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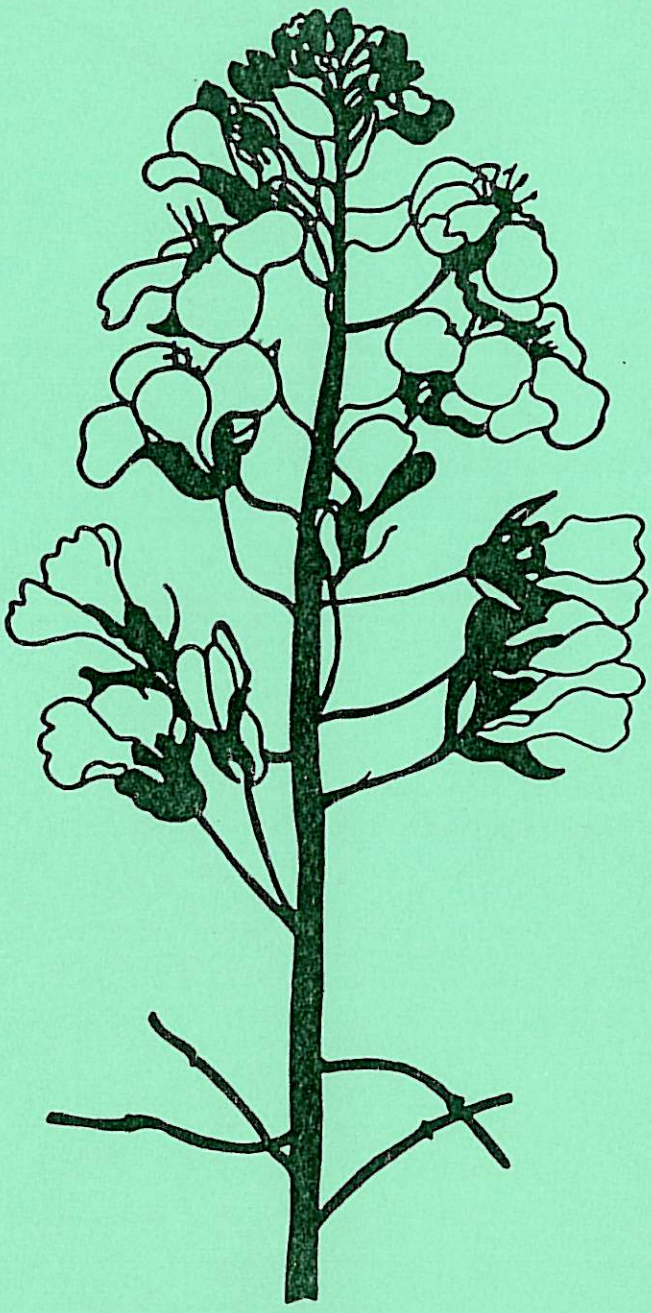

NOVEMBER 1987
EUCARPIA

CONTENTS

Editorial

Included with the Newsletter is an updated distribution list. As usual, we ask that you should notify us of errors in your address and of any names which may be deleted. Additionally, you may, on request, have your name removed from the next published list while continuing to receive the Newsletter. Copyright to the distribution list is reserved and it may not be used for commercial purposes without permission.

As an experiment this issue will be sent surface mail instead of airmail to certain destinations, giving a saving of 50% on mail charges and 25% of our total budget. Feedback from recipients is essential for us to evaluate this change in procedure - we want to know how long the Newsletter was in transit and the condition in which you received it.

As the number of articles submitted to the Newsletter continues to increase we have been forced increasingly to reject scripts which have not been prepared to the requested format or were received after the September deadline. This policy has enabled the Newsletter to be held to about the same size as last year and has helped to give it a more professional appearance without increasing the work involved in preparation.

Finally, if you were not at the 7th International Rapeseed Conference at Poznan, you might be interested to know that the background to the Newsletter was displayed in a poster presentation entitled 'Eleven years of Eucarpia Cruciferae Newsletter'.

