutes. Keep the pots in laboratory or open (whichever has lower temperature). It is desired that freezing treatment is completed before visible sunrise.

Maintain the txated plants under natural climatic conditions/alongwith control plants at field capacity for next about 10-15 days for recording observations in living and killed seeds. This period depends primarily upon the ambient temperature and humidity. A critical period has to be

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noted when there are apparent differences in living and killed seeds. The livings seeds are green and hard whereas the killed seeds turn dark brown and are watery. This period is very critical and visual judgement is the simple and best criterion. If observations are recorded too early, there may not be apparent differences in visible and killed seeds; if too late, all the seeds may turn brown. Freezing tolerance/susceptibility can be calculated from the per cent killed seeds.

$$\text{Per cent killed seeds} = \frac{\text{No.of killed seeds/siliqua}}{\text{No.of total seeds/siliqua}} \times 100$$

A variety will be freezing tolerant if less per cent of the seeds are killed by treatments. This method offers following advantages:

i) We need not grow the genotype in the pots. Non-experimental lines of genotypes grown in the field for other purpose may be used.

ii) Uniform stage may be maintained in a set of genotype to be treated during one time.

iii) Large population can be tested.

iv) Method is rapid, less expensive and less troublesome.

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INTERSPECIFIC HYBRIDIZATION IN BRASSICA JUNCIA× BRASSICA TOURNEFORTII USING OVARY CULTURE

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Interspecific hybridization provides a good tool to combine desired characters of two species. But usually the efforts fail due to incompatability barriars at different stages of development of embryo. Ovary culture could be an alternate way to save the interspecific hybrid. B.tournefortii is resistant to drought, Alternaria blight and white rust.The present study was undertaken to introduce these characters into B.juncea through hybridization and rescuing the hybrids using ovary culture.

Interspecific crosses were made between B.juncea cv.RH 30 and B.tournefortii at farm using usual emasculation and pollination method.After 5 to 10 days of pollination,ovaries were surface sterilized with 0.1 percent mercuric chloride for 5 to 7 minutes and thoroughly washed with sterilized distilled water three times.These were cultured on MS basal medium with different growth hormone combinations. The cultures were incubated in light at 26± 2° C. Within a period of 7 to 10 days ovaries started to elongate and bulged where the ovules were growing. Seed set was observed within 25 to 30 days.Seeds in mature pods ranged from one to three. In some pods no seeds were observed which may be due to failure of fertilization.

Culture response of ovaries was found dependent on days to excision of ovaries and different culture media tried (Table 1).It was observed that ovaries excised after 8 days of pollination were more responsive.Out of different media tried MS medium with IAA(2.0 mg/L), Kinetin (0.25 mg/L) Caesin hydrolysate(500 mg/L) was most effective to produce seeds. The ovaries excised before 8 days of pollination produced callus at the cut end and produced no seeds. The hybrid seeds were collected after 35 days of culture and will be evaluated for different agronomic traits in the field next year.

Table 1 Effect of different media on seed set in cultured ovaries.

Media	Total no. of ovary cultured*	Ovaries with seed set	Percent response	Average no.of seeds per siliqua
1 MS basal hormone free	150	48	32.0	0.25
2.MS+NAA(0.2mg/L) +BA(2.0mg/L)	128	72	56.3	1.30
3.MS+IAA(2.0mg/L) +Kin.(0.5mg/L)+ CH(500mg/L)	164	108	65.9	1.80
4 MS+BA(1.0mg/L)	142	61	42.9	0.45

* 8 days after pollination

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SEED TRANSMISSION OF SALT TOLERANCE IN REGENERANTS OF BRASSICA JUNCEA SELECTED IN VITRO

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We have earlier reported the existence of variation for tolerance to sodium chloride among somatic embryos of mustard, Brassica juncea (L) Czern & Coss (Cruciferae News Lr. 13 : 91, 1988). Such embryos tolerating 1·25% and 1·50% salt (equal quantities of sodium chloride and potassium chloride) have been selected, grown into plants, hardened in growth chamber and transferred to pots for maturity under natural conditions. The plants flowered normally and produced seed. Only one out of 54 plants was partially sterile. Selfed seed of the tolerant selections was collected and tested for salt tolerance. On half strength MS medium containing 1·25% sodium chloride the seed from tolerant selections has given germination as good as the control on salt free medium. The control seed fails to germinate on salt containing medium.

This study has shown that (i) In vitro selection for salt tolerance picks up genetic resistance and (ii) the resistance beside being genetic is stable and is transmitted through sexual cycle.

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IN-VITRO SELECTION OF PRIMARY EMBRYOS DERIVED from UV-TREATED MICROSPORES OF RAPID CYCLING *BRASSICA NAPUS* FOR HERBICIDE TOLERANCE

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We are currently evaluating microspore culture linked with mutagenesis (using ultra violet light (UV), X-rays and gamma radiation) as a means of generating novel variation using rapid cycling *Brassica napus* as a model. The present report concerns only the use of UV.

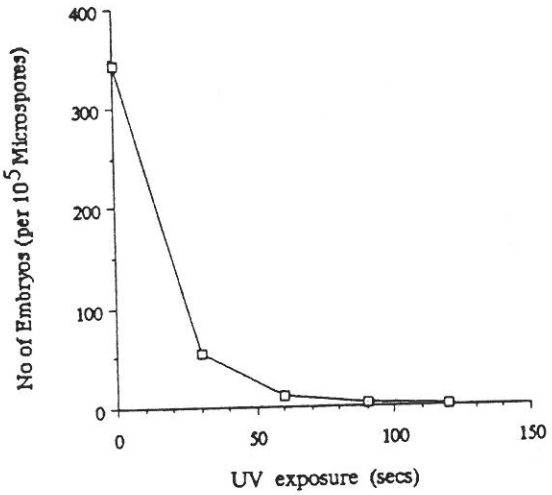

FIG.1. Embryo yield following irradiation of microspores of rapid cycling *Brassica napus* with ultra violet light.

The microspores were highly sensitive to UV(Ahmad et al, 1989), an exposure of 20 seconds being sufficient to give a 50 percent kill(Fig 1). Viability tests showed that death of the microspores was not immediate, but occurred during the 7 days of subsequent incubation. No evidence was seen of any morphological mutations induced by the UV-irradiation in embryos derived from treated microspores. A large number of regenerants has been established from embryoids and grown to flowering. These have set fertile seeds after selfing.

In preliminary experiments, the herbicide Chlorosulfuron is being used as an in vitro selection agent to identify mutants. Chlorosulfuron (CS), a product of E.I. du Pont de Nemours and Co., Wilmigton, Delaware, U.S.A., is sold under the trade name Glean^R . Tolerance to CS has been reported in yeast (Falco and Dumas, 1985), bacteria (Yadav et al, 1986), plants (Sebastian and Chaleff, 1987) and microspore culture systems(Swanson et al, 1988). Acetohydroyacid synthase (AHAS), which catalyzes the first step in the biosynthesis of the branched chained amino acids is the primary enzyme affected by CS in these organisms. Different tolerance mechanisms involving dominant, semidominant, and recessive mutations have been reported in the resistant plants (Swanson et al, 1988). To select for resistance to Glean primary embryos (2mm in length) were plated on MS medium (Murishige and Skoog, 1962) incorporating Glean, with 2% sucrose and solidified with 0.4% agarose. A kill curve was established and 100%

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embryo kill was obtained at 30 ug l^{-1} of Glean (Fig 2). This dose rate was used for subsequent screening.

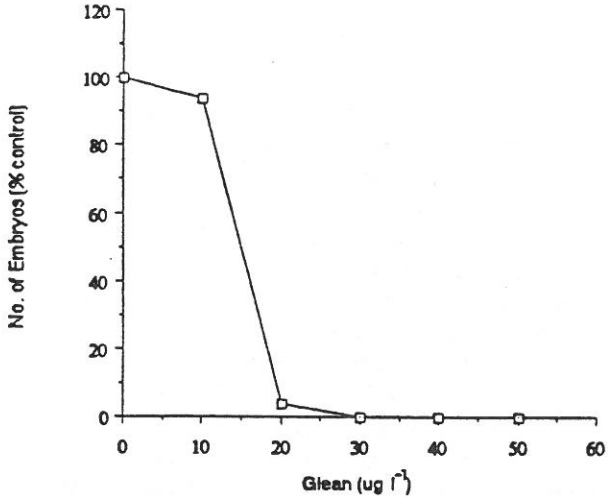

FIG.2 The kill curve for primary embryoids of rapid cycling *Brassica napus* three weeks after incubation on a medium containing herbicide Glean.

During the first selection cycle a number of embryos from various UV-exposures survived. However, there was no correlation between survival on Glean and exposure time to UV. In the second selection cycle all the surviving embryos from first cycle died except two which had been exposed to 30 seconds of UV. These were plated on a medium without Glean for the production of secondary embryoids. Six plants were regenerated, all of which had the characteristics of haploidy.Three plants were completely sterile. The other three produced some pollen and were selfed and have set seed. The progeny from these will be tested for Glean resistance. Indications are that the selection of embryoids will not give sufficient numbers for mutant detection. In further experiments therefore the selection agent is being incorporated into the microspore culture medium.

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RESPONSE OF THIN-LAYER FLORAL INTERNODE SECTIONS OF *BRASSICA OLERACEA* TO A RANGE OF SUCROSE AND MALTOSE CONCENTRATIONS.

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There has been recent interest in the use of maltose as an alternative to the more widely used sucrose as a carbohydrate source in plant tissue cultures (Kinnersley and Henderson, 1988). As part of our investigations into the stable and efficient regeneration of Brassicas from a range of explant sources and the applications of such techniques in genetic transformation studies a series of experiments investigating the use of alternative carbohydrate sources was undertaken. Longitudinal thin-layer sections were taken from floral internodes of glasshouse-grown plants of *Brassica oleracea* (accession number 87-001). The sections were surface-sterilised using the procedure of Brennan et al. (1989). The 5mm sections were blotted dry and placed cut-surface down on a series of media containing maltose or sucrose at levels of 30, 60, 90, 120 and 150 mM. The media comprised Murashige and Skoog (1962), 2µM BAP, 0.25µM NAA and 0.8% agar. Ten explants per cultivar were assessed at 7 day intervals and mean frequencies of shoot and callus production were calculated and tabulated below. All cultures were incubated at 25°C with a light intensity of 70 µe $m^{-2}sec^{-1}$ in a 16 h light, 8 h dark regime.

Table 1 - Effect of maltose concentration on shoot and callus frequencies.

medium	mean shoot frequency			mean callus frequency		
	7	14	21	7	14	21 (days)
30mM	0	0.13	0.17	0	0	0
60mM	0	0.63	1.00	0.10	0.38	0.38
90mM	0	0.13	0.17	0	0.13	0.13
120mM	0	0.63	1.33	0	0.50	0.50
150mM	0	0.25	1.33	0	0.38	0.38

It can be observed from Table 1 that the highest frequency of shoots observed is in the higher maltose concentrations, with the mid range treatment (90mM) giving the poorest frequency of shoot production. For callus production the highest figure was obtained from the 120mM treatment with no callus observed in the 30mM treatment.

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Table 2 - Effect of sucrose concentration on shoot and callus frequencies

medium	mean shoot frequency			mean callus frequency		
	7	14	21	7	14	21 (days)
30mM	0	0.75	1.67	0.20	0.38	0.38
60mM	0	0.25	1.33	0.10	0.38	0.38
90mM	0	0	0	0	0	0
120mM	0	0.88	2.67	0	0.75	0.75
150mM	0	0.13	0.33	0.10	0.50	0.50

From the data presented in Table 2 it can be seen that the 120mM treatment produced the highest frequency of shoot production with a mean figure of 2.67 per explant comparing with the maximum observed in the maltose series of 1.33 recorded in the 120 and 150mM treatments.
Similarly to the maltose data the mid-range treatment gave the poorest results (under the conditions employed) with no shoots or callus observed in this sucrose treatment at all.
Taking the combined data for the maltose and sucrose treatments gives mean shoot frequencies of 0.80 from maltose and 1.20 from sucrose, the data for callus frequencies is calculated as 0.28 from maltose and 0.40 from sucrose.

Experiments under same conditions using B.campestris produced only callus at varying frequencies throughout the assessment period.
The effects of carbohydrate source are thought to be both tissue and cultivar specific and further investigations into the mechanisms of action are in progress.

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IMPROVED EMBRYO RESCUE TECHNIQUE FOR INTERGENERIC HYBRIDIZATION BETWEEN *SINAPIS* SPECIES AND *BRASSICA NAPUS*

Regine Mathias

INTRODUCTION

Intergeneric hybridization is often used in breeding programs, mainly for the transfer of desired traits, e.g. pathogen resistance, from wild species to field crops. But often early embryo abortion prevents successful hybridization. The present paper describes an effective crossing and embryo rescue procedure that secures the production of intergeneric hybrids in the *Brassiceae*. The technique was developed for the crosses *Sinapis alba* x *Brassica napus* and *S. arvensis* x *B. napus*.

MATERIALS AND METHODS

Plants of the three species *S. alba*, *S. arvensis* and *B. napus* were grown in the greenhouse in a 20/15° C day/night temperature regime and a 16 h photoperiod in the fall of 1988. Buds were emasculated one day before flowering and pollinated twice the following two days. Pods were harvested 10-14 days after pollination and sterilized in 0.5% calcium hypochlorite + 0.1% sodium dodecylsulfate for 5 min. After three rinses in sterile deionized water pods were opened and fertilized ovules collected in a sterile petri dish. Ovules were cut transversally in halves under a stereomicroscope and the embryogenic sectors next to the hilum were transferred to 3.5 cm petri dishes with culture medium. The medium originally developed for microspore culture in *B. napus* (LICHTER 1982) was used, supplemented by an additional solid underlayer containing activated charcoal (MATHIAS 1988). Ovule sectors were incubated at 25° C in a 16 h photoperiod. After 7-14 days emerging embryos were transferred to 6 cm petri dishes with solid medium composed as above, but with only 2% sucrose and without activated charcoal. After further 2-3 weeks embryos were transferred to MS-medium (MURASHIGE and SKOOG 1962) with 2% sucrose and 1.25% agar in 50 ml culture flasks. Plantlets were transplanted to soil and covered with a plastic hood to prevent desiccation. After hardening they were treated with colchicine by submersion in a 0.34% solution for 2 h. Fertile inflorescences of hybrid plants were self-pollinated and either seeds harvested or ovule sectors cultivated *in vitro* to avoid embryo abortion.

RESULTS

Both intergeneric combinations were successful only with *Sinapis sp.* as the female parent and *B. napus* as the pollen donor; reciprocal crosses failed. Results of one crossing experiment are given in Table 1. Number of pollinated buds was not determined.

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Table 1: Number of harvested and fertile pods, fertilized ovules, embryos, primary hybrids and fertile amphidiploids from intergeneric crosses of *Sinapis alba* x *Brassica napus* and *S. arvensis* x *B. napus*

Combination	Pods total	Fertile pods	Fertile ovules	Emerging embryos	Primary hybrids	Amphidiploids
S. alba x *B. napus*	17	17	56	4	4	2
S. arv. x *B. napus*	34	9	19	2	2	1

DISCUSSION

The results indicate that harvesting hybrid pods 10-14 days after pollination might be too late, since only about 10% of the cultivated ovule sectors released embryos. Presumably the remaining ovules contained no or not viable embryos. In the cross *S. arvensis* x *B. napus* even 75% of all pods contained only shrivelled ovules, strongly indicating that early embryo abortion had taken place. For further experiments cultures will be started at earlier stages of development, e.g. 5-7 days after pollination. Nevertheless, the present technique is an effective tool for the production of intergeneric hybrids in the *Brassiceae*.

A similar procedure had been developed for interspecific crosses in the genus *Cuphea* (*Lythraceae*), showing high rates of embryo survival (MATHIAS et al. 1989). Evidently the culture of embryogenic ovule sectors in liquid culture medium allows to handle very young embryos. It conditions an effective nutrient exchange between developing embryos and surrounding culture medium. The solid underlayer serves as a nutrient reservoir and the included activated charcoal absorbs toxic components secreted by aborting tissue (JOHANSSON et al. 1982). The technique is supposed to be generally applicable to different species with minor modifications in e.g. sucrose content or incubation temperature.

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Embryo rescue of B. napus x Raphano Brassica hybrids

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Interspecific and intergeneric hybridization can be used to broaden the genetic base of cultivated species and for transfer of desirable agronomical traits from one crop to another through genome and cytoplasm substitution (Dayal 1986).

Raphanus (n=9) belongs to the subtribe Raphaninae. Intergeneric hybrids between Raphanus sativus x B. oleracea and Raphano Brassica x 2x and 4x Brassica species have been reported by Karpechenko (See Mizushima, 1980). Tang and Williams (1988) have studied the production of hybrids between rapid cycling Brassica sp. and Raphanus. Primary hybrids and amphidiploids between Raphanus sativus and the two diploid Brassica sp. B. campestris and B. oleracea have been produced having large vegetative vigour and very high field resistance to powdery mildew (McNaughton and Ross, 1978). However, they suffer from reduced fertility caused by genetic imbalance between parent genomes though partially homologous relationship between genomes of Brassica and Raphanus has been shown (Mizushima 1980).

Brassica napus (n=9) is the amphidiploid derived from the hybridization of B. campestris and B. oleracea. It is a very high yielding variety but losses in yield occur due to shattering of pods during harvesting. In order to attempt introduction of shattering resistance in B. napus from Raphanus, Raphano Brassica, obtained by crossing Raphanus with B. oleracea, was used as a bridging material and used as a male parent donor to B. napus.

The artificially fertilized B. napus ovaries started withering after 10-15 days when left on the plant. B. napus ovaries were, therefore, pollinated with Raphano Brassica and excised 5 days after fertilization and cultured on MS medium supplemented with Kinetin 1 ppm, Napthalene acetic acid 0.1 ppm, Gibberellic acid 1 ppm and Casein hydrolysate 10 ppm. After 15 days the ovaries were dissected out and the enlarged embryos cultured on the same medium. For further growth the hormone concentrations were reduced to one-tenth of the previous value and plants grown to maturity. 1% colchicine treatment of apical buds failed to give rise to doubled chromosome number. The hybrids had chromosome no. 37 and were male sterile.

About 10 viable plants were raised which showed intermediate morphological characteristics with leaves resembling B. napus and white flowers resembling Raphanus. DNA from B. napus, Raphano Brassica and the hybrids was analysed using B. campestris specific repeat DNA probe pA1-7 (developed in our laboratory) and Raphanus specific DNA probe p 337 (a kind gift from Prof. Delseny). The hybridization pattern showed the presence of both Brassica and Raphanus genomes in the hybrids. Wide hybridization may give rise to matromorphic plants (Eenink 1974). We obtained matromorphic plants of Moricandia arvensis and Eruca sativa by hybridization with Brassica sp. (data not reported here). The DNA analysis of the resultant hybrid is, therefore, important to establish that it is truly a hybrid with DNA from both parents.