A.B. WILLS T. HODGKIN

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Dr K F Thompson

Very sadly, Ken Thompson died on 21 September 1987 not long after his retirement in July 1986 from the PBI, where he had worked for almost 36 years. Having graduated at Cambridge, Ken began his career in plant breeding in 1947 on an ARC Research Scholarship, spending twelve months at each of three breeding establishments, the John Innes Horticultural Institution at Merton, the Plant Breeding Institute at Cambridge and Svalöv Agricultural Experimental Station in Sweden.

He joined the Institute in 1950 as a Scientific Officer working on potatoes. In 1951 he started working on marrowstem kale and concentrated on this crop from 1954. His work in collaboration with John Taylor using sporophytic self-incompatibility to produce the triple-cross hybrid Maris Kestrel, was recognised as a technological innovation by the granting in 1975 of a Queen's Award, and in 1976 Ken was given merit promotion to Senior Principal Scientific Officer.

In 1967 Ken was awarded the degree of PhD based on numerous published papers. In the same year he turned his attention to oilseed rape and true to character, having an eye and an interest in any novel aspect of a crop, developed the use of haploid plants as a method for producing inbred varieties of oilseed rape. The first PBI variety produced using the method was the spring rape variety Maris Haplona in 1975 followed by Fido in 1981. The culmination of his work using this method however, was the variety Mikado the first British bred winter rape. In his retirement, he carried on his breeding interest by acting as a consultant to the commercial company Cundy.

As well as expressing condolences to his family, colleagues the world over have expressed sadness at this loss and wish to recognise Ken's contribution to research and development in the field of plant breeding.

AUSTRALIAN PLANT BREEDING CONFERENCE
WAGGA WAGGA, NSW
JUNE 27-JULY 1, 1988

The Conference is the ninth in a series which began in 1946 with the Australian Conference of Cereal Breeders and Geneticists. It will be held at the Agricultural campus 10 km from Wagga Wagga - a city of 55,000 people located midway on the transport corridor between Sydney and Melbourne. Persons involved in agriculture, horticulture and silviculture and interested in any area of plant breeding and genetics, from both private and public sectors, are invited to participate in the Conference.

The Organising Committee has designed the format of the conference to embrace technical sessions and encourage informal meetings and discussion. All formal presentations will be made in non-concurrent sessions. Invited papers will be presented by prominent local and overseas speakers. Financial assistance is being sought to bring important speakers from overseas and to sponsor significant lectures/papers. Contributed papers are sought for poster presentation and inclusion in the Conference Proceedings, which will be available at the Conference. Selected papers will be presented orally.

Further information is available from Dr Barbara Read, Secretary, APBC, Agricultural Research Institute, Private Mail Bag, Wagga Wagga, NSW 2650.

ENGLISH TRANSLATION OF METZGER'S " ... KOHLARTEN ... " (1833)

NOW AVAILABLE

Hille Toxopeus and Emiel Oost

Johann Metzger's "Systematische Beschreibung der Kultivirten Kohlarten" (Systematic description of the cultivated Brassica species, Heidelberg, 1833) is a rare, but fascinating book on the diversity of Brassica crops in Western Europe some 150 years ago. The book has a very poor distribution and the text is printed in gothic script. Therefore, we have decided to produce, in collaboration with Irene Veerman (PUDOC, Wageningen), a full English translation of the book. To this translation we have added a short introduction on the importance we think this book has for the study of Brassica crop history, genetic resources and taxonomy.

Unfortunately, we are forced to charge Dfl 25,- (approx. $ 13,-) per copy to cover some of our costs. However, we are sure you will enjoy this book and find it well worth its price.

Stichting voor Plantenveredeling, PO Box 117, Wageningen 6140, THE NETHERLANDS.

PROCEDURES FOR THE DETECTION OF ISOZYMES OF RAPESEED (BRASSICA NAPUS AND B. CAMPESTRIS) BY STARCH GEL ELECTROPHORESIS.

Marian L. Thorpe, Louise H. Duke, and W.D. Beversdorf. 1987. University of Guelph Technical Bulletin TB OAC 887. 66 pp.

The techniques outlined can be used to detect up to 17 polymorphic enzymes extracted from cotyledons of 5 day old light-grown Brassica seedlings. Electrophoresis is carried out using a starch-sucrose gel and two histidine-citric acid buffer systems.

Starch gel electrophoresis can be used for rapeseed cultivar identification, for conformation of doubled haploid material, and for verification of rapeseed hybridity.

The bulletin includes details of sample and gel preparation, stains, zymograms of all recorded enzyme patterns, and discussion of the enzymes successfully stained for. A section on work attempted but unsuccessful is also included.

The bulletin is available for a cost of $5.00 from the senior author, Crop Science Department, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. The charge is to cover mailing costs, as well as a newsletter covering refinements and additions to the bulletin.

GERMPLASM RESOURCES OF BRASSICA AND RAPHANUS FROM SPAIN

F. Nuez, M.J. Diez, C. Ferrando, J. Cuartero, J. Costa

During 1984 and 1985 a project designed for collecting several vegetable crop species was conducted in Spain. The project was partially supported by the IBPGR/FAO and the Excma. Diputacion Provincial de Valencia. Brassicas and Raphanus were two of the species enclosed by it. From the beginning of the project 115 samples of Brassicas and 24 of Raphanus sativus have been collected. All of them were sent to the Institute of Horticultural Research, Wellesbourne, England, where they have been characterized and multiplied.

Further information on these accessions can be obtained from the authors.

Acknowledgements

We are extremely grateful to the Servicio de Extensión Agraria and to all those who have collected vegetable crop germplasm: M.S. Catalá, M.L. Gómez-Guillamón, C. Cortés, G. Anastasio and P. Fernández de Cordova.

Biotechnology Dept. Politechnic University of Valencia, Camino de Vera S/N, Valencia, SPAIN.

A cultivar group classification of Brassica rapa L., update 1987.

H.Toxopeus, H.Yamagishi and E.H.Oost.

This paper is the latest update of our effort towards a satisfactory, scientific infraspecific classification of B.rapa L., applying the concept of the cultivargroup (Toxopeus and Oost, 1985).
The second author and coworkers (1985) published the classification of the leafy vegetables of B.rapa used in Japan and commented on the preliminary proposal for a cv group classification (Toxopeus et al, 1984).
He worked at the SVP Wageningen in the first three months of 1987, amongst other things, to become more familiar with the system of cv group classification. The following, updated and expanded version of the cv group classification of B.rapa L is the result of our deliberations.

Table 1: Cultivar group classification for B.rapa L. update 1987.

name of cv group	shared character(s)	use
Vegetable Turnip	turnip	vegetable (turnip)
Fodder Turnip	upright rosette of large leaves	fodder (leaves and/or turnip)
Winter Turnip Rape	biennial	oilseed
Spring Turnip Rape	annual	"
Yellow Sarson	annual, yellow seeds, siliques usually multivalved	"
Chinese Cabbage	heading, petioles winged	vegetable (head)
Pak Choi	non-heading, petioles conspicuous, fleshy, not winged	vegetable (leaves)
Mizuna	many tillers	vegetable (leaves)
Taatsai	flat rosette of many small dark green leaves	vegetable (leaves)
Leaf Turnip	non heading	vegetable (leaves)
Saishin	early bolting (inflorescence loose)	vegetable (inflorescence)
Brocoletto	inflorescence enlarged and compact	vegetable (inflorescence)

Explanatory notes

For better distinction between cv groups we have added, under the column "use" in the table, the part(s) of the plant that is (are) used.
Where the notes of the former version (Toxopeus and Oost, 1985) are valid they will not be repeated here.
To keep the classification clear and convenient cv groups have been created that are of more than local or national importance, unless a shared character makes them stand out very clearly such as in cv groups **Mizuna**, **Brocoletto** and **Yellow Sarson.**