This A1 hybrid was back crossed with B. napus and a few seeds were obtained. The plants are also being multiplied in vitro by micropropagation to obtain a sufficient number of plants. They will be backcrossed with B. napus for several generations to screen for shattering resistance under field conditions.

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EXOGENOUS FACTORS AFFECTING EFFICIENCY OF *BRASSICA NAPUS* RESYNTHESIS BY MEANS OF *IN OVULE* EMBRYO CULTURE.

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Recently Sacristan & Gerdemann (1986) and Diederichsen & Sacristan (1988) demonstrated *in ovule* embryo culture to be a suitable way to overcome interspecific incompatibility in the genus *Brassica*. We used this technique to produce self-incompatible *B. napus* by crossing *B. oleracea* and *B. campestris* lines homozygous for different S-alleles. In order to improve the culture medium, the effect of the following factors on the efficiency of resynthesis was tested.

a. sucrose concentration (10, 30 and 80 g l^{-1})
b. casein hydrolysate (0, 200 and 500 mg l^{-1})
c. gelling agents (8 g l^{-1} Difco-Bacto Agar vs. 2,5 g l^{-1} Gelrite, Gellan Gum)

The trials were carried out with different *B. oleracea* x *B. campestris* and reciprocal crosses from our resynthesis program. Ovules of one pod were distributed equally between the different concentrations or gelling agents respectively, to avoid single-pod effects. MS-11 with no hormones was used as the basic medium. The results are given in table 1:

	No. of pollinated buds A	No. of ovules cultured	No. of hybrid plants B	$B/A \times 100$
1 a. Sucrose concentration (16 crosses)				
10 g l^{-1}	49	243	42	85.7
30 g l^{-1}	49	249	35	71.4
80 g l^{-1}	49	245	25	51.0
1. b. Casein hydrolysate (17 crosses)				
0 mg l^{-1}	38	345	34	89.5
200 mg l^{-1}	38	345	30	79.0
500 mg l^{-1}	38	352	31	81.6
1. c. Gelling agents (26 crosses)				
Difco-Bacto	50	536	23	46.0
Gelrite	50	536	45	90.0

Sucrose concentrations higher than 10 g l^{-1} are not advantageous for ovule culture in *Brassica*. Our investigation was carried out with ovules removed 22-28 days after pollination. Younger ovules (Kameya & Hinata 1970) or ovules

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of other cruciferous species like *Capsella bursa-pastoris* (Monnier & Lagriffol 1985) have been found to need higher sucrose-concentrations, probably to suit their special osmotic requirements.

Casein hydrolysate, often found useful for ovary culture of interspecific *Brassica* hybrids (Inomata 1985), proved to be ineffective in the range of the tested concentrations.

Compared to Difco-Bacto Agar, Gelrite significantly increases the rate of hybrid plants. This might be due to a higher purity or the lower concentration of this gelling agent. Koda et al. (1988) found Gelrite superior to other gelling agents for plant regeneration from protoplasts of red cabbage.

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HIGH FREQUENCY REGENERATION FROM CRUSHED APICAL BUDS IN *Brassica juncea* L.

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Selection of desired somaclonal variants requires high frequency regeneration in desired crop species.

To get high frequency of regeneration in *B. juncea* cv. RH30 was undertaken as a model system. The terminal buds were isolated from 7 to 10 days old seedlings. The buds were crushed to give multiple injuries and were cultured on two different modifications of MS medium[1], namely Y medium (MS + 0.05 mg/l NAA) and Z medium (MS + 0.2 mg/l NAA + 2 mg/l Kinetin). Callus obtained from the crushed buds gave 136.67% regeneration on Z medium. This may be because primordial buds get dispersed in the medium and initiate more adventitious buds which subsequently form multiple shoots.

The regenerated young shoots were recultured with or without callus on these media. Their basal ends produced callus which in turn differentiated into multiple shoots[2]. After three recultures both on Z and Y medium 102 plants were obtained from a single cultured bud. So the total regeneration percentage at the end of third reculturing was as high as 13,940.34 per cent. The regenerated plants showed morphological as well as cytological variation[3].

Above procedure of high frequency regeneration would greatly boost the selection of desired genotypes of *B. juncea* from somaclonal variants.

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A COMPARISON OF THE REGENERATION EFFICIENCIES OF DIFFERENT EXPLANT SOURCES USING A RAPID-CYCLING ACCESSION OF BRASSICA OLERACEA.

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The use of rapid-cycling Brassica lines has great potential in genetic transformation studies, the short generation time representing a valuable experimental tool (Williams and Hill, 1986). Key factors in the Agrobacterium mediated transformation of Brassica are efficient regeneration systems and the choice of explant to target the transformation process to meristematic tissue. In this investigation four explant sources were analysed for their potential regeneration efficiencies. The explant sources used were 2mm internodal sections bisected longitudinally, 4.0mm diameter leaf discs, 2mm longitudinal petiole sections and 5mm thin-layer sections taken from the floral internodes. All material was taken from in vitro grown plants. The media used was either D1 - comprising Murashige and Skoog(1962), 2% sucrose, 10mg/l BAP and 1.0 mg/NAA or PS1 - comprising Murashige and Skoog, 2% sucrose, 4.0 mg/l BAP and 0.5 mg/NAA. Both media were supplemented with 0.8% Difco agar. Ten explants per plate were set up, the plates sealed with Nescofilm and all plates kept at 25 C in a 16:8 hour light:dark regime with a radiant flux density of 70umol. m sec . After three weeks the plates were assessed for shooting efficiency per treatment calculated as number of shoots per plate divided by number of initial explants to give comparative data. The results are presented below:-

	Medium PS1	Medium D1
internodal sections	10.70	7.20
leaf discs	0.42	0
petiole sections	1.00	0
floral sections	1.25	2.50

Callus was also formed on up to 75% of the leaf discs, petioles and floral explants but not from internodal sections on media PS1. On medium D1 callus was formed on up to 75% of all the explants tested. Roots were recorded from petiole explants on both media.
The results suggest that under the conditions employed the use of longitudinal internodal sections is the most efficient rapid regeneration system and highly suitable for use in transformation studies.

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HAPLOID PRODUCTION IN RAPID-CYCLING BRASSICA CAMPESTRIS AND B. NAPUS

F.N. Aslam, M.V. MacDonald and D.S. Ingram

Rapid-cycling populations of the major Brassica species have been developed in the University of Wisconsin-Madison and are now well established as models for research in genetics and plant pathology. However, little is known of the tissue culture potential of this material. The present study was concerned with the production of haploids of rapid-cycling Brassica campestris and B. napus by anther culture and microspore culture.

Initially methods were developed for successful anther culture (Aslam, MacDonald & Ingram, 1989a). In the case of B. campestris, best results were obtained when: anthers were excised from 1.6-2.00 mm buds with the pollen at the mid-uninucleate stage of development and were cultured on a solidified Brassica anther culture medium of Keller & Armstrong (K); and incubation was at 35°C for one day, followed by 30°C for one day and then continuous incubation at 25°C. The response to anther culture of B. napus was generally very poor compared with that of B. campestris, but the best results were obtained when: anther donors were grown at 18°C with continuous light; anthers were excised from 2.1-2.5 mm long buds with the pollen at the mid-uninucleate stage of development; anthers were cultured on a liquid formulation of K medium; and incubation was at 35°C for one day, followed by 30°C for six days and then continuous incubation at 25°C.

Once a suitable protocol for anther culture had been established, a study was made to determine the feasibility of producing, by inbreeding and selection, lines of rapid-cycling B. campestris with defined potential for anther culture (Aslam, MacDonald, Loudon & Ingram, 1989). Since the base population of rapid-cycling B. campestris is self incompatible, inbreeding was achieved by a combination of bud-pollination and the application of pollen to the cut surfaces of decapitated stigmas. Three inbred generations were raised, and in each generation plants were selected for high or low ability for anther embryogenesis. The proportion of viable pollen present in anthers, as indicated by a fluorochromatic reaction and a germination test, was also determined at each stage. Lines of rapid-cycling B. campestris with clearly defined high or low potential for anther embryogenesis were isolated in these experiments. Within each line, however, continuous variation was always observed. Pollen viability and anther efficiency were not correlated. Although inbreeding depression caused a significant decrease in pollen viability over the three generations, there were no obvious deleterious effects on anther efficiency. In general, over the three generations of inbreeding, no segregation in plant morphological characters was observed, although many developmental abnormalities were seen in the third inbred generation and there was a marked reduction in the number of seeds set. No association between plant vigour and high or low anther efficiency was noted.

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All plants regenerated from anther embryoids of rapid-cycling B. campestris were haploid. By treating anther-derived embryoids, axillary buds and whole plants with colchicine, dihaploid plants were produced, but these failed to set seed after self pollination. The diploid nature of the plants was confirmed, however, when they produced normal seeds after cross pollination with plants of the base population.

It was concluded that although the possibility existed for the selection of genetically defined lines of B. campestris with respect to anther culture potential, this was rendered impracticable by the self incompatibility of the species. It was therefore decided to repeat the selection experiments using B. napus, a self compatible species. In contrast to the observations for B. campestris, the anther culture potential of the plants of successive inbred generations of B. napus remained uniform, and anther efficiency was poor. This negative response to selection may have been due to an absence of variation with respect to anther culture ability in the base population, resulting from the self fertility of the species. Cytological studies of cultured anthers of B. napus indicated that in each generation there was a poor correlation between pollen induction and embryoid production.

In an attempt to improve the yield of haploid embryoids of B. napus, isolated microspore culture was carried out according to the methods of Keller (pers.comm.), which were similar to those of Chuong & Beversdorf (1985). This was found to be much more efficient than anther culture: anther efficiencies of up to 166,000 per thousand anthers used were recorded for microspore culture, compared with a maximum of 476 with anther culture of the same plant (Aslam, MacDonald & Ingram, 1989b). In experiments designed to ascertain the reasons for such differences, an inhibitory effect of the anther wall on the anther embryogenesis of B. napus was observed, and embryoid yields were improved by centrifuging buds prior to anther extraction to simulate the effects of the centrifugation which is a component of the microspore extraction procedure.

The culture of isolated microspores was found to be useful for the study of patterns of development of haploid embryoids. Comparative experiments showed that the addition of activated charcoal to the culture medium encouraged normal development in microspore-derived embryoids.

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FACTORS AFFECTING EMBRYO PRODUCTION FROM MICROSPORE CULTURE OF BRASSICA NIGRA (KOCH)

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Black mustard haploids can be obtained by anther (Govil et al, 1986, Leelavathi et al, 1987) or microspore culture (Hetz and Shieder, 1989). This latter could be more promising for haploid production. Experimental design was the same as that used for rapeseed (Lichter, 1982). The genotype used was a german population-variety. A preliminary study aimed at establishing a precise definition of factors determining embryo production from microspores.

1. Conditions of plant growth temperature

Five experiments under varying temperature conditions produced the following results : field, 5/10°C, 10/15°C no embryo, green house 0.25 to 1 embryo/bud, 20°C 6 embryos/bud. Sixtheen hours photoperiod was used under controlled temperature.

2. Definition of optimum stage for beginning culture

The most favourable stage for beginning culture is that immediately preceding the first pollen mitosis. This was detected by staining with DAPI (4, 6 Diamidino - 2 Phenylindole) for counting number of nuclei per microspore (Coleman and Goff, 1985). For more precise definition we used DAF (fluoresceine diacetate) for determining the size of the vacuole (Knox, 1984), which attains a maximum before the first pollen mitosis.
A correlation could be established between cytological and morphological observations ; optimum stage has been found for petal lenght/stamen lenght ratio of 1/2 to 1 on plants cultivated in the field. Even if correlation is always good, it is essential to redefine the petal/stamen lenght corresponding to the optimum stage each time conditions of cultivation are modified.

3. Deviation towards embryogenesis by temperature stress after beginning of culture

The stress chosen to make the microspores deviate from their normal maturing process was a thermal shock. Intensity and duration of the shock were varied. (Table 1).
A thermal shock of 7 days duration at 32.5°C gave the best results. However, the heterogenous nature of results from microspores grown in petri dishes under identical stress conditions led us to examine another parameter : microspore concentration.

4. Concentration of microspores in culture

Microspore concentration was assessed by counting on Malassez cells. The results obtained for the different concentrations studied are shown in Table II.
The optimum concentration is thus $1.5.10^5$ microspores/ml.

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5. Role of active charcoal

Only the addition of a drop of active charcoal solution (1 %) (Lichter, comm. pers.) allowed us to obtain embryos. In the absence of this stimulus the process is blocked at the 4 to 8-cell stage. Its seems to act as a toxin trap and thus improve organogenesis.

These experiments suggest that it is possible to obtain B. nigra embryos by microspore culture, with a production rate of 5 to 6 embryos per bud. Regeneration of plants seems to present some difficulty, and is currently under study.

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Table I : Embryo production per bud after different thermal shocks (test carried out on 192 buds)

Duration	12 hours	24 hours	7 days
30°C	0.25	1	1.25
32,5°C	-	0	5.66

Table II : Embryo production per bud as a function of initial microspore concentration (experiment carried out on 256 buds)

Microspore concentration per ml	1.10^5	$1.5.10^5$	2.10^5
Number of embryos per bud	0	5.75	0.25 à 1

Direct Embryogenesis and Plant Regeneration through Microsporeculture of *Brassica nigra*

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Haploid plants are useful tools for the selection of desirable characteristics in plant breeding programs. The production of haploid plants of *Brassica napus* from isolated microspores or via anther culture is well documented (Keller et al. 1977, Lichter 1982). In 1989 Lichter reported that it is possible to obtain haploid plants from *B. nigra* through microspore culture. The aim of this study was also the induction of embryogenesis from isolated microspores of *Brassica nigra* and the regeneration to plants.

Donor plants of *B. nigra*, lines 460 and 2051, were cultivated in the greenhouse. The temperature ranged from 24° C at day to 18 ° C at night with a photoperiod of 16 h. Two times a week the plants were fertilized with a 0.2 % solution of a N/P/K fertilizer. The plants grew up during spring and the first buds developed in May.
Buds of about 1.5 mm length were collected before the first blossom opened and were surface sterilized for 15 min with 5 % natrium hypochloride, washed three times with sterile destilled water. For cold treatment the buds were transferred into 60 mm Petri dishes containing 5 ml of a 12 % sucrose solution supplemented with 1000 mg/l glutamine and 100 mg/l inositol and were stored at 4 ° C for three days (Lichter 1982). For all experiments only anthers possessing uninucleate microspores were used.
From 40 buds, microspores were isolated (Lichter 1982) and suspended in 4 Petri dishes (35 mm diameter) containing 1.5 ml modified culture medium according to Lichter supplemented with 12 % sucrose, 0.5 mg/l NAA and 0.05 mg/l BAP (Mathias 1987). The obtained density was about 2,2 x 10^5 microspores/ml.
For induction of embryogenesis the microspores were incubated in darkness at 32 ° C for 2 days. Afterwards they were transferred into an incubator at 25 ° C and 3000 lux light intensity.
Embryoids were further cultivated in liquid MS medium supplemented with 2 % sucrose but lacking any hormones. Embryoids in a further stage and shoots were cultivated on solid hormone free MS medium supplemented with 2 % sucrose and 0.8 % agar. After root regeneration shoots were cultivated in soil and transferred to the greenhouse.

The development of embryoids from *Brassica nigra*, line 2051, was similar to that found in the related species *B. napus* (Lichter 1982). At the third day of culture many microspores were rounded up and looked like protoplasts and first divisions could be observed. After 6-8 days some of the divided microspores formed nascent embryoids. Ten days later these embryoids formed heartshaped greenish structures.

In one experiment, 139 embryoids developed from about 2.2 x 10^5 microspores (240 anthers/40 buds). The embryoid/anther ratio was 0.58.
Three weeks after starting the experiments, 32 of the 139 embryoids were nearly 3 mm in diameter and showed a clear bipolarity. In this stage the 32 embryoids were subcultivated in a liquid MS medium, and 10 days later, the embryoids were placed on a solid MS medium. After about 3 months 13 shooted clones could be regenerated. The shoots were cut off and after root regeneration on hormonfree agar medium, they were transferred into soil. The cytological analysis revealed that all 13 clones were diploid.

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As shown in Table 1, the reproducibility of the experiments was weak. Embryoid formation was only observed in two out of seven experiments.

Table 1: Number of embryoids, subcultured embryoids and clones formed by microspore culture with *B. nigra*, line 2051. Results of experiments carried during July/September are shown.

Culture start	Cold treatment days	Cell divisions	Embryoids	Subcultured embryoids	Clones
01.07.	-	+	3	3	-
09.07.	3	+	139	32	13
14.07.	3	+-	-	-	-
14.09.	-	-	-	-	-
26.09.	3	-	-	-	-
27.09.	-	-	-	-	-
30.09.	-	-	-	-	-

+ : Cell-divisions observed in high rate
+- : Cell-divisions observed in low rate
- : No cell-divisions

Within the first 3 days the microspores of line 460 behaved similar as found for line 2051. The microspores rounded up and some showed first cell divisions, but after six days no further development could be obtained.

Very essential for the success of microspore culture was the physiological stage of the donor plants and the uninucleate stage of the microspores. Also the influence of the season has to be considered. The most efficient culture time seems to be in spring. The successful microspore cultures (Tab. 1) derived from plants which were grown up during May/June. The high light intensity and the lower temperature at this time may be advantageous in comparison to the conditions in August/September. Cold treatment for 3 days at 4° C was helpful (Lichter 1982) to increase the rate of embryoids but most important in microspores culture experiments is the genotype of the donor plant as also demonstrated in this study with the *B. nigra* lines 2051 and 460.

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PLANT REGENERATION FROM BRASSICA OLERACEA VAR. ITALICA (BROCCOLI) PROTOPLASTS

M Girmen, R Backes and J Grunewaldt

Introduction

Since the first success of Kartha et al. (1974) a wide range of Brassica species could be regenerated out of protoplasts. As broad as the genotypic variability used are the different protoplast sources, namely leaf mesophyll, hypocotyl, and root.
Among the cultivated Brassica, Broccoli has a very short generative reproduction cycle. Together with its cross ability with most of the economic important Brassicae, Broccoli is an excellent tool for genetic engineering. Regards cell organelle or direct gene transfer the regeneration of plants out of protoplasts is, however, a prerequisite.
As regeneration of Broccoli plants out of leaf (Glimelius 1984) and cotyledon (Robertson and Earle 1986) protoplasts is known, we report in this communication on the regeneration of Broccoli plants out of hypocotyl protoplasts.

Materials and Methods

Seeds of cv. Cruiser were sterilized for five minutes with 3 % Na-hypochlorite and 0.2 % Tween in water solution, rinsed three times in sterile water and sown in Petri dishes containing an agar medium with the Murashige and Skoog macro and micro elements plus 30 g/l sucrose. Plants were raised in the dark at + 26° C within 7 days.