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The new cv group **Leaf Turnip**, combines cultivars of rather small crops like komatsuna, "non heading Chinese cabbage", zairainatane (all three non bulbing), kabuna (bulbing, but only the leaves are used for long term pickling), raapsteeltjes (Dutch leafy vegetable of ± 6 weeks old plants) and turnip greens.
This means that formerly proposed cv groups **Komatsuna** and **Turnip Greens** have been cancelled.
Furthermore, we have decided to change the former name of the cv group **Pe Tsai** into **Chinese Cabbage**, the latter being more widely known and giving less rise to confusion.

For the time being we have decided to drop the provisional cv group **Rapola**. This group was proposed to set aside the "double low" (low erucic acid and glucosinolate contents) cultivars from the cv group **Spring Turnip Rape**. The name Rapola is likely to cause confusion with the commercial name "Canola" for "double low" rapeseed from Canada. This seed is a mixture from **Spring Turnip Rape** (B.rapa L) and spring rape (B.napus L.) cultivars.

The name **Mizuna** has been confirmed for the cv group including cvs of two Japanese crops: mizuna with pinnate leaves and mibuna with entire leaves.
The inclusion of **Taatsai** (formerly provisonally proposed: Taku Tsai) and **Saishin** as cv groups is based on the fact that cultivars of these groups are extensively grown in many countries in eastern Asia.
Many of the cvs of **Saishin** look like early bolting forms of **Pak Choi**, and may be seen as a parallel variation of kailan or Chinese kale (B.oleracea L.).
Brocolleto was kept as a cv group, although it consists of a rather small number of cultivars. However, the large compact flowering head of the cultivars, like in broccoli, is a very distinctive character and there seems to be an increasing interest of breeders for this group.

General remarks

As has been stated before, a cv. group classification is dynamic and designed to deal adequately with new developments, one of which is the growing importance in Japan of a crop of the name "non-heading Chinese cabbage". For the time being it has been classified in the cv group **Leaf Turnip**. Should this crop, or a certain form of it, expand and exceed local significance it would be given cv group status with a name yet to be decided.

The continued improvement of the cv group classification of B.rapa L., and therefore its usefulness, depends on a continuing discussion: so please keep sending us your comments.

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TOWARDS SYNTHESIS OF X RAPHANOERUCA

Narsinha Dayal

Since the classical synthesis of Raphanobrassica by Karpechenko (1927), intergeneric hybridization in the subtribe Brassicinae have been attempted on several occasions by researchers in order to provide new genetic diversity for breeding of cruciferous crops and to exploit the intergeneric hybrids as genetic bridge for transferring agronomically useful characteristics from one crop to another through genome and cytoplasm substitution (Dayal, 1986). More recently, Matsuzawa and Sarashima (1986) have been able to obtain 8 Eruca sativa x Raphanus sativus seedlings through ovary culture technique in order to raise chromosome addition and substitution lines via hybrid progenies.

Here we report a cytogenetical study on the intergeneric hybrid between a nontuberous and early flowering mutant of radish, R. sativus and a local variety of ' Taramira ', E. sativa. Raphanus crossed with much difficulty with Eruca. Out of 110 pollinations made, only 12 siliquae with 8 small and shrivelled seeds could be obtained. On germination they gave rise to three plants only. These plants in general were intermediate between Raphanus and Eruca in morphophysiological characteristics. They had bushy habit with profuse branching, long tap root and white flowers. Flowering in hybrids was much delayed but it continued for a longer period in comparison to their parents. On the whole, leaf and floral characters of Eruca showed dominance over those of Raphanus. Both vegetative and reproductive phases in hybrids were quite prolonged.

Preliminary cytological characteristics of these hybrids were quite interesting. There was a complete collapse of the meiotic system. Pollen mother cells at metaphase I showed a total failure of chromosome pairing with mostly univalents and occasional 1-3 bivalents. Anaphase I was most irregular showing abnormal and unequal segregation of chromosomes, bridges and laggards. At pollen tetrad stage, frequency of diads (85%) and monads and triads (10%) were higher than that of tetrads (5%). The hybrid plants had only 2-5% good pollen.