Hypocotyls were harvested, cut into 3 to 4 cm long segments, and incubated in the dark at + 33° C for 16 to 20 hours in an incubation medium. This consists out of the KM 8p macroelements, organic acids and sugars (Kao and Michayluk 1975), the B5 microelements and vitamines (Gamborg et al. 1968), 0.5 % cellulase Onozuka R-10, 0.1 % Macerozyme and 70.1 g/l mannit. The medium was filter sterilized (0.2 µm), the pH adjusted to 5.7 and the osmolarity to 525 mOs/kg H_2O. After digestion protoplasts were released by meshing, followed by a three times washing and centrifugation at 150 g for 5 minutes in 50 mmol sucrose, 300 mmol $CaCl_2$, 252 mmol KCL, 2 mmol NH_4NO_3 and 1 mmol KH_2PO_4.
Protoplasts were plated in 0.25 % agarose in Petri dishes at a concentration of 3 x 10^4/ml on the protoplast incubation medium supplemented with 0.4 mol glucose, and per liter 1.0 mg 2,4-D, 1.0 mg NAA, 0.5 mg BAP and 0.5 % agarose.
The incubation in the dark was for 8 days at + 33° C. Before transferring the Petri dishes into 1,000 lux light conditions 2.5 ml of callus induction medium (Table 1) were added but removed after 2 hours.

Micro calli were harvested after two weeks and transferred to the callus induction-medium and cultivated for 3 weeks. After this, calli of about 2 to 3 mm were transplanted on plant regeneration-medium (Table 1) and subcultured at + 26° C and about 2,000 lux in a 16 hours day length. Regenerated shoots were transferred on MS medium without hormones.

Results and Discussion

The isolated hypocotyl protoplasts entered first cell divisions within two days. After another eight days micro calli appeared which reached the size of 2 to 3 mm in three weeks. The transplanted micro calli be-

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came green on regeneration medium and shoots appeared in about 26 % of green calli eight weeks after protoplast isolation. Multiple shoot formation was observed in about 17 % of green calli. As far as analyzed the regenerates develop into normal appearing plantlets.

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Table 1. Callus induction and plant regeneration-media

Basic medium:

Macroelements	mM
$NaH_2PO_4 \times H_2O$	1.087
KH_2PO_4	0.367
$CaCl_2 \times 2\ H_2O$	6.121
KNO_3	24.725
NH_4NO_3	3.123
$(NH_4)_2SO_4$	1.891
$MgSO_4 \times 7\ H_2O$	1.014
$FeSO_4 \times 7\ H_2O$	0.1
Na_2 EDTA	0.1

Microelements according to B 5

Vitamines according to B 5

Growth regulators:

Phytohormones	Callus induction-medium	Plant regeneration-medium
2,4-D	0.45 µM	0
NAA	2.68 µM	0.29 µM
GA_3	0	0.57 µM
BAP	4.44 µM	0
Zeatin	0	9.12 µM
Sucrose	100.0 mM	29.21 mM

CALLUS FORMATION AND PLANT REGENERATION FROM PROTOPLASTS OF RAPID-CYCLING *BRASSICA CAMPESTRIS* L.

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Plantlet regeneration from protoplasts of *Brassica campestris* remains difficult, although progress has been made in determining the optimal growth regulator sequence (Yamagishi et al., 1988). Recently we have developed a simpler and more reliable culture system for regeneration of protoplasts of *B. oleracea*. Protoplasts are plated on a filter membrane over a *B. campestris* feeder layer (Lentini et al., 1986), which in turn has been plated on solidified media from the Pelletier series (Pelletier et al., 1983). In preliminary experiments this method also has permitted easy and reliable formation of callus from protoplasts of *B. campestris*, as well as regeneration of a plant that has flowered *in vitro* for almost 4 months.

In the experiment described here, the plant material is a self-compatible line of rapid-cycling *B. campestris* (CrGC#66). Media B, C, E and F of the Pelletier series were used in sequence, all solidified with 0.22% gelrite (Scott Labs). Seeds were surface-sterilized and grown on gelrite-solidified LS medium (Linsmaier and Skoog, 1965) with 3% sucrose in the dark for five days. Hypocotyls were then harvested and protoplasts isolated as in Jourdan et al., 1989. A total of 7.55×10^5 protoplasts were obtained from 895 mg of hypocotyl tissue. 2.55×10^5 protoplasts were resuspended in 0.5 ml medium B of the Pelletier series and pipetted onto a filter membrane (Millipore # AABG 047 SO, Type AA, 0.8 µm pore size). This membrane was on top of a cell-suspension of *B. campestris* that had been plated onto gelrite-solidified medium B several days earlier. After ten days in the dark, the membrane was transferred to a new feeder layer of the *B. campestris* callus plated on medium C and placed in the light (80 $\mu E/m^2/s$). Two weeks later, it was transferred again to a new feeder layer on medium C. After two to four more weeks, individual calli of approximately 1 mm diameter were picked off the membrane with forceps and placed on medium E, upon which growth was rapid. A total of 198 calli were transferred individually (colony formation of ~0.1%). Most of the calli were subsequently transferred to medium F (approximately 7 weeks after protoplast isolation).

Nine weeks after protoplast isolation, a very pale green shoot bud was visible on the top side of one of the calli (which also showed root morphogenesis). Although this was the only callus to form a shoot, 80 calli (40%) exhibited root morphogenesis. The shoot was transferred to gelrite-solidified LS medium with no growth regulators, in a glass baby food jar with a plastic lid (Magenta Corp.), 15 weeks after protoplast isolation. A dense mat of roots was produced, but leaf production was limited to two small strap-like leaves, followed by many flower stalks. Flower buds were visible by 17 weeks after protoplast isolation. A few flowers showed somewhat normal development, but most were aborted early, perhaps due to ethylene build-up in the jar (Lentini et al., 1988). Several siliques were formed, although no seed was produced (*in vitro* pollination was not attempted). To date, 8 months after protoplast isolation, the plantlet is still growing and producing flower buds *in vitro*, although vigor has declined somewhat; the shoot, now divided into several clumps, has not regenerated new roots after they were cut off the second time, and the flowers are aborting earlier, with no more siliques being formed. The shoot is transferred to new LS medium approximately once every 4 weeks. All other calli were discarded by 4 1/2 months after protoplast isolation, as they began to turn brown with no sign of shoot morphogenesis.

Similar experiments using this material and another line (atrazine-resistant *B. campestris* ssp. *oleifera* cv "Candle") have resulted in comparable callus formation from protoplasts. The greatest benefit of this modified culture method seems to be in the early stages of cell colony development. Previous work with regeneration of *B. campestris* in our laboratory, in somatic hybridization experiments involving hypocotyl protoplasts of the

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cultivar "Candle" (Robertson et al., 1987; Jourdan et al., 1989), resulted in much poorer early growth. Using *B. campestris* alone in control experiments, few colonies developed and no plant regeneration was obtained (Jourdan and Earle, 1989). (Interestingly enough, several *B. campestris* parental escapes did regenerate from a fusion experiment). The series of media developed by Pelletier et al. had also been used almost without modification (Robertson and Earle, 1986) in the previous experiments, but with the sequence through medium C involving liquid culture in 24-well plates, and no membrane or feeder layer used.

Although in current experiments only one *B. campestris* plantlet has been regenerated, it is likely that the regeneration efficiency would be improved by adjusting the growth regulator content of the media for the callus proliferation and shoot induction stages for the specific requirements of *B. campestris*. Efficient shoot regeneration from protoplasts of rapid-cycling *B. campestris* would be of use in basic studies and as a breeding tool.

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IN VITRO CULTURE OF BRASSICA OLERACEA L. VAR. CAPITATA (K.K. CROSS)

Neera Pradhan and S.B. Rajbhandary

Brassica oleracea L. var. capitata is used as vegetable. The seeds were of F1 generation and were imported from Japan. Since tissue culture offers a possibility of obtaining a large number of clonal plants, we have attempted to clone the hybrid plants via organogenetic pathway. This paper deals with the formation of multiple shoots from hypocotyl culture with subsequent non-sterile rooting in sand and field establishment.

Seeds were surface sterilized with 0.1 ppm mercuric chloride solution for 10 minutes and grown aseptically on MS medium (Murashige and Skoog, 1962). After 10 days of incubation, hypocotyl explants were cultured on MS medium containing different supplements. After 10 to 12 weeks of explant culture, multiple shoot proliferation was observed in MS medium supplemented with BAP 1.0 ppm and NAA 0.01 ppm. The number of shoots varied with the NAA concentration. In NAA 0.1 ppm and BAP 1.0 ppm the number of proliferated shoots varied from 10 to 15 whereas in NAA 0.01 ppm and BAP 1.0 ppm it varied from 25 to 30.

Rooting was done by treating the excised microshoot pieces in 0.1 ppm indolacetic acid for 5 to 10 minutes. The treated shoots were rooted in non-sterile sand box. The sand boxes were covered with polythene sheets to maintain high humidity. Roots were visible in 10 to 12 days after transfer to sand. The rooted plants were successfully established in the field. Head formation was observed in more than 90 percent of the plants.

So this method provides an excellent means of mass propagation of hybrid plants. The direct non-sterile rooting of multiple shoots apparently makes the production of tissue cultured plants cost effective.

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CYTOPLASM SUBSTITUTION INCREASES REGENERATION RESPONSE IN TWO ALLOPLASMIC BRASSICAS

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Our earlier work has shown that both nuclear and cytoplasmic genes influence regeneration in the Brassicas (Narasimhulu et al, 1988; 1989). In continuation of this work we have investigated regeneration responses of two alloplasmic Brassicas synthesised for evaluating male sterility resulting from nuclear cytoplasmic interactions. The alloplasmic Brassicas used in the study combined nucleus of B. campestris cv. Pusa Kalyani or that of B. juncea cv. Pusa bold with the cytoplasm of B. oxyrhina. Comparison of regeneration responses were made for the alloplasmic lines, their respective parental controls and amphidiploid of B. oxyrhina and B. campestris Regeneration responses were assessed on MS medium supplemented with four hormone combinations that evoked regeneration in unsubstituted B. campestris cv. Pusa Kalyani. Mean regeneration responses recorded on the conclusion of the fourth week in these media indicated that alloplasmic B. campestris regenerated with approximately three fold frequency compared to that of B. campestris cytoplasm. Similarly regeneration in B. juncea was over 50% higher compared to that in unsubstituted parental line. B. oxyrhina and amphidiploid of B. oxyrhina x B. campestris failed to regenerate. The failure of amphidiploid to regenerate suggests that the inhibitory effect of nuclear genes of B. oxyrhina cannot be neutralized by the positive influence of B. oxyrhina cytoplasm. The present study demonstrates the potential use of cytoplasm substitution for improving regeneration frequency in recalcitrant systems

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IN VITRO PROPAGATION OF MALE STERILE CHINESE CABBAGE FOR HYBRID SEED PRODUCTION

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Chinese Cabbage is one of important vegetables in China. High yield,uniformity and high resistance have been achieved through the development of F1 hybrids. A new method for hybrid seed production is described.

F1 hybrid has been produced by using self-incompatible lines or genetic male sterile lines. Although hybrid production using S-alleles has been highly successful, continuous selfing(bud pollination) results in low vigor of inbred parents, and the maintenance and multiplication of parental lines is laborous and time consumming. Some genetic variations may cause the self-incompatibility unstable. For these reasons, we have used single recessive genetic male steriles to produce F1 hybrids(Niu et al. 1980). Parent seed can be easy to obtain by sib-mating sterile plants with fertile plants of the same line; their parents are more vigorous. However, hand-roguing of half male-fertile plants from female lines is required prior to the onset of bee pollination. It is not only laborous but also low in seed yield.

In order to overcome the above problems, an in vitro technique for F1 seed production has been developed. The method has many advantages: 1) High quality of 100% hybrid seeds is ensured because the population of female line is from a single male sterile plant, the male sterility is absolutely stable; 2) The parental plants are more vigorous and uniform, they don't exhibit depressed vigor and genetic variations; 3) It is easy to obtain male sterile plants, so there are rich parental resources; 4) The parent lines can be produced in large scale under controlled conditions; 5) Genetically identical plants can be produced from a male sterile clone. Therefore,it is possible to produce hybrids in early generation of selfing lines.

88-13 is our best basic medium among 9 experimental media. The results of experiments with various combinations of NAA,IAA,2.4-D,6BA and KT gave their optimum components and concentrations in media.

All culture can be maintained at 18-24 C/day, 10-15 C/night and 10 hours light a day at 2000 Lux with cold white fluorescent tubes.

Seeds of male sterile AB lines must be sterilized with 70% alcohol for 5-30 seconds, then with liquor benzalkoni bromidum for 5-6 minutes and with HgCl2 for 8 minutes, finally thorougly rinced 3-4 times with sterile distilled water.

The seeds are transplanted to basic medium containing 3.0-5.0 mg/L 6BA, 0.1-0.5 mg/L NAA germinating for a week.

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Seedling shoot tip explants are used to initiate shoot culture. Explants are inoculated onto basic medium containing 2.0-4.0 mg/L 6BA and 0.1-1.0 mg/L NAA. A subculture can be conducted per 10-20 days.

Shoots from the stock culture are transferred to basic medium containing 1.0-4.0 mg/L 6BA; 0.5-1.0 mg/L NAA for rooting. After 15-30 days, the rooted plantlets can be transplanted to plastic pots filled with mix of vermiculite and garden soil under 25 C/day, 10 C/night.

Seeds from male sterile AB line will segrate 50% fertile plants. Therefore we must determine whether the clone is male sterile or fertile. 5-10 rooted plantlets for each clone are vernalized at 5 C for about 30 days, then transplanted to the plastic pots for flowering. Only male sterile clones are maintained.

F1 hybrid seeds were produced by using in vitro propagation of male sterile chinese cabbage in 1989. The parents were male sterile clone from 'Xiao Bao Kou' AB line, an early type and E9 inbred line.

In order to determine the optimum trasplanting date in Beijing, the plantlets were transplanted to plastic pots on Jan. 21, Feb. 4 and 16, 1989 respectively. Some of them were transferred to greenhouse for a week, then moved to seedling bed with glass covers and windbreaks. The others were directly put on the seedling beds. Seeds of pollen plants were sown on Jan 20 in the seedling beds. There were some differences between clones from same male sterile AB line. But plants from same clone were highly uniform in morphology.

The seedlings were transplanted to the field with seed plant:pollen plant at 3:1 ratio and 40cmX40cm spacing on Apr. 5. The F1 hybrid seeds were harvested in June 25. About 3000 plants produced 56 Kg seeds, though some of plants were not transplanted to the seedling beds at an appropriate time.

Vegetatively propagating male sterile chinese cabbge for seed production is a pratical method. We have got a patent for this new technique in China. Athough the cost of in vitro propagation was temporaryly higher than tranditional methods, there is a great potential to reduce the cost by simplifying media and culture conditions. We are propagating different male sterile lines for seed production in large scale.

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ROOTING OF EXCISED LEAVES IN OILSEED CROPS

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Excised, rooted leaves of mungbean are used in our laboratory to select for powdery mildew resistant segregants in the breeding programme (Reddy et al 1987). The possibilities of using thismethod for other pulse crops has also been reported (Reddy et al 1989). The rooting of groundnut branches is widely practised (Ashri and Golden 1964). This paper reports rooting of excised leaves of threeoilseed crops namely sunflower (Helianthus annuus L.),mustard (Brassica juncea (L) Czern and Coss.) and rapeseed(B. napus Linn.).

Leaves at different stages were collected from the crop grown in the experimental field of this centre. The best results were obtained when the leaves with petioles cut at the base with a sharp blade were inserted in test tubes filled with tap water and held in position with a cotton plug. Data on the number of leaves rooted, days required for rooting and their survival were recorded (Table-1).

Table-1.Rooting and survival of excised leaves in sunflower, mustard and rapeseed

Culture	No.leaves cultured	Leaves rooted	Days for rooting	No.days leaves survived
Sunflower	25	25	9-45	45-60
Mustard	25	22	8-15	35-50
Rapeseed	25	20	10-17	40-60

In sunflower, the 20th leaf from the base from 54 day old plants, at preflowering stages rooted in 18 days . The older lower leaves did not root while the upper, younger leaves first developed callus and later on produced roots in about 28 days. In mustard, 5th to 7th leaves from 40 day old plants, at preflowering stage rooted in 8-15 days. In rapeseed and sunflower the rooted leaves could survive upto 60 days whereas in mustard, they survived upto about 50 days.

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During these 40-60 days the reproductive ability and life span of aphids on mustard leaves were studied under the laboratory conditions and cultures with relative tolerance to aphids could be identified (to be published). Similarly these leaves can be used to screen for resistance to foliar diseases.

The major advantages of the excised leaf method are: 1) it requires only limited controlled environment space, 2) the interaction of other pests and diseases are minimised, and 3) while the yield and yield components of single plants are evaluated in the field,their reaction to foliar diseases and pests can be tested in the controlled environment.

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INDUCTION OF *AGROBACTERIUM* TUMOURS ON SEEDLINGS AND *IN VITRO* PLANTLETS OF *BRASSICA NAPUS*.

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Seedlings and plantlets of three commercial cultivars of *B.napus* were infected with *Agrobacterium tumefaciens* strain C58 (wild type).
Seeds were surface-sterilised and germinated on basal Murashige and Skoog medium. After 7 days the cotyledons and shoot tip were excised and the remaining hypocotyl inoculated on the cut-surface with an overnight culture of *A.tumefaciens* using a sterile loop. Thirty seedlings per cultivar were set up. *In vitro* grown plantlets (ten of each cultivar) were pricked several times below a node with a sterile hypodermic needle dipped in an overnight culture of the bacteria. All cultures were incubated at 25 oC for six weeks.

Results
Tumour induction after six weeks co-culture:-

cultivar	no. seedlings	(%)	no. plantlets	(%)
Bienvenu	20	66	3	30
Mikado	18	60	2	20
Rafal	10	33	5	50

The results show that the wild-type *Agrobacteria* strain is capable of infecting all three cultivars tested, but there is a degree of variation observed.
Tumours from the plantlets were excised and maintained on media containing no hormonal supplements, and the tissue was tested for the presence of nopaline by the methods described by Lichenstein and Draper (1985). The resultant electropherograms were further tested for confirmatory evidence using the visual analysis methods of Yang *et al.*(1987) which involved heating the electropherograms at 85 oC in a convection oven, redipping in 0.02% phenanthroquinone and drying. Nopaline was found to be present in at least three samples from each cultivar tested.

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PROTEIN HARVEST INDEX IN OILSEED RAPE

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Nitrogen (N) is essential for plant growth and production of plant proteins. Similarly, N in the form of protein in oilseed rape is important in cattle feeding. Plant Breeding in oilseed rape is strongly directed towards the improved meal quality. Rapeseed meal contains about 40% protein (dry matter basis) with a well balanced amino acid (Josefesson and Muhlenberg, 1968). Protein harvest index- a new criteria has been proposed for the selection of high protein yielding genotypes. An attempt has been made to study the protein harvest index of eight diverse genotypes and their possible crosses.