The hybrid plants did not set seeds when pollinated with Raphanus and/or Eruca. However, they gave 4 seeds on manipulated self-pollination and are carefully kept for further studies.

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INTERSPECIFIC HYBRIDS BETWEEN BRASSICA CAMPESTRIS AND B. MONTANA BY OVARY CULTURE IN VITRO

Nobumichi INOMATA

In previous papers, many F_1 hybrids between B. campestris and wild-related species of B. oleracea, B. bourgeaui, B. cretica, were obtained by ovary culture in vitro (Inomata 1985a, b, 1986). It was suggested that B. bourgeaui and B. cretica became gene sources for the breeding of B. napus. For further enlargement of a gene source of B. napus, hybrid production between B. campestris and B. montana was intented. B. montana is one of wild-related species of B. oleracea. The present paper deals with the results on the production of interspecific hybrids between B. campestris and B. montana, and on cytology of the F_1 hybrids.

The materials used in the present experiment were B. campestris subsp. chinensis cv. Seppaku-taina and subsp. pekinensis cv. Nozaki-hakusai No. 2, and B. montana 89 which was collcted in Gorges-du-Loup near Nice, France (Snogerup, personal communication). The ovaries in the cross of B. campestris x B. montana were cultured in vitro four days after pollination. The culture method and condition were the same as a previous paper (Inomata 1978) and the culture medium was also the same as a previous paper (Inomata 1985a).

The results are shown in Table 1. Number of ovaries explanted in the medium was 50 in each cross combination. Many developing embryos and seeds were obtained and they further cultured in the medium. Production rate of the hybrids was better in the cross between subsp. chinensis x B. montana than that in the cross between subsp. pekinensis x B. montana. Mean production rate of the hybrids in the present experiment was worse than that in the cross of B. campestris x other wild-related species of B. oleracea, B. bourgeaui, B. cretica (Inomata 1985b, 1986). Morphological characteristics of leaf was intermediate between B. campestris and B. montana. Pollen fertility was examined in 26 F_1 hybrids. No pollen fertility was observed in almost hybrids. Mean pollen fertility was 0.78% in both cross combinations.

The first meiotic division was examined in 10 F_1 hybrids having 19 chromosomes. The results are shown in Table 2. Seven hybrids were examined in subsp. chinensis x B. montana. Three hybrids were in subsp. pekinensis x B. montana. Mode of the chromosome configuration at PMCs was $9_{II}+1_I$ in both cross combination. The frequency of $9_{II}+1_I$ and $1_{III}+8_{II}$ was 49% in total cells observed. Other types of the PMCs in the present experiment increased more than that in the previous works of B. bourgeaui and B. cretica (Inomata 1985b, 1986). These F_1 hybrids may be useful for the breeding of B. napus like those of the F_1 hybrids between B. campestris and wild-related species of B. oleracea, B. bourgeaui, B. cretica.

Acknowledgment

I would like to thank Dr. S. Snogerup at the Department of

College of Liberal Arts & Sci., Okayama University, 2-1-1 Tsushima-Naka, Okayama 700, JAPAN.

Systematic, University of Lund, Sweden for providing the seed of B. montana. The present work was supported partly by the Grant-in-Aid (No. 61560007) for Co-operative Research from the Ministry of Education, Science and Culture, Japan.

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Table 1. Production of interspecific hybrids between Brassica campestris and B. montana by ovary culture

Cross combination (B. campestris x B. montana)	No. of capsules examined (A)	No. of embryos further cultured: Late torpedo	walking-stick	Full-grown embryo	No. of seeds obtained	No. of hybrids obtained (B)	B/A x 100
subsp. chinensis[1] x B. montana	44	17	32	18	14	54	122.7
subsp. pekinensis[2] x B. montana	44	6	14	15	1	15	34.1
Total or mean	88	23	46	33	15	69	78.4

1: cv. Seppaku-taina x B. montana 89. 2: cv. Nozaki-hakusai No. 2 x B. montana 89.