Materials and Methods: The material comprised of eight genetically diverse genotypes of Brassica napus and were crossed in a 8 X 8 diallel set of crosses excluding reciprocals. The parents and hybrids were grown in a randomized block design with three replications. The nitrogen in the seed and straw was estimated by Kjeldhal method. Seed and straw nitrogen was multiplied by 5.7 to obtain seed and straw protein per cent. The protein harvest index was calculated by using the following formula :

$$\text{Protein harvest index (PHI)} = \frac{\text{Seed yield X Seed protein}}{\text{Straw yield X Straw protein + Seed yield X Seed protein}}$$

Results and Discussion: Out of the eight parents studied, ISN-129 had the heighest (42.20%) whereas the parent Pol-6 had the lowest mean protein harvest index. The crosses viz., Bronowski X ISN-129, Bronowski X Topa and Lores X Pol-6 had 45.34, 46.65 and 47.70% mean protein harvest index, respectively. The analysis of variance clearly indicated the significance of different treatments. The parent ISN-129 can be used in the hybridization programme for the development of high protein yielding genotypes.

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OIL STORAGE PROTEINS IN THE CRUCIFERAE AND OTHER OILSEEDS

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Storage lipids are deposited in the reserve tissues of many plants during seed or fruit development. Examples of such tissues include the cotyledons of oilseed rape, the endosperm of castor beans, and the mesocarp of avocado pears. In the case of the Cruciferae, the normal lipid storage tissue is the cotyledon of the developing seed. The mechanism of storage triacylglycerol deposition in developing cotyledons of radish, mustard and rapeseed and the role of oil-body proteins has now been studied (1).

The accumulation of storage lipids normally begins at the end of the cell division stage of embryogenesis, ie about 2-3 weeks after anthesis (2). The storage oil is initially laid down as relatively large spherical droplets which lack an osmiophilic limiting membrane. The appearance of these large oil bodies occurs several weeks before the onset of storage protein synthesis and there seems little doubt that the two processes are under different forms of developmental regulation. Towards the end of storage oil accumulation and somewhat after the onset of storage protein synthesis, the oil bodies simultaneously acquire an osmiophilic outer membrane and become reduced to 30-40% of their original size. The dimensions of the oil bodies are species dependent. For example, in rapeseed the oil bodies are deposited initially as 1.4um diameter droplets (average size) which are reduced to 0.4um diameter at seed maturity, while in ustard the respective dimensions are 0.6um and 0.25um. The mechanism of this size reduction is unknown but it is associated with the appearance of a specific class of proteins, the oleosins, which are associated exclusively with the limiting membrane of mature oil bodies.

Oleosins are found in all lipid storing tissues in plants but have been studied most extensively in the Cruciferae. Oleosins in the Cruciferae are only found in developing cotyledons where they are synthesised late in seed development, ie slightly after the major seed storage proteins such as cruciferin and napin and well after the deposition of storage oil bodies (2). This implies that oleosins do not play a part in storage oil formation per se but that they are required for the packaging of oil bodies in the mature seed. Ultrastructural studies are consistent with the view that oleosins are synthesised on rough endoplasmic reticulum and possibly inserted directly onto the surface of the immature oil body.

The oleosins of 15 species of the Cruciferae have been isolated and in each case the major components were polypeptides of 18-20 kDa (3,4). Antibodies raised against the 18-20 kDa oleosins of any given crucifer will cross react specifically with similar oleosins from any other species of crucifer. This implies that the oleosins from different crucifers are structurally related to each other, a view that is reinforced by data from proteolytic mapping studies. Oleosins from rapeseed and radish have now been partially sequenced and a cDNA for a rapeseed oleosin has been cloned and sequenced. These data show that oleosins from the two species are indeed extremely similar at the level of their primary amino acid sequences (5).

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Protein structure predictions indicate that the oleosins from rapeseed and radish resemble the major oleosin from maize - a 16.5 kDa protein - in consisting of a central 70 residue hydrophobic sequence flanked by polar N- and C-terminal domains (5). The central hydrophobic region is extremely unusual for a lipid-associated protein in that it is very strongly predicted to consist of β-strand structure. The N-terminal domain contains a region predicted to consist of a strongly amphipathic α-helix. These two regions probably constitute the lipid-binding sites of the oleosins and would therefore be responsible for the interfacial properties of such proteins. The tertiary structure and the role of oleosins in the stabilisation of oil-in-water emulsions is now being studied by a variety of biochemical, chemical, and physical techniques.

Antibodies raised against oleosins from the Cruciferae have also been used in cross-reactivity studies with lipid-associated proteins from other plant families, human serum, and animal adipose tissue. These studies have shown that oleosin-like proteins can be found in all of the lipid storing plant that were investigated. For example, in species of the Compositae, such as sunflower and safflower, the major oleosins are 20kDa proteins, while in Leguminosae, such as soybean and pea they are 24kDa proteins. Oleosins have also been identified in the Oleaceae (olive), Euphorbiaceae (castor bean), Solenaceae (tobacco), Lauraceae (avocado) and even in monocotyledons, such as wheat and maize. More recently, oleosin antibodies have been shown to cross react with human serum apolipoproteins and with hamster adipose tissue apolipoproteins. Further studies are now under way to investigate the structural basis of this cross-reactivity between lipid-associated proteins from such a diverse range of organisms.

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GLUCOSINOLATE CONTENT AND COMPOSITION OF EIGHT BRASSICACEAE SPECIES

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Introduction

This study was conducted to determine the range of total glucosinolate concentration and composition in eight species of the Brassicaceae family. Glucosinolate concentration in paired leaf tissue and mature seed samples from individual plants were compared to determine if a correlation exists between leaf and seed glucosinolate content.

Materials and Methods

Accessions from the University of California-Davis collection were planted in the University of Idaho greenhouse on 21 November 1985. Species in the collection included *Eruca sativa* Mill., *Brassica campestris* L, *B. nigra* (L) Koch, *B. carinata* Braun., *B. tournefortii* Gouan, and *B. hirta* Moench (*Sinapis alba* L). Accessions of *B. campestris* L., *B. oleracea* var. *botrytis* L., and *B. oleracea* var. *capitata* L. from the USDA Plant Introduction Stations at Ames, Iowa, Pullman, Wash., and Geneva, N.Y. that had previously been screened for glucosinolate content with the glucose sensitive test tape method and rated as 5 (high) were selected for more detailed analysis (3).

The first four leaves below the primary raceme were removed from two plants in each of 40 lines in the UC-Davis collection that had been grown in the greenhouse. Plants were sampled at full bloom just prior to pod fill. Tissue samples were frozen immediately with liquid nitrogen and stored at -20 C until freeze-dried for glucosinolate analysis. Glucosinolate content was also measured in mature seed from each plant. The glucosinolates in these accessions and in the seed of the selected USDA accessions were quantified using a modification the TMS-GC method as described by Daun and McGregor (1).

Results and Discussion

The glucosinolate composition of the accessions varied widely between species (Table 1). Each species contained one or two primary glucosinolates and traces of other minor glucosinolates. Glucosinolate composition in the seed and leaf tissue were generally similar within a species; however, correlations between leaf and seed glucosinolate levels were non-significant (R = -0.35 to 0.60) (2). The glucosinolate concentration in the leaves was always lower than that in the seed. The similarity between leaf and seed glucosinolate composition suggests that genetic regulation remains relatively constant throughout the plant and the life cycle.

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Table 1. Mean glucosinolate content in seed meal from selected lines of seven species in the USDA and the UCD collections and in leaf tissue from selected lines of five species in the UCD collection.

Species	n	Glucosinolate Composition					total gluc.
		allyl	3-butenyl	2-hydroxy-3-butenyl	4-methyl-thiobutyl	*p*-hydroxy benzyl	
Seed		----------µmoles gram^{-1} ----------					
B. campestris	36	12.3	234.5	2.5	trace	-	249.3
B. carinata	12	319.9	3.8	7.5	-	-	331.2
B. hirta	8	2.3	trace	7.1	-	288.7	298.1
B. nigra	5	397.6	trace	-	-	-	397.6
B. oleracea var. *botrytis*	15	25.7	1.0	5.0	3.6	-	35.3
B. oleracea var. *capitata*	25	20.9	1.5	7.7	9.4	-	39.5
Eruca sativa	5	trace	trace	trace	314.4	-	314.4
Leaves							
B. campestris	10	1.2	20.3	trace	-	-	21.5
B. carinata	12	9.0	1.0	trace	-	-	10.0
B. hirta	8	trace	trace	trace	-	7.7	7.7
B. nigra	5	14.8	1.4	-	-	-	16.2
Eruca sativa	5	trace	-	-	1.0	-	1.0

Comparison of Near-Infrared Reflectance Analyses with GC Analyses of Glucosinolate Concentration in Rapeseed

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Near infrared reflectance spectroscopy (NIRS) has been proposed as a more efficient means of determining the concentration of glucosinolates in rapeseed (1). The NIRS procedure would reduce both cost and time required for quantitative analyses of glucosinolates of rapeseed. Seed samples of rapeseed were analyzed in duplicate for glucosinolate content using both the NIRS and the trimethylsilyl procedure to compare results across several genotypes and production environments.

Near infrared reflectance spectroscopy (NIRS) measurements were made with a Pacific Scientific Neotec 6250 Scanning Reflectance Monochromator interfaced with an IBM Personal System/2 Model 60 computer. Whole rapeseed samples were packed into sample cells with a near infrared transparent quartz cover glass. Reflectance (R measurements (log 1/R) were completed (2-nm wavelength intervals) from 1100 to 2500 nm, and were averaged over two scanning periods of 45s. The spectroscopic data were related to the laboratory determined glucosinolate values using modified stepwise linear regression procedure from the wavelength selection program CAL (Infrasoft International). The calibration procedure consisted of a computer search for the wavelengths within each mathematical treatment of the log 1/R measurements that gave the best linear relationship of glucosinolate concentration with a function of the reflectance data. Criteria for selection of the best productive equation was described by Westerhaus (3). Gas chromatographic glucosinolate analyses (GC) were conducted on ground seed using a modified procedure described by Daun and McGregor (2).

NIRS and GC values were compared using regression analyses. Those samples with GC values below 60 µmoles/g of defatted meal of total glucosinolates were designated as a low population, and those with greater than 60 µmoles/g were designated as a high population. In the high population, the regression coefficient was R^2 = 57.4** (Figure 1), which indicates that this procedure would be moderately effective as a selection tool and would allow an estimate of quantitative glucosinolate values.

In the population of relatively low glucosinuolate rapeseed, the regression value was much higher (R^2 = 80.6**) (Figure 2). With the level of association observed in this study, the NIRS procedure would provide a fairly accurate initial screening procedure. The regression values obtained in this study were much lower than those reported earlier for transformed data (1). NIRS could also be used to separate Canola and non-Canola quality seedlots; however, the procedure needs further refinements before it can be used for establishing official crop grades.

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Figure 1. Regression analyses of NIRS and GC glucosinolate concentration in 94 seedlots of rapeseed with high levels of glucosinolates.

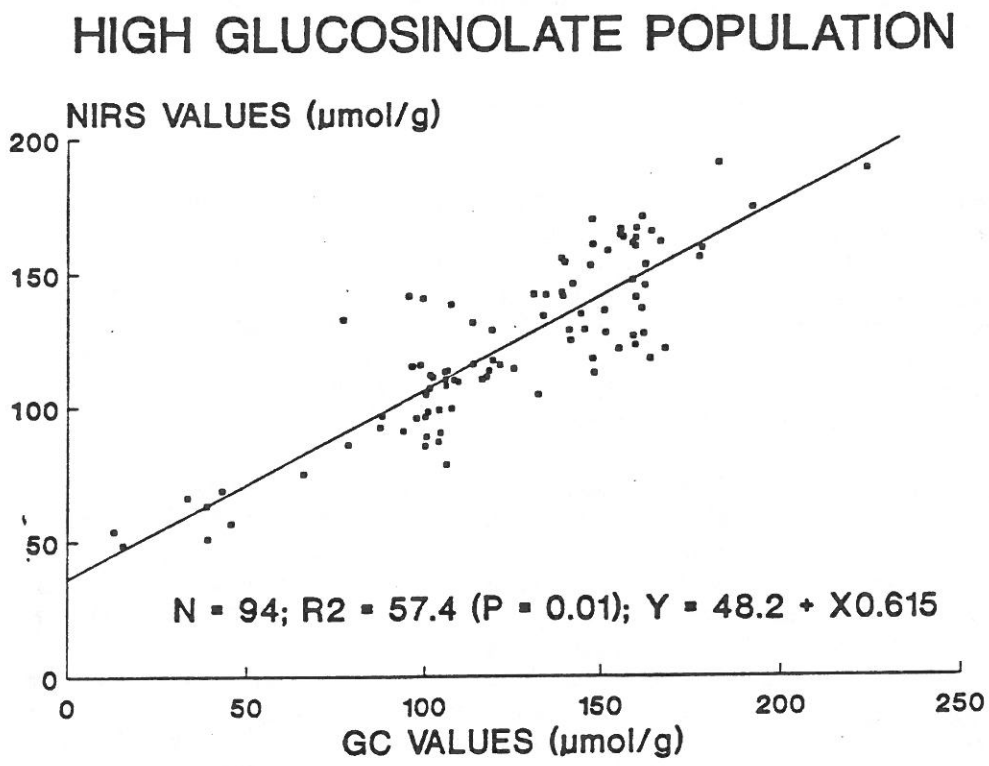
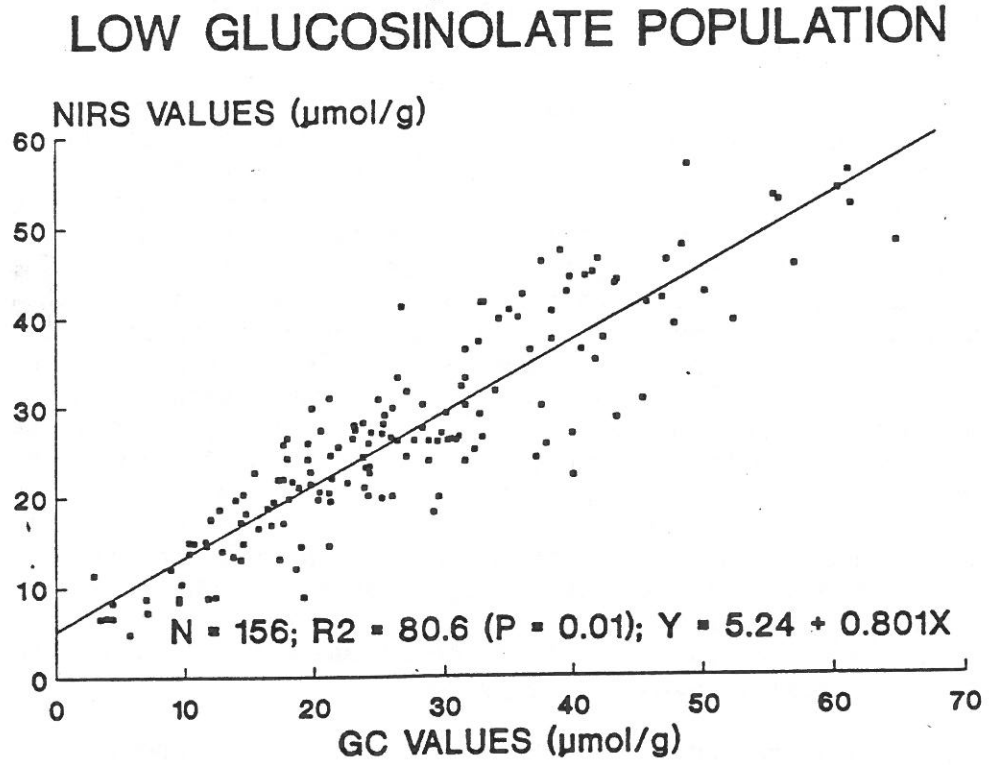


Figure 2. Regression analyses of NIRS and GC glucosinolate concentration in 156 seedlots with low levels of glucosinolates.



WOUND-INDUCED CHANGES IN THE GLUCOSINOLATE CONTENT OF CRUCIFEROUS PLANTS.

V.M. Koritsas, J.A. Lewis and G.R. Fenwick

Glucosinolates, or the products of their hydrolysis by the thioglucosidase, myrosinase, are known to play a major role in the complex interactions between crucifers and their potential herbivores, pathogens, competitors and symbionts. Despite the considerable activity in the study of insects, pests and fungal pathogens which cause wounding of glucosinolate-containing plant tissue, little is known about the effects of such damage on the glucosinolate content of the plants.

PROCEDURE: Plants of oilseed rape (cv. Rafal), were raised under controlled conditions and infested with the cabbage stem flea beetle (P. chrysocephala) when the fifth leaf was emerging. Individual laboratory plants of oilseed rape and also brown mustard (B. juncea cv. Trowse), black mustard (B. nigra cv. Sutton), white mustard (Sinapis alba) and kale (cv. Fribor) were physically damaged by puncturing the petiole with a hyperdermic syringe, and some infected with Pseudomonas obtained from the surface of P. chrysocephala. Field grown oil seed rape plants (cv. Rafal), naturally infested, were also included in this study (Koritsas et al., 1989). The appropriate plant parts (petioles, laminae) were excised from groups of ten plants, combined and freeze dried. Samples were stored at -20°C until extracted and analysed by HPLC for individual glucosinolate content.

RESULTS: Infestation of oilseed rape plants with P. chrysocephala under field or laboratory conditions showed marked changes in the levels of total and individual glucosinolates (Tables 1, 2). These changes, which were also found, in the lamina reflected a large increase in the levels of the indole glucosinolates, especially glucobrassicin and its 1-methoxyderivative (neoglucobrassicin). The effect of puncturing plants, with or without bacteria, also results in the accumulation of indole glucosinolates (Table 2) albeit to a lesser extent than was apparent in the beetle-infested tissue. The results of puncturing oilseed rape petiole or lamina tissue 1, 4, 8 or 16 times with a hypodermic is shown in Figure 1. Damage increased total glucosinolate content of young petioles (4.18 mg g^{-1} → 6.74 mg g^{-1} freeze dried tissue) and levels of individual compounds varied markedly; once again an increase in indole glucosinolate content was apparent. The mustard plants examined exhibited a different response to wounding than did those of oilseed rape (Table 3). Whilst large increases in total glucosinolate content were observed following damage, this was primarily a result of the accumulation of sinigrin (B. nigra, B. juncea) or sinalbin (S. alba).

DISCUSSION: Damage produced by cabbage stem flea beetle induces physiological responses in plants leading to the accumulation of certain glucosinolates. Previous reports of tissue damage affecting glucosinolate content (Butcher et al., 1976; Lammerink et al., 1984) suggest that this may be a general response to pathogen infection and insect damage, but the earlier workers used analytical methods which

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failed to provide detailed information of individual and total glucosinolate contents. In rapeseed plants indole glucosinolates were markedly increased following infestation, or damage, and work is in progress to determine whether such changes are linked to indole phytoalexin accumulation (Takasugi et al., 1986). Such compounds have also been recently reported in B. juncea (Devys et al., 1988). The significance of the findings reported here have yet to be fully assessed. The findings do however call into question the reliability of analysing whole plant glucosinolates as a means of assessing or predicting insect/pathogen resistance or susceptibility. Moreover they also suggest that the biological activity and relative importance of indole glucosinolates may be greater than hitherto considered.

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TABLE 1. Glucosinolate content of infested and non-infested petioles of field grown oilseed rape (cv. Rafal) plants.

Glucosinolate content mg g^{-1} Freeze dried weight (f.d.w.)			
Aliphatic[a]	Aromatic[b]	Indole[c]	Total
2.11 (0.36)	0.08 (ND)	0.42 (1.11)	2.61 (1.53)
12.16 (6.02)	0.24 (0.20)	0.45 (2.50)	12.85 (8.75)
18.88 (14.88)	0.36 (0.36)	1.77 (6.51)	21.08 (21.80)

Figures in brackets relate to infested tissue.
[a] gluconapin, progoitrin, gluconapoleiferin, glucobrassicanapin
[b] gluconasturtiin
[c] glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin

TABLE 2. Comparison of infestation, wounding and bacterial infection on glucosinolate content of laboratory-grown oilseed rape (cv. Rafal) petiole.

	Glucosinolate content (mg g^{-1} f.d.w.)			
	Aliphatic[a]	Aromatic[b]	Indole[c]	Total
Control petiole	3.56	0.42	0.36	4.43
Infested petiole	2.22	1.18	12.88	16.33
Wounded petiole	4.59	1.30	2.22	8.11
Wounded/infected petiole	3.81	1.34	4.91	10.09

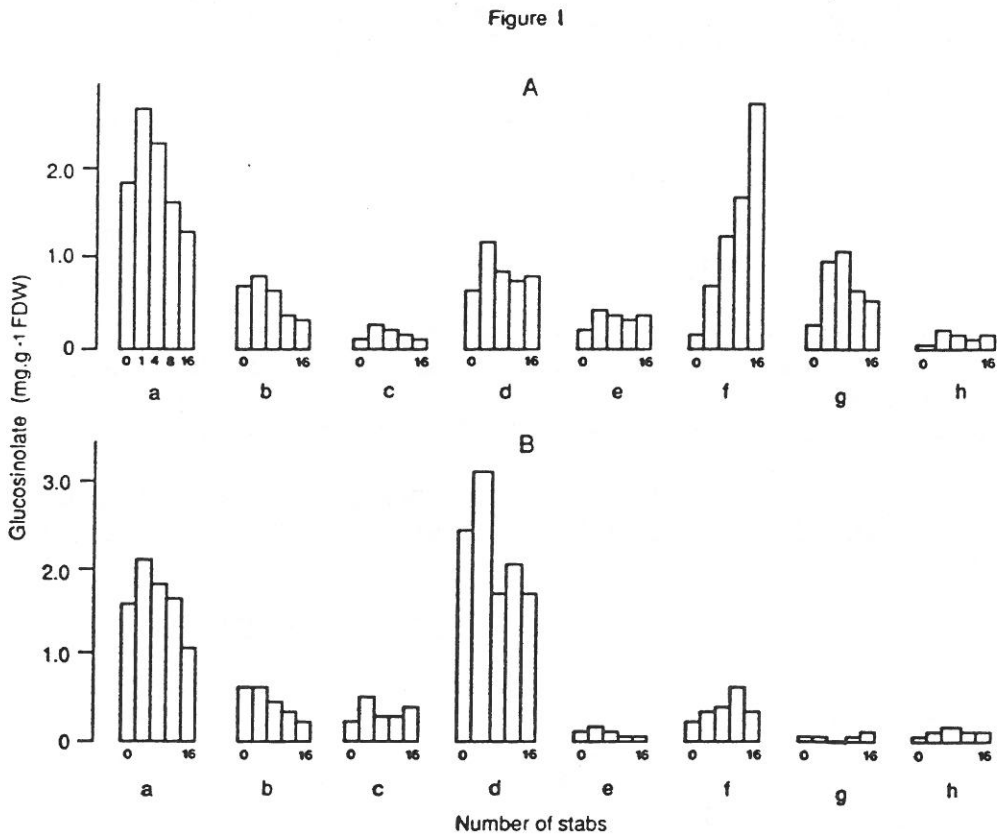
[a,b,c] as in Table 1.

TABLE 3 Response of laboratory-grown mustards and kale to physical damage.

Species	Glucosinolate content (mg g^{-1} f.d.w.)			
	Aliphatic	Aromatic	Indole	Total
S. alba	-	3.34 (9.28)	0.02 (0.14)	3.37 (9.42)
B. nigra	6.71 (10.71)	0.77 (0.46)	0.01 (0.11)	7.50 (11.30)
B. juncea	3.96 (10.46)	0.32 (0.48)	0.04 (0.25)	4.30 (11.20)
B. napus	3.92 (5.37)	0.40 (0.53)	0.12 (0.96)	4.45 (6.85)

Figures in brackets relate to petioles punctured 16 times with a hyperdermic needle.

FIGURE 1. The effect on the individual glucosinolate levels of puncturing laboratory grown oil seed rape tissue 1,4,8 or 16 times with a syringe. Young Petiole (A), Young Lamina (B). With the following glucosinolate side chains; 2-hydroxybut-3-enyl, (a), but-3-enyl (b), 2-hydroxypent-4-enyl (c), pent-4-enyl (d), Phenylethyl (e), Indolyl-3-methyl (f), 1-methoxyindolyl-3-methyl (g) and 4-methoxyindolyl-3-methyl (h).



ISOLATION OF ZERO GLUCOSINOLATE F1 HYBRIDS OF RAPESEED

S. K. Gupta K.S. Labana And K.L.Ahuja

Plant Breeding in rapeseed is today strongly directed towards the improved meal quality. Rapeseed meal contains about 40-43% protein (dry matter basis) with well balanced amino acid. However, the value of the meal as a protein source for the animal feeding is limited by presence of sulphar containing compound glucosinolates. Recently, a growing concern about the quality of the meal or cake has been felt in the Indian subcontinent and this has led to the identification of zero glucosinolate cultivars in Brassica napus An attemp has been made to screen some of the F1 hybrids for zero glucosinolates.

Materials and Methods:

Nine Brassica napus genotypes representing 5 different countries of wide geographical background were analysed for glucosinolate content. All genotypes were crossed in a diallel fashion excluding reciprocals. Twenty eight F1s and parents were sown in a randomized block design with three replications. Initially 3 to 5 individual seeds of each of the hybrid was analysed using simple tes-tape method (Mc Gregor and Downey, 1975) and the variation in glucosinolate for each of the hybrid is recorded. Later the hybrid showing contrasting variation were picked up for the estimation of total glucosinolates using the method of Brzezinski and Mendelwski, 1984.

Results and discussion:

Amongst the genotypes studied Bronowski had the lowest glucosinolate content (8-10 μ moles/g).The hybrids, Ashai X Christa, Ashai x Topa, Christa X Topa, GSL-1 X Topa Bronowski X Topa and Lores X Pol-6 had 12.2, 11.4, 11.4, 11.2, 14.2 and 15.7 μ moles/g glucosinolates, respectively.Further more,these hybrids will be used in breeding programme as a gene source for the production of low/zero glucosinolate meerial in rapeseed.

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STUDY ON QUALITY CHARACTERS OF RAPESEED GERMPLASM

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Four hundred and one rapeseed accessions were collected from rapeseed main growing area (including the Sichuan basin, Guizhou plateau, the middle and lower Yangtze valley, and the coast of southern China). The performance, correlation and geographical distribution trends of quality characters for rapeseed were observed and studied. The results were as follows:

1. The rapeseed germplasm with good quality is quite rich in rapeseed main growing area of China. The contents of oil, protein and glucosinolate varied from 48.74 to 30.36%, 33.78 to 17.50%, and from 267.07 to 3.71 umol/g, respectively. The average contents were 39.48%, 26.67%, and 153.53 umol/g, respectively. The erucic acid content ranged from 57.33 to 0.18% and the mean content was 17.23%. The content of linoleic acid varied from 33.38% to 11.20%, and the average was 17.23%. The oleic acid content varied from 66.18 to 6.7%, and the average was 21.03%. The content of palmitic acid varied from 8.89 to 1.54%, and the average was 3.96%. The fibre content varied from 12.06 to 4.74%, and the average was 7.99%.

Some cultivars with good quality were screened by means of quality traits analysis. There are 15 cultivars with high oil content (45%), 12 cultivars with high protein content (30%), 7 cultivars with low glucosinolate (30 umol/g), 19 cultivars with zero erucic acid (1%), 25 cultivars with low erucic acid (5%), 8 cultivars with high erucic acid (55%), 5 cultivars with high linoleic acid (30%) and 11 cultivars with high oleic acid (60%).

2. There was a highly negative correlation between oil and protein, and a positive correlation between oil and total oil plus protein. Selection for total of oil plus protein can raise oil and protein content simultaneously. The correlation of glucosinolate with oil or protein was not significant. The correlations of fibre with oil, protein, erucic acid, linoleic acid and oleic acid are all not significant, There were no significant correlations between erucic acid and oil, between erucic acid and protein in rapeseed (Brassica napus L.) and mustard (Brassica juncea L.).

3. There were obvious geographical differences for oil, protein, erucic acid and oleic acid contents among regions. The rapeseeds grown in Guizhou Plateau and the lower Yongtze valley were higher in oil content. The rapeseeds grown in the Sichuan basin and the middle Yangtze valley were higher in protein content. Rapeseeds in middle Yangtze valley were lower in erucic acid and higher in oleic acid content. The

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oil and erucic acid content were higher in high latitude or high elevation region than low latitude or low elevation region. Otherwise, the protein and oleic acid contents of high latitude or elevation region were lower than low latitude or low elevation region. All the glucosinolate, linoleic acid and fibre contents had no distinct geographic distribution trends.

POTENTIAL REDUCTION IN AUSTRALIAN CANOLA QUALITY FROM WEEDY CRUCIFER CONTAMINATION

P Salisbury, R Mailer and J Sang

Recently released Australian canola cultivars have been of extremely high quality. In 1988/89 trials, these cultivars had an average total glucosinolate concentration less than 16 μmoles/g oil-free meal (equivalent to 5-10 μmoles/g of canola glucosinolates), with less than 1% erucic acid.

However, a small number of trial sites in recent years have highlighted the potential damage of crucifer weeds to canola quality. Glucosinolate concentrations as high as 120 μmoles/g and erucic acid contents of 14% have been measured in canola cultivars from trial plots.

A recent survey of Australian crucifer weeds has shown that most have high glucosinolate and erucic acid concentrations and would therefore reduce the quality of canola if present in samples. Several of these weeds are present in Australian canola growing regions. Many species (e.g., *Sisymbrium* spp., *Hirschfeldia incana*) are smaller than canola and would be removed during harvesting. Others, such as *Raphanus raphanistrum*, are harvested as larger pod segments and can be separated later. While these species reduce canola yields as a result of competition, they tend not to significantly contaminate samples and reduce quality.

Crucifer weed species which have a comparable seed size to canola and cannot easily be removed from samples are of major concern. Such species include *Sinapis arvensis*, *Conringia orientalis* and *Brassica tournefortii*. The seed quality of these species is presented in Table 1.

Table 1. Mean quality of crucifer weeds

Species	No. lines	Oil content (%)	Erucic acid (%)	Total glucosinolates (μmoles/g meal)
Brassica tournefortii	8	32.2	47.8	121
Conringia orientalis	2	32.2	25.6	140
Sinapis arvensis	5	30.0	38.8	196

Of the species, *Sinapis arvensis* is the most widespread in Australian canola growing regions. It is very similar to canola seed in size and appearance and virtually impossible to separate. The degree of quality reduction of canola samples expected with different levels of *Sinapis arvensis* contamination is presented in Table 2.

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Table 2. Reduction in quality of canola cv. Shiralee with *Sinapis arvensis* contamination

% seed contamination with *Sinapis*	Oil content (%)	Erucic acid (%)	Total glucosinolates (μmoles/g meal)
0	41.2	0.4	15.9
5	40.6	2.3	24.9
20	39.6	8.1	51.9
50	35.6	19.6	106.0

While oil content is reduced only marginally with low levels of contamination, erucic acid and glucosinolate concentrations are greatly increased.

HPLC and GCMS analyses have shown that the major seed glucosinolates in these three weedy species are: 3-methylsulphinylpropyl-(*Brassica tournefortii*), 2-propenyl-(*Conringia orientalis*) and *p*-hydroxybenzyl-(*Sinapis arvensis*) glucosinolates. The analysis of only canola glucosinolates would not detect this contamination and would therefore give a misleading quality result. This highlights the advantage of a total glucosinolate method such as a glucose release method (Mailer 1989) in preference to the canola standard.

It is evident that crucifer weeds have the potential to seriously downgrade the quality of Australian canola crops. Herbicide resistant cultivars to allow in-crop control of these weeds, or management to remove them in different stages of the cropping rotation, are required to minimise their damaging effects.

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The Precision of Oilseed Rape Cultivar Evaluation Trials in Western Canada

J.E. Brandle

Cooperative testing represents the final stage in the evaluation of breeding lines from the various oilseed rape (Brassica napus L.) breeding programs operating throughout Canada. The precision of entry means in the individual trials of the cooperative test must be sufficient to detect differences between breeding lines and checks, and is therefore of major significance to plant breeders. In the Western Canadian cooperative test, individual trials are grown using randomized complete block designs with four to six replications at anywhere from 22 to 31 locations. Plot size and harvest area varies according to the location. The objective of this study was to determine if the level of precision of entry means in the Western cooperative test was adequate to detect the differences between breeding lines and checks.

Four years data (1985-88) from the Western Canola Co-op Test 'A' were used with the permission of the test co-ordinator, Dr. K. Downey Agriculture Canada, Saskatoon. Trials were conducted in the Provinces of Manitoba, Saskatchewan and Alberta, Canada. The coefficient of variation (CV %) required to detect a given difference was calculated using the method outlined by Lin and Binns (1984), with P=0.8, α=0.05 and r=4. The number of entries in each trial was assumed to equal 20. For each year-location combination, the difference between Westar and the best breeding line was calculated and expressed as a percentage of the trial mean. Westar was the standard against which all breeding lines were measured at the time the trials were grown. The frequency distributions of coefficients of variation and percent differences (D %) for each year are presented in Tables 1 and 2.

The average difference between the best breeding line and Westar was found to be 14.3 %, the CV required to detect this average difference is 8.0 %. The average CV was found to be 15.7 % . This level of precision is adequate for differences of 20 % or greater, which occur in only 26 of the 110 year-location combinations. Therefore, precision of cultivar means must be increased if differences on the order of 14 % are to be detected. Increasing the number of replications would be the simplest means of improving precision. However, the use of incomplete block designs or increased plot size would also assist in reducing CV's.

Given the large number of locations that are used for the cooperative tests, any of the previously mentioned methods of decreasing CV's may also result in substantial increases in costs. Brandle and McVetty (1988) did not detect any significant cultivar x location interaction in their work, indicating a degree of redundancy in the Manitoba locations used in that investigation. Analyses of variance of two subsets of the Western cooperative test also resulted in a non-significant cultivar x location interaction (Brandle unpublished). Therefore, the cost of increasing precision in the Western cooperative test could be offset by decreasing the number of locations used for testing. The cultivar x year interaction was significant in all of the above mentioned examples, indicating that a reduction in the number of years used for testing would not be advisable.

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Table 1. Distribution of coefficients of variation for the Western Canola Co-op Test 'A'

			CV %				
Year	#Loc.	Mean	5-10	10-15	15-20	20-25	>25
1985	27	15.7	2	10	9	3	3
1986	30	15.6	7	12	3	7	1
1987	31	14.3	4	17	8	1	1
1988	22	17.3	5	8	3	2	4
Total	110	15.7†	18	47	23	13	9

† average of year means

Table 2. Distribution of differences between the best breeding line and Westar, expressed as a percentage of the trial mean, for the Western Canola Co-op Test 'A'

			D %						
Year	#Loc.	Mean	0	0-5	5-10	10-15	15-20	20-25	>25
1985	27	12.3	6	4	6	2	4	1	4
1986	30	13.7	5	5	2	8	5	0	5
1987	31	11.8	8	5	5	3	2	3	5
1988	22	19.5	1	0	5	5	3	2	6
Total	110	14.3†	20	14	18	18	14	6	20

† average of year means

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PROSPECT OF *BRASSICA NAPUS* CULTIVATION IN BANGLADESH

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The oleiferous *Brassica*, an important source of vegetable fats, is mainly represented by rape *(B. napus* L.), turnip rape *(B. campestris* L.) and mustards (*B. juncea* Czern and Coss and *B. carinata* Braun). Among them, *B. napus* is more productive may be due to its better physiological constitution (Kariya and Tsunoda 1972, 1973, cit. Tsunoda, 1980) and or being subjected to an advance breeding technique in Europe and Canada. However, *B. napus*, being a crop of temperate region remains constantly in vegetative stage or too late to be a crop in Bangladesh. In an attempt to increase vegetable oil production in Bangladesh, breeding and introduction of *B. napus* was considered a realistic approach. With this aim the A genome of carefully selected early representatives of *B. campestris* (AA) and *B. juncea* (AABB) and C genome of *B. alboglabra* (CC) and *B. carinata* (CC) were used for introgression with the analogous genome of *B. napus* (AACC), cv. Olga from Sweden. *B. campestris, B. juncea* and the late maturing *B. alboglabra* and *B. carinata* seem to be equally effective for introgressing appropriate earliness for Bangladesh (Zaman, 1989).

Breeding Programme on *B. napus* which gained momentum in Bangladesh after this investigation comprises a good number of lines which are now at different stages of varietal development process. Significantly and consistently higher yield was observed in some of the selected lines both in regional yield trials and farmers plot trials (Table 1) at different regions of the country. Erucic acid content in some of these lines is also low along with tolerance to major diseases (*Alternaria sps*) and pest (aphid). In addition, *B. napus* especially nap 3, can stand temporary water logged condition. It can be sown from first week of October to last week of November with good performance and yield but optimum time of sowing for higher yield is mid-October to mid-November in Bangladesh. Seed rates from 5-12 kg/ha did not show any significant variation in yield. Similarly, line sowing (15-30 cm) and broadcasting have equal influence on yield. Therefore, broadcast method of sowing, a common practice for

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Brassica cultivation in the country may be suggessted. Like other oilseeds (e.g. Turnip rape and Sesame), *B. napus* has an inherent character of shattering pods at maturity. It is mainly influenced by indeterminate flowering habit of the crop. To ensure economic yield, the highest oil content and to avoid shattering loss, *B. napus* can be recommended for harvest when 60-80 per cent pods become yellow. In cent per cent yellow pod stage, reduction in oil content occurs which may be due to reversion of fats and oil to carbohydrate by glyoxalate cycle.