Table 2. Chromosome configuration at first meiotic division of F_1 hybrids between Brassica campestris and B. montana with 19 chromosomes

Cross combination (B. campestris x B. montana)	No. of PMCs observed (%)	First meiotic division: $_{I}I^{+8}_{II}$	$_{I}I^{+7}_{II}{}^{+2}_{I}I$	$_{I}I^{+1}_{I}$	$_{II}{}^{+3}_{I}I$	Other types
subsp. chinensis[1] x B. montana	230	43 (18.7)	12 (5.2)	68 (29.6)	6 (2.6)	101 (43.9)
subsp. pekinensis[2] x B. montana	94	31 (33.0)	5 (5.3)	17 (18.1)	1 (1.1)	40 (42.6)
Total or mean	324	74 (22.8)	17 (5.2)	85 (26.2)	7 (2.2)	141 (43.5)

1: cv. Seppaku-taina x B. montana 89. 2: cv. Nozaki-hakusai No. 2 x B. montana 89.

ALLOPLASMIC LINE OF CHINESE CABBAGE

Y. Matsuzawa, S. Suto and M. Sarashima

Alloplasmic line have great potential for plant breeding and is routinely bred via interspecific and intergeneric hybrids followed by backcrossing. On this purpose, works were carried out to get Chinese cabbage(Brassica campestris, 2n=20, AA genome) with the cytoplasm of black mustard(B. nigra, 2n=18, BB genome).

At first 6 F1 hybrid plants were reared in B. nigra x B. campestris; according to Matsuzawa(1983), 49 embryos were extracted from 2236 flowers pollinated and cultured in vitro to overcome the intergeneric barrier. Three of them were studied cytologically and shown in Table 1. They showed the pairing type of (0-5)II+(8-16)I in MI and had 5-12 chromosomes in MII of PMCs and ascertained to be the amphihaploid of BA genome constitution. Two F1 hybrid plants have seed fertility only in open pollination. Successive four F2 plants were also analyzed in PMCs and estimated to be sesquidiploidal with BBA genome(Table 2). Seed fertility of two F2 plants was shown in Table 3. They produced some seeds not in open pollination but in backcrossing by some lines of B. campestris. Recurrent backcrossing may offer the alloplasmic line of Chinese cabbage with black mustard cytoplasm. Studies on the evaluation of B. nigra cytoplasm for the breeding of B. campestris are in progress.

Table 1. Chromosome configulation in the meiotic division of PMCs of F1 amphihaploid hybrids(2n=18) between B. nigra and B. campestris

Hybrids	No. of cells observed	Pairing in MI				
		1II+16I	2II+14I	3II+12I	4II+10I	5II+8I
NiC 84 F1-1*	20		7	10	3	
NiC 84 F1-2	20	1	5	6	7	1
NiC 84 F1-3	34	1	2	11	9	11

Hybrids	No. of cells observed	Chromosome numbers in MII cells								
		5	6	7	8	9	10	11	12	13
NiC 84 F1-1	32		2	5	6	8	5	5	1	
NiC 84 F1-2	33			2	8	10	12	1		
NiC 84 F1-3	40	1	0	5	11	8	11	3	1	

* B. nigra L143 x B. campestris ssp. rapifera cv. Shogoin-kabu

Faculty of Agric., Utsunomiya University, 350 Mine-machi, Utsunomiya 321, JAPAN.