Table 1. Comparative performance of *B. napus* in Bangladesh

Variety/ line		Yield* (kg/ha)	Relative position	Difference in days to flowering	Difference in days to maturity	Oil content	Erucic acid%	Alternaria reaction
Tori-7	*(campestris)*	1000	100	0	0	43	50	8
Sonali	,,	1182	118	+13	+16	45	52	6
nap 1	*napus*	1369	137	+ 4	+ 7	45	40	3
nap 3	,,	1454	145	+10	+16	43	39	2
nap 8501	,,	1233	123	+ 6	+12	44	-	2
nap 8509	,,	1537	147	+ 3	+ 8	45	9	3
nap 8559	,,	1493	143	+ 3	+ 7	44	-	3
Rai 5	*juncea*	1265	127	+10	+20	39	-	2

* Average of fourty trials in three years. Days to flowering for Tori 7 is 26 days and maturity is 73 days.

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EFFECTS OF BAGGING ON POD AND SEED DEVELOPING IN RAPESEED

Du Xin

Rapeseed (Brassica napus L.) is a cross-pollinating crop, bagging for isolation is the key link in its breeding. But the seed produced by bagging has a lower rate of emergence and weak growth of the seedling, often resulting in severe seedling death, which impedes the breeding advance. Usually, it is considered qualitatively that the seed is affected by bagging, but there are few reports about the range or level of such effects and the possible approach to overcome them. Therefore, it is necessary to make a thorough study of the effects of bagging in the development of pod and seed in rapeseed, to provide a basis for breeding and the physiologic research.

MATERIALS AND METHODS

Twnety-five plants of rapeseed (B. napus) line 87-51 were chosen randomly in the field before flowering for the treatment of bagging for self-fertilization (SF); fifteen plants of 87-51 as the material line for bagging for cross-fertilization (CF) by line 148 as the paternal plant; five plants of 87-51 for the open-fertilization (CK) treatment. The bagging lasted 7, 15, 20, 30 and 57 days (harvest stage).

RESULTS AND DISCUSSION

1. Effects of bagging on pod and seed development in rapeseed

The results indicated that bagging has a marked effect on pod and seed development in rapeseed. Pod size, seed diameter and weight, emergence vigour and the rate of emergence decreased with bagging duration. Bagging affected the development of pod and seed by affecting the light, temperature, moisture and ventilation inside the bag. Photosynthesis in the green pod, flower stem and some upper leaves were affected by bagging. The photosynthetic products of these organs make a great contribution to nutrient accumulation and physiological activity during the development of pod and seed. Temperature and moisture increase inside the bag and bagging promotes plant respiration and reduces the accumulation of dry matter. Bagging also changes the ventilation and reduces the CO_2 concentration inside the bag.

2. The different effects of bagging between SF and CF in rapeseed

It is shown that there are different effects of bagging between SF and CF in rapeseed from the results. The differences are displayed mainly in the level and time that pod and seed development is affected. In most of the characters, obvious effects of bagging can be found on 7th day, for SF, as compared to about 15th day for CF, probably due to a shorter time of fertilization for SF than for CF. Meanwhile, the lesser effect of bagging on CF than on SF is possibly because the heterosis of CF can partially offset the effect of bagging.

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3. Choosing the suitable time of bag removal

How to overcome or reduce the harmful effects of bagging on pod and seed development in rapeseed? Choosing the best time to remove the bag is one of the effective methods to off-set or reduce the harmful effects on pod and seed development. Based on the result, it is better to take the bag off on the 7th day for SF, but a little later for CF, about 10th-15th day.

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VARIABILITY OF *P. BRASSICAE* POPULATIONS AND OF HOST *B. OLERACEA* POPULATIONS FOR RESISTANCE BREEDING.

F. ROUXEL* ; G. THOMAS**

Introduction :

Clubroot, due to *Plasmodiophora brassicae* Wor., is the major disease of vegetable cruciferous crops in France. In absence of really efficient methods to limit infections, resistance breeding appears as the main way to get free to the important losses in the field. This investigation concerns two aspects :

- studies on variability of *P. brassicae* populations in Brittany, in connection with cauliflower types and crop rotations
- studies on variability of *Brassica oleracea* landraces for clubroot resistance.

Materials and methods :

1 - test for crucifer susceptibility to *P. brassicae*

a) Inoculation consisted on pouring a resting spore suspension into a steamed soil in which 5 day-old seedlings were grown. The final inoculum density was 10^6 sp/ml soil. The test was performed in glasshouse conditions (t° 18-22 °C - high humidity).

b) Evaluation is done after 6 week growth.
Infected plants were divided into 4 classes according to disease level on root infection basis. A disease index was calculated by summation of the coefficients (0 - 0,25 - 0,5 - 1) affecting plant class frequency.

2 - *P. brassicae* isolates

a) Study on pathogen variability.
20 isolates were sampled in the two main areas concerned by cauliflower crop in Brittany :
8 concerning Ille et Vilaine district : Eastern Brittany, (autumn cauliflower)
12 from Finistere district : western Brittany. (winter cauliflower)

b) Study on B. oleracea variability.

Among the various French isolates discribed above, 2 have been chosen for Brassica tests: 1 from western Brittany (ECD code 17/15/15) ; 1 from eastern Brittany (ECD code 16/14/31). This choice has been done according to results on P. brassicae study.

3 - Plant material

a) Study on pathogen variability : The 15 hosts of the ECD series were used and results were expressed according to the coding system described by BUCZACKI and al (1).

b) Study on B. oleracea variability : 64 landraces (grown and bred by farmers for local use) coming from various areas of France were chosen corresponding to different crop types :

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B. oleracea var. acephala (Kale) : 26 landraces
B. oleracea var. capitata (cabbage) : 14 landraces
B. oleracea var. botrytis (cauliflower) : 12 landraces
B. oleracea var. gemmifera (B. sprouts) : 12 landraces

Results :

1 - Variability of the pathogen

No real difference was observed among the 20 isolates in connection with the two geographic origins (fig.1). Only *B. campestris var-pekinensis* was susceptible in the *B. campestris* group. In the *B. napus* group, DC 101 and DC 130 hosts differentiated some isolates without any origin relation. The 5 B. *oleracea* hosts appeared very mixed, according to previous observations (2).

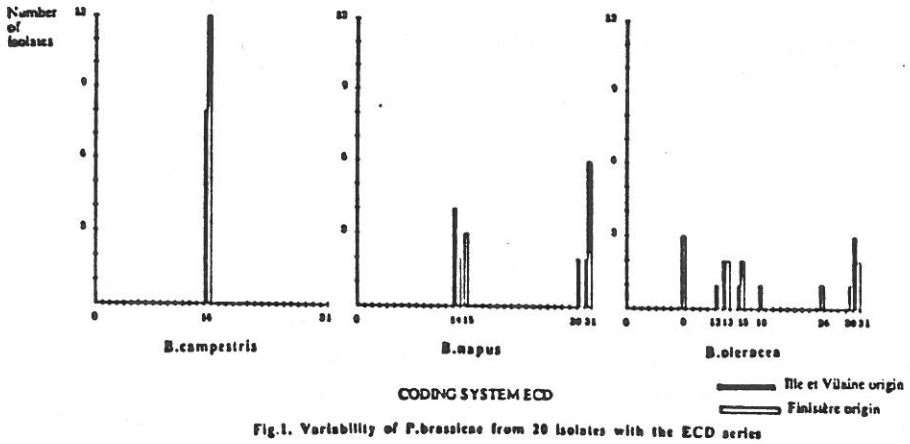

Fig.1. Variability of P.brassicae from 20 isolates with the ECD series

2 - Variability of B. oleracea landraces

A large variability is assessed among the local types involved, based on mean disease index evaluation. A general tendency can be presented showing differences between culture types. In the range below, forage kale and cabbage populations show meanly better general behaviour than others, cauliflower being the most susceptible type involved.

Kale	Cabbage	B sprout	Cauliflower	
60,5	86,8	90,00	96,7	mean disease index
[23,6 - 87,5]	[76,3 - 97,5]	[86,2 - 97,5]	[90,9 - 100]	[externe values]

Conclusion :

This work on variability shows that a limited series of isolates can be used for genetic assessment without taking into account the loca spreading of the parasite. Besides, according to the limited number of resistance sources, principally for B. oleracea, a larger investigation must be done on local variability principally turned on kale and cabbage types which seem more efficient.

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RECESSIVE INHERITANCE OF RESISTANCE TO CLUBROOT IN BRASSICA OLERACEA.

Roeland E. Voorrips and Dirk L. Visser

Many accessions of B.oleracea with known or reputed resistance to clubroot (caused by the soil-borne fungus Plasmodiophora brassicae) were collected in 1986-1988. They were tested for resistance to a dutch population of P.brassicae (ECD 16/3/30) which appears to be widespread in the Netherlands. A seedling test was utilized in which seeds were sown in 4 x 4 cm pots using sterilized potting compost (pH approx. 6.0) and immediately inoculated with 2 ml of a spore suspension of 10^7 spores/ml. After 6 weeks the symptoms on the root systems were classified in 4 categories, from 0 (no symptoms) to 3 (heavy club formation, more than 50 % of the root system affected). A disease index ranging from 0 (completely resistant) to 1 (very susceptible) was calculated as follows:

$D.I. = (n_1 + 2*n_2 + 3*n_3) / ((3 * (n_0 + n_1 + n_2 + n_3))$,

where $n_0 \ldots n_3$ denote the number of plants in each category.

Plants from the most resistant accessions were selfed and crossed with Septa, a very susceptible cabbage cultivar (ECD 14) and with each other in an incomplete diallel. All progenies were tested for resistance as described above. In table 1 the results are presented of resistance tests of the selected accessions, and of the I1's (selfings) and F1's (crossings with Septa) of one single plant of each accession. Since the seedling test was not yet developed at the time of planting, those plants were not individually selected for resistance to clubroot. Their level of resistance could be estimated later from the resistance of their I1's. The level of resistance in the original accessions of which they were part was determined in a separate experiment.

Table 1. Disease indices of selected accessions of B.oleracea and of I1's and F1's with Septa.

genotype		accession		I1		F1	
		# plants	D.I.	# plants	D.I.	# plants	D.I.
W77208	Resistant Detroit	34	0.39	27	0.22	10	0.97
W77255	Larson	26	0.03	16	0.40	33	0.99
W87030	Böhmerwald	43	0.16	36	0.25	32	1.00
W87031	Bindsachsener	35	0.27	37	0.93	36	1.00
Br87011	Badger Shipper	108	0.19	23	0.28	40	1.00
Bo78203	Petibor F1	10	0.07	31	0.58	39	0.94
W87016	inbred line (F5)	40	0.15	35	0.41	80	0.97
W87024	Septa	220	0.98				

Since most original accessions showed considerable variation with respect to clubroot resistance, and because the parent plants were not individually selected for resistance, the resistance of the I1's is in most cases different from the mean resistance of their original accessions. In one case (W87031, Bindsachsener) the parent plant was presumably susceptible, resulting in a completely susceptible I1. I1's resulting from other plants in this accession of Bindsachsener, however, had lower disease indices.

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All F1's with Septa are completely susceptible. Since the parent plant was partially resistant in all cases except Bindsachsener, as is concluded from the resistance of the I1's, this means that in all cases the resistance is expressed in a recessive manner.

Not all F1's between two resistant accessions have been tested so far. However, in most cases these F1's are moderately to highly susceptible; only the F1 of Resistant Detroit x Larson is resistant (D.I. 0.15). Assuming recessive inheritance of resistance, this shows that the selected accessions do not have many resistance genes in common. This indicates that by intercrossing several of these accessions, followed by inbreeding and selection of multiple recessive segregants, it may be possible to obtain a higher level of resistance.

DEGRADATION OF CLUBROOT RESISTANCE IN CHINESE CABBAGE EFFECT OF TEMPERATURE AND DAY-LENGTH

YASUHISA KUGINUKI, HIROAKI YOSHIKAWA and SUSUMU YUI

Clubroot of Chinese cabbage (Brassica campestris L. var. pekinensis)caused by Plasmodiophora brassicae Woronin is a very common and serious disease. At the beginning of breeding for clubroot resistance, Yoshikawa et al(1981) established a simple and efficient inoculation method 'Insertion method' for the early selection, and by using this method some highly resistant cultivars were found in European turnip. Crossing Chinese cabbage with the resistant turnip, commercial Chinese cabbage cultivars with clubroot resistance have been released in Japan. However, these resistant cultivars were found not to be sufficiently resistant in some fields and locations. So this experiment was undertaken to clarify the cause of this degradation in its resistance.

Materials and Methods

Varieties : Siloga, 77b (resistant turnip), breeding material No.1, No.4 (resistant Chinese cabbage) and Sin-azumasanto (susceptible Chinese cabbage)

Artificial inoculation 'Insertion method' (H.Yoshikawa, 1983) was applied. Observations on clubbing were carried out six weeks after inoculation. The degree of infection was rated on a 0(no infection) to 3(severe clubbing). The number of plants in grade 1 was multiplied by 20, in 2 by 80, in 3 by 100 and the sum of the products was divided by the total number of plants to give the disease index.

Experiment 1. Effect of temperature and day-length on clubbing

Plants were grown in a phytotron at the combination of 3 levels temperature (20,25 and 30°C) and two levels day-length (12 and 16 hours). Light intensity was 15000lux in all treatments. Each treatment consisted of 30 plants.

Experiment 2. Effect of selection at high temperature

Resistant plants were selected under 30°C(12h of day)-25°C(12h of night) in a phytotron. Then these progenies were inoculated at temperature between 35(high)-20(low)°C in a glasshouse. Each treatment consisted of 60 plants.

Results and Discussion

Typical clubbing appeared on susceptible plants about six weeks after inoculation. Throughout the all conditions (Table 1 and 2), there were high percentage of diseased plants (D.P.) and high disease index (D.I.) in susceptible check cultivar, Sin-azumasanto. On the other hand, D.P. and D.I. were incresed at 30°C in the resistant varieties (Table 1). This degradation at higher temperature was observed in the resistant source cultivars as well as the breeding lines, although some plants were still resistant at 30°C. In Table 2, the progenies which were screened under high temperature condition showed higher resistance than the original lines.

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From these results, it is considered that degradation of clubroot resistance in Chinese cabbage was partly caused by high temperature condition. However, some plants still maintained resistance to clubroot under high temperature condition (Table 1) and this resistance was heritable (Table 2). If selected under high temperature condition, more highly resistant lines could be bred. It is necessary to clarify the mode of inheritance on clubroot resistance under high temperature condition.

Table 1. Effect of temperature and day-length on the incidence of clubroot

Temperature / Day length / Varieties	20℃		25℃		30℃	
	12h	16h	12h	16h	12h	16h
77 b	4[a]	5	7	4	24	14
Siloga	0	1	4	0	54	18
Breeding material No.1	4	7	1	10	14	7
Breeding material No.4	41	33	28	37	66	45
Sin-azumasanto	100	98	99	100	100	99

a D.I.: Disease index

Table 2. Effect of selection clubroot resistsnce under high temperature condition[a]

Varieties	D.P.[b]	D.I.[b]
Siloga	10	6
Breeding material No.4	33	32
Breeding material No.4 -S.1[c]	0	0
Breeding material No.4 -S.2[c]	6	5
Sin-azumasanto	100	100

a 35(high)-20(low)°C in a glasshouse
b D.P.: Percentage of diseased plants, D.I.: Disease index
c Progeny of selected plants of Breeding material No.4
Pathogen for inoculation was isolated from clubbed root of susceptible cultivar(S-1) and resistant cultivar(S-2)

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PROSPECTS OF BIOLOGICAL CONTROL OF CLUBROOT

I Djatnika

Some control methods of clubroot, caused by *Plasmodiophora brassicae*, have been done. Most of them used chemicals, while biological control of the pathogen has never been found.

Isolation of microbes from soil which have capabilities to control *P. brassicae* have been doing since 1980. The result showed that fungi isolate no. Cen.Csi.B.2.1 (seem like *Haplosporangium* or *Mortierella*) could reduce the clubroot intensity on greenhouse from 90.3% to 45.7% (Djatnika, 1987).

Inoculation of the isolate into the soil reduced disease index better than other methods, except for root dipping (Murtafingah, 1987). A microplot (open air) experiment showed that in dry season on limed soil, the isolate could decreased disease index from 89.9% down to 84.5%, but in wet season could not (Djatnika, 1988a). In another experiment, the isolate could suppressed better than lime treatment (2 tons/ha) or other microbes (i.e. *Gliocladium* sp. and *Chaetomium* sp.) (Djatnika, 1988b) The isolate, in a field test, is only able to control *P. brassicae* in non-limed soil (Djatnika, 1989).

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Resistance Sources to *Phoma lingam* and *Alternaria brassicae*

J. Zhu and A. Spanier

For a general overview about available genetic resistance sources six *Brassica* species and two species of *Sinapis* were tested. Cotyledon and stembase infection were applied with *Phoma lingam* pycnidiospores,whereas only cotyledon infection was performed with *Alternaria* spores. In each species two to three varieties or subspecies with 12-15 plants each were inoculated by 10 µl spore suspensions, as follows:

Pathogen	Infection method	Suspension density (spores/ml)
Phoma lingam	cotyledon, with injury, drop	10^6
	stembase, with injury, injection	10^7
Alternaria brassicae	cotyledon, no injury, drop	10^3-10^4

After inoculation all plants were kept under cover of plastic foil in the greenhouse. Disease symptoms were scored 10 days after inoculation for *Phoma* cotyledon infection, after two months for stembase infection and after 8-10 days for *Alternaria* cotyledon infection, using scales divided from 1 (no symptom) to 9 (collapse of cotyledon/plant). The experiments were repeated two times. The average infection rate calculated for each species varied as follows:

Species	Cotyledon/*Phoma*	Stembase/*Phoma*	Cotyledon/*Alternaria*
B. nigra	1.8	3.0	7.6
B. carinata	2.9	3.4	6.7
B. juncea	3.0	3.1	7.9
B. campestris	5.0	3.8	6.8
B. oleracea	8.9	5.9	7.8
B. napus	8.9	5.2	8.7
S. arvensis	2.8	3.0	8.8
S. alba	2.4	3.5	4.8

The results indicate the following rankorder of resistance (from highly resistant to susceptible) for Phoma:

B. nigra =S. arvensis >B. juncea =S. alba =B. carinata >B. campestris >B. napus =B. oleracea;

for Alternaria:

S. alba >B. campestris =B. carinata >B. nigra =B. oleracea =B. juncea >S. arvensis =B. napus.

In agreement to Sacristan und Gerdemann (Plant Breeding 97, 304-314, 1986) *B. carinata* and *B. juncea* were found to offer resistance against *Phoma lingam.* But *B. nigra* and *S. arvensis* exhibited even better resistance means against *Phoma.* Further genetic research should therefore be directed to these species. Presently the only resistance source to *Alternaria brassicae* is represented by *S. alba* as shown above.

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RESISTANCE OF SOME BRASSICA OLERACEA L. PLANT INTRODUCTIONS TO DOWNY MILDEW, PERONOSPORA PARASITICA.

J Hoser-Krauze, E Łąkowska-Ryk, J Antosik

Plant response to the pathogen was tested under controlled conditions at the cotyledons stage during the first decade of November 1989.

Afterwards the resistant plants /class 0-1/ were tested again at the stage of 4-5 true leaves in the half of December.

Reproduction of the pathogen and inoculation conditions were done according to the method of Williams /4/. Cotyledons as well as leaves were inoculated through spraying with Polish isolate of broccoli cv. fungus suspension at the concentration of 10^5 spores per 1 ml of water. The infection degree of the studied plants at the cotyledons and leaves stage was determined visually according to the 6-grade scale of Williams /4/ from 0 to 9.

Accessions of PI have been previously tested and described as resistant by Dickson /1/.

As the resistance control were used two selfed F_7 broccoli lines possessing the resistance of cotyledons and true leaves. These lines have been derived from the breeding material /Hoser-Krauze 2/. The resistance at the cotyledons stage of these two lines was proved by Moss in 1987 /3/. The susceptible control was Polish broccoli cv. Piast.

From the obtained results /tab.1/ the following PI accessions proved their resistance to the Polish isolate of downy mildew: PI 263056, PI 263057, PI 357374, PI 418984, PI 418985, PI 418986, PI 418987, PI 418988.
In our opinion these Plant Introductions can be used as the resistance sources to downy mildew.

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Table 1. Response of Brassica oleracea plants to downy mildew.

PI No.	No. of plants	Percentage of plants					
		Cotyledons stage			4-5 true leaves stage		
		R class 0-1	T class 3	S class 5-9	R class 0-1	T class 3	S class 5-9
189028 var. botrytis	18	5	-	95	100*		
208478 var. botrytis	14	28	22	50	100		
231210 var. botrytis	18	28	22	50	100		
245013 var. capitata	20	80	-	20	100		
246063 var. capitata	17	94	-	6	100		
246077 var. capitata	23	56	-	44	100		
263056 var. capitata	17	100	-	-	100		
263057	15	100	-	-	100		
357374 var. capitata	15	100	-	-	100		
418984 var. capitata	17	100	-	-	79	14	7
418985 var. capitata	17	100	-	-	100		
418986 var. capitata	18	100	-	-	100		
418987 var. capitata	14	100	-	-	100		
418988 var. capitata	17	100	-	-	100		
5 R control	33	100	-	-	100		
7 R control	48	100	-	-	100		
Piast - broccoli cv. S control	34	-	-	100	-	-	-
					plants not survived after infection at the cotyledons stage		

R - resistant, T - tolerant, S - susceptible.
* / The resistant plants at the cotyledons stage were tested again at the true leaves stage.

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NEW SOURCES OF RESISTANCE TO DOWNY MILDEW IN BRASSICA NAPUS SSP OLEIFERA

N. I. Nashaat & C. J. Rawlinson

Downy mildew *(Peronospora parasitica* (Pers.) Fr.) is the most frequently recorded disease of winter oilseed rape *Brassica napus* ssp. *oleifera* in the United Kingdom (Evans, *et al.*, 1984; Gladders, 1987). Some cultivars are very susceptible at the seedling stage (Anon., 1987, 1988), but the disease is not thought to limit yield except, rarely, when seedlings are killed (Davies, 1986). Consequently, selection and breeding for resistance is not given a high priority at present. By contrast, elsewhere in northern Europe (Sadowski, 1989) and Asia (Kolte, 1985) severe infection can cause regular, significant yield loss. However, the status of downy mildew in much of Europe may change with the recent predictions of climate warming (Parry, Carter & Porter, 1989) and the possibility of milder, wetter winters in Europe would also be likely to favour downy mildew.

So far, the only reported major gene for resistance to downy mildew in *B.napus* ssp. *oleifera* was identified in the spring rape cultivar Cresor, but this resistance was overcome by two sexual progeny isolates derived from a homothallic isolate of *P. parasitica* (Lucas *et al.,* 1988).

We have screened a wide range of germplasm (*B.napus* ssp. *oleifera*) collected from sources in the UK, Canada, China, France, Germany and the USA. The screening was carried out, at the cotyledon stage, to four isolates of *P.parasitica*, using a method described by Nashaat and Rawlinson (1990). One of these isolates (obtained from J.A. Lucas, University of Nottingham) was virulent on cv. Cresor. When the germplasm collection was screened for resistance, it was noted that some cultivars showed distinct variation in the level of resistance to downy mildew. However, we have identified two groups of new sources for resistance. The first group includes four genetically uniform genotypes/cultivars and 18 selected lines from different genotypes/cultivars, carrying resistance equivalent to that in Cresor. The second group consists of at least three selected lines from different genotypes/cultivars. These lines were resistant to all four isolates indicating new major gene(s) for resistance different from that expressed in Cresor. Both groups included spring and winter types. Also, new double low winter rape genotypes (low glucosinolate, low erucic acid) are included in the first group. Further work continues on this material to breed truly homozygous lines from the populations to further widen the base of available sources of resistance. The identity of these sources will be published elsewhere.

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SCREENING OF CAULIFLOWER GERMPLASM LINES AGAINST DOWNY MILDEW (Peronospora parasitica).

VIJAY MAHAJAN, H.S. GILL AND RAM SINGH

Epiphytotics of downy mildew-DM (Peronospora parasitica) PP has become a limiting factor both in vegetable and seed crops of cauliflower in the hills and north Indian plains. Kontaxis (1977) estimated crop loss at $ 100,000, due to downy mildew epiphytotic on broccoli in California. Lund and Wyatt (1978) found that the downy mildew can be a significant cause of post-harvest spoilage of cauliflower. The fungicides which can control the disease to some extent are costlier, hazardous to health, require special equipment and attention for its application, which the poor and marginal vegetable growers of India can not afford. The use of resistant varieties in such cases is the best alternative to tackle the problem.

The first step in a breeding programme for resistance against diseases is to locate the source of resistance. With this aim in view the available germplasm of cauliflower was screened against downy mildew.

As Peronospora parasitica is an obligate parasite, it is difficult to culture the fungus on artificial media. The inoculum was collected from the leaves of diseased plants and spore suspension was made at concentration of 10^5 spores/ml of water, and was sprayed twice at an interval of 10 days. The field was also kept wet at the the time of spraying. Three observations were taken at an interval of 10 days on the basis of fungal growth and percentage leaf area affected on the dorsal side of the leaf. First rating was done 9 days after the spray of second spore suspension, on the onset of the disease. Lines were scored on the following numerical scale.

Numerical scale	Percentage leaf area affected	Disease reaction
0	0	Immune
1	1 - 10	Highly resistant
2	11 - 25	moderately resistant
3	26 - 50	Moderately susceptible
4	51 and above	highly susceptible

Intermediate numerical grades were also given while taking observations wherever needed.

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On the basis of observations at 10 days interval, it was noticed that disease increased progressively from 10 to 30 days in susceptible lines while in immune/highly resistant lines no further spread was observed. BR-2 was scored to be immune and cc(12-c) and selection $6F_2$-3-5-1-1 as highly resistant to this disease. These lines flowered and set seed in north Indian plains. cc(12-c) has been reported to be self-incompatible and is being exploited in heterosis breeding programme. Its F_1 hybrids. besides giving higher yield, will also possess resistance to downy mildew, as resistance is dominant over susceptibility in the F_1 hybrids. Curd to plant ratio can also be improved by using resistant selection $6F_2$-3-5-1-1 in the hybridization programme which possesses high curd to plant ratio. Lines E.C. 177283, E.C. 191179, Novembrina, kibo Giant and Mero Giant were also classified. under immune and highly resistant groups. Some of these lines did not form curd and some did not set good seed under Delhi conditions. Lines 330-5, 452-10, Prem's Agrahani, HRG-1, 269-6-9, Pusa Himjyoti and selection $9F_2$-1-3-18-19 were found to be extremely susceptible recording the disease rating of 4. Those lines which recorded the highest disease rating at the time of first observation can be used as infector rows. The susceptible lines with good economic characters can be improved by using them as recurrent parent in the hybridization programme.

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INFLUENCE OF THERMO-HYDRO AND POTENTIAL EVAPOTRANSPIRATION ON WHITE RUST EPIDEMIC OF MUSTARD

B.S. LAKRA AND G.S SAHARAN

White rust caused by Albugo candida (Lev.) Kuntze is a serious bottleneck in augmenting mustard (Brassica Juncea) production in India. Its infection causes symptoms on leaves (local infection) and inflorescence (staghead infection). Where later is more pronounced and destructive in nature.

MATERIALS AND METHODS: To measure the progression of white rust in relation to prevailing environmental conditions cv. Prakash (highly susceptible) was sown in the last week of October, 1985 and 1986 in field where mustard was sown year after year. The white rust incidence and progress was measured at 12 days interval using ratng scale described by Lakra (1987). The different meteorological paramenters viz. mean temperature (MT), mean relative humidity (MRH), potential evapotransapration (PET) and dewfall (DF)were recorded at meteorological observatory situated at a distance of 500 m and correlated for their influence on disease progression. The amount of water which is lost to the atmosphere by the green plant (PET) was also taken into account since it plays vital role in providing moist film of water on host surface. Higher DF and lower PET provide moist conditions for longer duration. The DF (mm) on upper surface at 50 cm height was taken into account keeping the hight of mustard plant in view.

RESULTS AND DISCUSSIONS: In the present investigations the disease severity in leaf phase was recorded above 60% by the end of December and in staghead above 20% by the end of February. When disease severity and progression was correlated with the prevailing environmental components, it was concluded that white rust progressed at a faster rate with DF>.100 mm, MRH> 65%, MT 10-18 C and PET<60. White rust epidemic of mustard predominently depended upon, DF>.100 mm which may be attributed to provide water droplets/free film of water on host surface (pre requiste for sporangial germination of A.candida - Lakra and Saharan; 1988) and high RH. Favourable thermo-hydro conditions (DF>.100 mm, MT 10-18 C and MRH>65%) with PET<60 causes high amount of condensation in the form of water droplets/ moist film for longer duration on host surface which serve as a generation house for secondary infection by imparting and facilitating multifold sporangial germination. Lakra and Saharan (1988) also observed maximum (77.2%) sporongial germination in vitro in water at 12-14 C. Kolte et al. (1986) reported a positive correlation of rain with development of stagheads of white rust. This study showed that from these factors we can calculate and modulate the potential period of white rust epidemic at any location.

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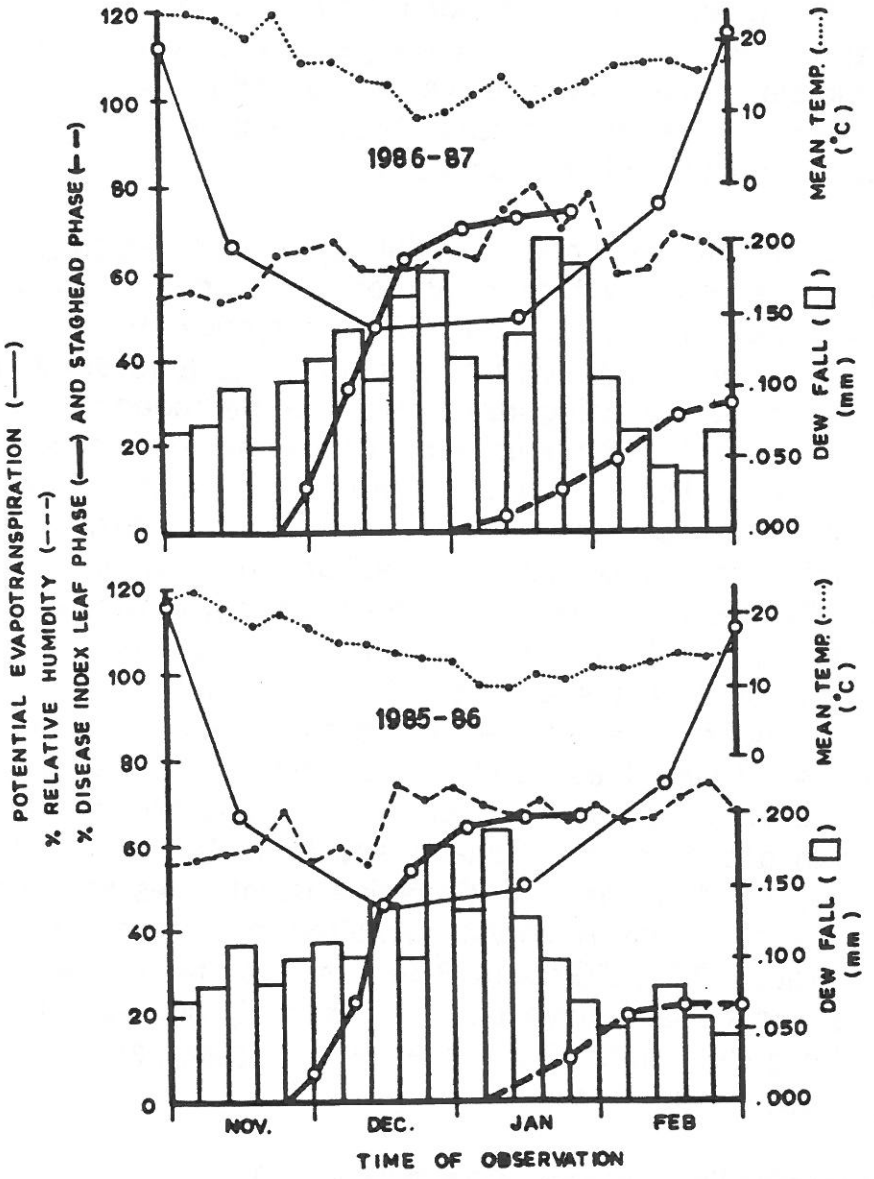

INFLUENCE OF THERMO-HYDRO AND POTENTIAL EVAPOTRANSPIRATION ON WHITE RUST OF MUSTARD EPIDEMIC DURING 1985-86 AND 1986-87.

Pathogenicity grouping of Leptosphaeria maculans isolates based on three cultivars of Brassica napus. **A. Mengistu, S. R. Rimmer, E. Koch and P. H. Williams.**

Although the existence of aggressive and non-aggressive isolates of Leptosphaeria maculans (Desm.) Ces & de Not. (anamorph - Phoma lingam [Tode ex Fr.] Desm.) has been extensively documented (1, 2, 3), knowledge regarding the variability in pathogenicity among aggressive isolates is incomplete. The genetic basis of the variability in L. maculans is poorly understood, primarily because it is difficult to obtain sexual recombination of the fungus in vitro.

As part of a study into the pathogenic variability in Leptosphaeria maculans (Desm.) Ces & de Not. from different geographic regions, we studied the pathogenicity of 39 isolates from N. America, Europe and Australia against a range of Brassica napus var oleifera (oilseed rape) cultivars. Isolates could be categorized into four pathogenicity groups (PG) based on differential pathogenicity on cotyledons of Westar, Quinta, and Glacier (Fig. 1). PG1 isolates can be distinguished by lack of virulence to Westar. PG2 isolates are virulent only on Westar but tend to give slightly more susceptible interaction phenotypes on Quinta than on Glacier. PG3 isolates are virulent on Westar and Glacier and intermediate on Quinta. PG4 isolates are virulent on all three cultivars. These three cultivars are able to discriminate between aggressive and non-aggressive isolates and between three groups of aggressive isolates. Using these cultivars as differentials we have examined about 70 single ascospore isolates from oilseed rape debris from Saskatchewan and Manitoba, Canada and from Western Australia and New South Wales, Australia. All Canadian isolates tested were PG2 types whereas isolates from Australia exhibited pathogenicity characteristic of groups PG2, PG3 and PG4. Isolates from Western Australia, Europe and Canada have been successfully crossed in vitro and studies on the genetic control of virulence in L. maculans and resistance in B. napus is underway.

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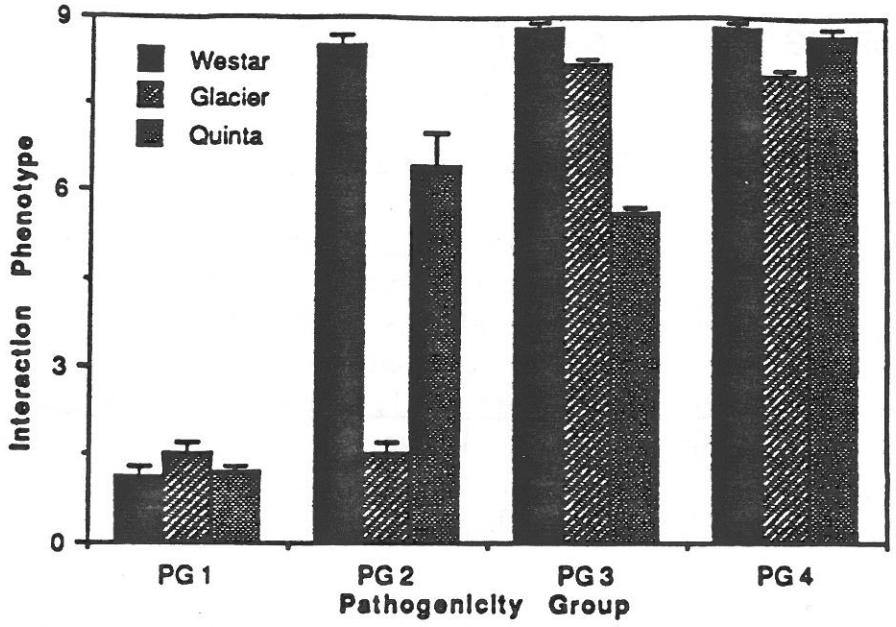

Fig. 1 Pathogenicity grouping (PG) for Leptosphaeria maculans based on differential interaction phenotype of Westar, Glacier, and Quinta. Plants were rated on 0-9 scale where 0= no darkening around wound and 9= rapid tissue collapse at about 10 days post inoculation of cotyledons (4).

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NEW SOURCES OF RESISTANCE TO BLACK ROT AND THEIR INHERITANCE

Z H Guo, M H Dickson, and J E Hunter

Black rot (BR), caused by the bacterium Xanthomonas campestris pv. campestris is a serious disease of cruciferous crops wherever the climate is warm and humid. Efforts have been made by breeders to develop host resistance to control the disease. Many resistant cultivars of cabbage (Brassica oleracea L. var. capitata) have been released in recent years. However, the need for new sources of resistance remains pressing and information related to BR resistance in other species rather than B. oleracea is very limited.

Two B. napus accessions, PI 199947 and PI 199949 exhibited a level of resistance close to immunity (HR). They were challenged by five strains representing the diversity of this pathogen. Another three accessions, PI 199950, PI 273640, and, PI 357374 showed medium level of resistance (MR). In addition, two accessions of B. campestris, B 162 and B 171 from the AVRDC showed moderate level of resistance. These may be valuable sources of resistance for intra or interspecific introgression.

To elucidate the inheritance of the sources of resistance, both intra and interspecific crosses between accessions representing different levels of resistance of the two species were made. The parents, reciprocal F_1's, F_2's, and backcrosses to both parents were tested using wound-colony inoculation for B. napus, and interspecific progenies and wound-suspension inoculation for B. campestris. These procedures are going to be described elsewhere.

The high resistance of B. napus exhibited complete dominance in F_1 generation of both intra and interspecific crosses whereas the medium resistance showed recessiveness. No different reactions were observed between reciprocal F_1 offsprings of any cross.

In the backcross PI 199947 (HR) X CC55 (S) to PI 199947 (HR), all BC_1 plants were resistant, whereas the backcross to CC55 (S) resulted in segregation ratio which fitted 1:1. Segregation in the

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F_2 population gave a ratio well fitted 3 resistant to 1 susceptible. These results suggested a single dominant gene difference between these two parents. The segregation ratios derived from cross PI 199949 (HR) X 357374 (MR) was similar to that of cross PI 199947 (HR) X CC55 (S), again indicating one single gene difference between the parents.

In cross PI 273640 (MR) X CC55 (S), offsprings of backcrosses to both parents were all susceptible. The F_2 population yielded a segregation ratio of 1 medium resistant to 3 susceptible. The medium resistance was explained by a homozygous recessive modifier. The symbol Br is assigned to HR, bm to the modifier. Therefore, the genotypes for the three phenotypes HR, MS, and S will be: Br___, brbrbmbm and brbrBm_ respectively.

In the F_2 generation of interspecific crosses between B. campestris and B. napus, the segregation for resistance and susceptibility did not give the expected ratio maybe because of assortative recombination of chromosomes during the gamete formation. Almost no segregation of morphological charaters was observed, may be due to the dominance of the morphological characters of B. napus and their linkage with factors for fertility.

The moderate resistance found in B. campestris demostrated quantitative inheritance. The results did not allow the calculation of heritability because of the relatively small variance of the F_2 population. The degree of inbreding of the parents were not clear with regard to this character.

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The behaviour of three *Alternaria* species in relation to leaf penetration of hosts and non-hosts

N. McRoberts and J.H. Lennard

Relatively few studies have been made of the behaviour of *Alternaria brassicae* and *A.brassicicola*, especially in relation to factors determining pathogenicity and plant resistance. Results are presented here of an examination of the behaviour of *A.brassicae*, *A.brassiciola*, and *A.alternata* on leaf surfaces of single cultivars of oilseed rape (OSR), wallflower (WFR), and wheat (WHT).

Leaf disks (9mm diameter) were taken from first leaves of test plants, inoculated with 0.2ml of spore suspension (5000 spores/ml), and were incubated in continuous light in sealed petri dishes containing 0.5% water agar with 80ppm benzimidazole added. Two days after inoculation the disks were partially cleared in hot, 200% W/V chloral hydrate, and rinsed twice in sterile distilled water. The leaf disks were stained and mounted in a 1ug/ml aqueous solution of D.A.P.I., and examined under U.V. light. The behaviour of fifty conidia and resulting primary hyphae was examined on each of six replicate leaf disks for each fungus/plant combination.

Percentage germination (Fig. 1) was not found to vary significantly for any *Alternaria* species between the three plant species. However, *A.brassicae* gave higher percentage germination than *A.brassicicola* and *A.alternata* on all three plants. *A.alternata* gave higher germination than *A.brassicicola* on WFR.

Stomatal penetration (Fig.2) was observed only for *A.brassicae* and *A.brassicicola* and with theses species only on OSR; frequency of stomatal penetration was low for both *A.brassicae* (10.0%) and *A.brassicicola* (3.0%). Primary hyphae of *A.brassicae* and *A.brassicicola* penetrated directly on OSR and WFR, but not on WHT (Fig.3). In the case of *A.brassicae* 44.0% of primary hyphae penetrated directly on OSR while 6.7% penetrated WFR directly. With *A.brassicicola* 18.0% and 0.8% of primary hyphae penetrated directly on OSR and WFR respectively. *A.alternata* was not observed to penetrate directly on any plant. A percentage of primary hyphae from each of the *Alternaria* species terminated in swollen structures (Fig.4). With *A.brassicae* and *A.brassicicola* terminal swellings (TS) were often associated with, though did not occur exclusively at, sites of direct penetration on OSR and WFR. With *A.alternata* TS occurred only on WHT and here at a relatively low frequency (5.0% of primary hyphae).

The results of this study provide evidence that features of the behaviour of *Alternaria* pathogens of *Brassica* species are affected by interactions occurring at the plant surface. Germination was not significantly inhibited or enhanced for any of the *Alternaria* species by any of the plant species tested here. This finding and those of similar previous studies (McKenzie, Robb and Lennard, 1988) suggest that inhibition of germination is not a feature of either "host" or "non-host" resistance in the plants examined. *A.brassicae* and A.brassicicola displayed enhanced ability to penetrate directly and via stomata, and to form TS on OSR and WFR as compared with their ability to do so on WHT. This pattern of behaviour suggests that both species are able to differentiate "host" from "non-host" surfaces; however the means by which this differentiation occurs is still unknown.

Features of the behaviour of *A.brassicae* noted here have been previously observed: Tewari (1986) noted that *A.brassicae* was able to penetrate *Brassica napus* leaves directly without prior appressorium formation, Tsuneda and Skoropad (1978) found that *A.brassicae* penetrated *B.napus* either via stomata or directly, and that appressoria were formed at random sites on the leaf surface. Behaviour of *A.brassicicola* was found here to be generally similar to that of *A.brassicae*, and in addition it was found that the behaviour of these two species showed some specialisation to the cruciferous plants tested, as compared with the behaviour of *A.alternata*. Although phytotoxins have been implicated in

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the pathogenicity of both *A.brassicae* and *A.brassicicola* toward *Brassica* species (Hodgkin and MacDonald, 1986; Bains and Tewari, 1987)) the results of this examination have indicated that resistance to these pathogens may be partially dependant on the outcome of interactions occurring at the plant surface.

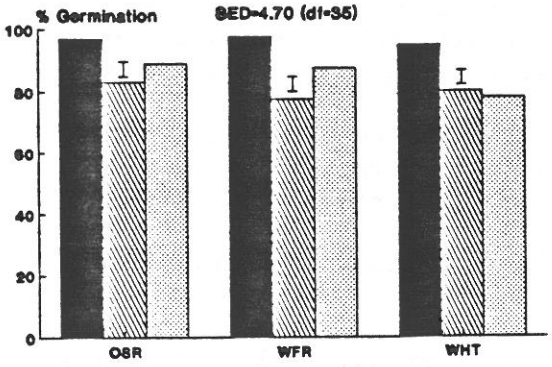

Figure 1

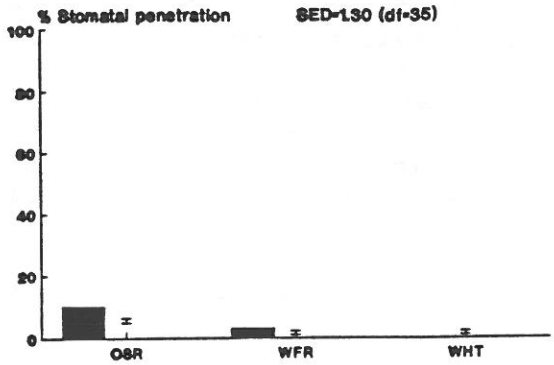

Figure 2

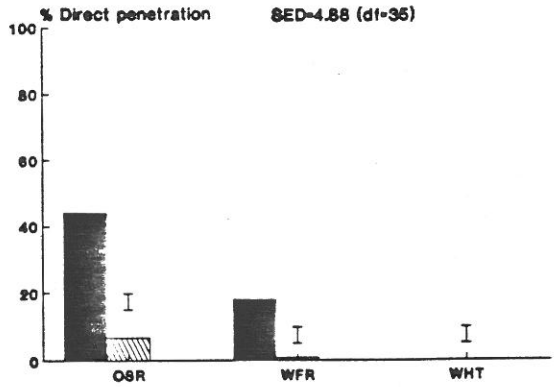

Figure 3

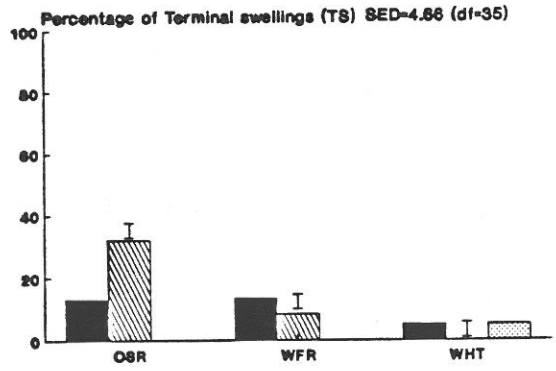

Figure 4

Spore germination rate (Fig. 1) and surface behaviour of primary hyphae from germinated spores (Figs. 2-4) for three *Alternaria* species on oilseed rape (OSR), wallflower (WFR), and wheat (WHT).

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RESPONSE OF CABBAGE SEED WEEVILS (*CEUTHORHYNCUS ASSIMILIS* PAYK.) TO RAPE PLANT ODOUR IN THE FIELD AND IN THE LABORATORY.

K.A. Evans, L.J. Allen-Williams and I. Simpkins.

Cabbage seed weevils, ***Ceuthorhyncus assimilis*** Payk., are insect pests of oilseed rape; their larvae eat the developing seeds in the pods and fungal infection of the pod can occur via the larval exit hole. Weevil feeding and oviposition punctures in the pod wall are utilised by another rape pest, the brassica pod midge (***Dasineura brassicae*** Winn.) for oviposition.
The seed weevil overwinters as an adult and due to crop rotation emerges in a non-host crop. Location of oilseed rape is thought to involve attraction to visual (yellow rape flowers) and olfactory stimuli.
The role of rape plant odour in host-plant location was studied in a field situation and in the laboratory.

Plastic trays painted yellow and baited with an alchohol (Industrial methylated spirit) extract of rape leaves or flowers (50% w/v) attracts more weevils than traps baited with alchohol alone (Table 1).

Table 1. Mean No. of insects/baited trap between 28th April and 5th May 1989*

Insect	Emergence site			Rape crop		
	Control	Rape leaf	Rape flower	Control	Rape leaf	Rape flower
Male weevil	0.38	61.38	43.38	2.25	11.13	11.63
Female weevil	0.13	17.25	18.50	0.88	1.63	3.13

* period of maximum numbers of weevils in traps.

Using an electroantennogram (EAG), the responses of the sensory receptors on insect antennae to olfactory stimuli can be measured and recorded. The EAG setup (based on that described by Guerin & Visser, 1980) consisted of an amplifier, a recording and an indifferent electrode, both sheathed by a capillary tube containing 0.1M KCl as the conducting fluid. The recording electrode was inserted into the head cavity of a decapitated weevil, and the indifferent electrode on the tip of the antenna. The response of the antennae of male seed weevils to rape leaf odour is shown in Figure 1. The rape leaf odour was obtained by trapping the volatiles from crushed rape leaves (variety Ariane) on "Tenax GC", and eluted from the adsorbant with hexane. This extract was then concentrated, evaluated by gas chromatography (Table 2) and 10 µl of known concentrations of this extract (diluted in hexane) was pipetted onto glass filter paper, placed into a 1 ml syringe and injected into an airstream that carries the puff of odour over the insect antenna.

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The response is gauged by comparing it to the response to a standard of 1% cis-3-Hexen-1-ol using the formula

$$\frac{R}{S_1 + S_2} \times 100\ \%$$

where R is the response (mV) to the test compound, S_1 the response to the previous stimulation with 1% cis-3-Hexenol, and S_2 the response to the next stimulation with 1% cis-3-Hexenol (after Guerin & Visser, 1980).
The responses obtained (Fig. 1) indicate a distinct dose response to rape leaf odour.

Further work on the trapping of seed weevils will be carried out using traps baited with compounds identified from rape leaf and flower odour, which produce strong responses in the EAG assay.

Table 2. Composition of rape leaf odour extract used in male seed weevil EAG's (equivalent to 100%)

Compound	Concentration (%)
trans-2-Hexenal	0.0089
sec-Butyl isothiocyanate	0.0019
cis-3-Hexenyl acetate	0.1051
cis-3-Hexen-1-ol	0.1380
3-Butenyl isothiocyanate	0.0007
4-Pentenyl isothiocyanate	0.0027

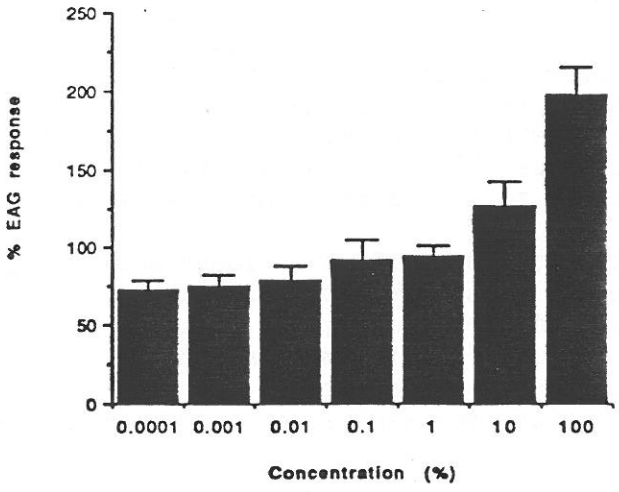

Fig. 1. EAG response of male seed weevils to a rape leaf extract. Vertical lines refer to standard errors of the mean (n=6).

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THE PRELIMINARY STUDIES AS TO A VIRUS DISEASE AFFECTING CAULIFLOWER AND CABBAGE PLANTS IN TURKEY

S.Erkan D.Esiyok B.Eser

Cauliflower and cabbage are two popular and important vegetable crops extensively grown in many regions of Turkey over the areas of about 4 000 and 16 000 ha, respectively (1). The most commonly cultivars are Snowball Y, Brio osenia, Winner osenia, Igloo and Iglory for cauliflower and Yalova I, Bayrakli and Edirne for cabbage.

During the past two growing seasons it was found out that a viruslike disease had been a serious problem in the production areas of these two plants over especially Western parts of Turkey. Symptoms of the disease on both plants were often noticed until the plants were half-grown or later. The affected cauliflower plants firstly showed pronounced vein clearing in young leaves which then developed into pale green or yellowish vein banding. As the leaves matured, a clearly defined large irregular dark-green banding of main veins was seen, contrasting with paler intervening areas. The leaves of diseased plants could be puckered or distorted. Infected cauliflower plants slightly stunted. Because infection of disease checked the growth of heart leaves which were often curved, the curd coming could be unprotected from frost and sunshine. Heads from affected plants tended to be of poor quality. In cabbage, the symptoms were similar to those on cauliflower in general, but the disease caused narrower yellowish banding of leaf veins and darker green banding was less evident. The diseased cabbage plants were also stunted and produced smaller heads than normal ones.

In the present study, we have tried to identify the agent of disease involved. So, leaf specimens were collected from cauliflower and cabbage plants with virus-like symptoms in some locations. The sap from them served as inoculum for mechanical transmission of disease agent to certain test plants. The agent involved was maintained and propagated in Brassica campestris subsp. rapa and for the determination some physical properties of the agent the same plant species was used as assay plants. The data from these studies are presented in Table I.

Following the mechanical inoculation of test plants all isolates from cauliflower and cabbage proved to be of the same type, causing a variety of symptoms. So, all isolates were regarded as isolates of the same virus. The symptoms on test plants and the results from the physical properties gave the first indication that the studied disease was due to infection by a virus, cauliflower mosaic virus "CAMV". Also, the findings obtained from our experiments corresponded to those reported for this virus in some studies (2,3).

The present virus disease on the plants of cauliflower and cabbage is shown for the first time in Turkey by this work. For this reason, now it has been continued the studies for identifying clearly the virus by the serological assays and the observations in the electron microscope. On the other hand, as it was observed that the virus could be readyly transmitted by aphids in fields it is planned to perform the experiments on the transmission of virus by some aphids.

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Table I. Symptoms produced by the virus from cauliflower and cabbage on certain test plants and the physical properties of the virus in question.

Test plants (1)	Symptoms (2)
Brassica campestris subsp. pekinensis	VC, M, LD,Stu, V
B. campestris subsp. rapa	CS, CM, LD, V
B. napus Var. oleifera "Optima"	CS, M, V
B. oleracea var. botrytis "Snowball Y and Brio osenia"	VC,M,GVB,LD,E,Stu,V
B.oleracea var. captitata "Yalova 1"	VC,YVB,LD,V
B.oleracea var. italica "Charade and Shogun"	VC,GVB,LD,V
Datura stramonium	NS, V
Matthiola incana var. annua	CM, V
Nicotiana clevelandii	NS, M, V
Dilution end point (DEP)	1/1000-1/10000
Thermal inactivation point (TIP)	70-75° C
Longevity in vitro (LIV)	4-5 days

(1) Attempts to sap-transmit the virus a range of test plants including Capsicum annuum, Chenopodium amaranticolor, C.quinoa, Gomphrena globosa, Lycopersicon esculentum, Nicotiana glutinosa, N.rustica, N.tabacum "Samsun and Xanthi" and Solanum melongena were unsuccessful.

(2) Abbreviations used in Table are as follows:

C	chlorotic	M	mosaic,mottle	V	virus recoverable
E	enation	N	necrotic	VB	vein banding
G	green	S	spots	VC	vein clearing
LD	leaf distortion	Stu	stunting	Y	yellow

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STUDIES ON THE HEREDITARY REGULARITY OF RESISTIBILITY OF CHINESE CABBAGES AGAINST TURNIP MOSAIC VIR-LIAONING STRAIN No.1

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Abstract

Through investigations, we have obtained a result that the resistibility of chinese cabbages (brassicae pekinensis) to the Turnip Mosaic Virus-Liaoning Strain No.1 (TuMVln-1) was a incomplete recessive characters,at least it was controled by more than five pairs of minor genes, and it was also influenced grestly by the sensitive genes of circumstance, it has more significant characteristics of quantitative inheritance.

In the experiments of backcross,it shown very clear nuclear inheritance, and has close relationships with cytoplasms. According to the analytic compatibility,we have got that the variance analysis of combining ability in general (g.c.a.)and combining ability in special(s.c.a.)were all shown most significant level,gca/sca=13.28, that the additive effect was in main position.thus,when some one work for the breeding of virus resistance,that the rotational crossing or self-bred must be chosen by them,it could be easierto get high resistant varieties,and they must take care of both parents would have high resistant qualities, when in selecting of superior crossing combinations, besides, they must also pay attentions to the difference of resistance in both parents.

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