Table 2. Chromosome configulation in the meiotic division of PMCs in the F2 plants between B. nigra and B. campestris

Hybrids	No. of cells observed	Pairing in MI			
		7II+12I	8II+10I	9II+8I	10II+6I
NiC 84 F1-1-0p-1	15		3	6	6
NiC 84 F1-2-0p-1	15		10	4	1
-2*					
-3	15	1	2	7	5

Hybrids	No. of cells observed	Chromosome numbers in MII cells					
		11	12	13	14	15	16
NiC 84 F1-1-0p-1	15	2	3	6	2	1	1
NiC 84 F1-2-0p-1	17		1	8	6	1	1
-2							
-3	22	2	6	8	4	2	

*This plant have 2n=44 showing a pairing type of 1IV+16II+8I in MI and 21-25 chromosomes in MII cells.

Table 3. Seed fertility of F2 plants of NiC 84 F1-1-0p-1 and NiC 84 F1-2-0p-1 in crosses with B. campestris cultivars

Pollen parents	NiC 84 F1-1-0p-1 Flowers pollinated	Pods developed	Seeds	NiC 84 F1-2-0p-1 Flowers pollinated	Pods developed	Seeds
C-* 3	40	5	8	42	32	41
C- 7	40	36	156	40	35	68
C- 8	41	39	185	44	39	90
C-13	45	43	186	44	36	124
C-29	45	37	169	41	30	84
selfing	36	6	0	42	25	22
open	-	129	122	-	216	290

*C- 3: ssp. pekinensis cv. Chitose, C- 7: ssp. pekinensis cv. Kyoto No. 3, C- 8: ssp. pekinensis cv. Matsusima Sinnigo, C-13: ssp. pekinensis cv. Hiratsuka, C-29: ssp. rapifera cv. Shogoin-kabu.

Reference

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A SCHEME FOR ALIEN CHROMOSOMAL TRANSFER IN BRASSICA THROUGH ANTHER CULTURE

S.LEELAVATHI, V. SIVA REDDY AND S.K. SEN

We have drawn a scheme for the production of chromosomally balanced homozygous oil yielding Brassica plants with chromosome numbers ranging from basic genome to amphidiploid status (Fig. 1). As evident from the scheme, the microspores of interspecific hybrids contain as the result of meiosis at least one complete genome of A/B/C and partial genome of A/B/C depending upon the species involved. As a case study, we utilised two sets of interspecific hybrids, viz., B. napus (AACC, 2n=38, strain H-1110-1) x B. campestris (AA, 2n=20, var. Pusa Kalyani) and B. napus x B. juncea (AABB, 2n=36, var. Pusa Bold) for anther culture.

The uninucleated microspores of these F_1 hybrids were utilised for in vitro plant regeneration following essentially the method of anther culture, developed by us (Leelavathi et al., 1984,1987). The morphologies of the regenerated plants (A_1 plants) were distinct from the F_1 hybrids. The A_1 plants were small and weak when compared to the F_1 hybrids (B. napus x B. campestris) maintained through shoot tip culture and transferred to soil along with the A_1 plants. Altogether 53 plants were regenerated of which 7 were analysed. The leaves of five A_1 plants closely resembled B. campestris but contained the traces of waxiness like B. napus. The leaves of these plants were longer and delicate and four of them contained red tips on the anthers, a character of B. napus. On the other hand, leaves of the remaining two plants resembled B. napus closely and one of them contained red tip on the anther. Cytological analysis of A_1 plants at diakinesis stage revealed that two B. napus type and one B. campestris type of plants contained 17 chromosomes. The remaining four B. campestris type of plants contained 16 chromosomes. The observations made from the anther culture of the other interspecific hybrid (B. napus x B. juncea) were in the same line. None of the regenerated plants set seeds upon their transfer to soil.

Although our success at the preliminary stage has been limited, the results have demonstrated that production of aneuhaploids (n=16 and 17) is practically possible which is in line with our proposed scheme. Once these aneuhaploids are diploidised, they are expected to be stable chromosomally and breed true. Since amphidiploidy in Brassica is a stable chromosomal status naturally found, diploidisation of the aneuhaploids should not pose much problem with regard to chromosomal stability.

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BD-32, Salt Lake City, Calcutta - 700064, INDIA.

ALIEN CHROMOSOMAL TRANSFER IN BRASSICA THROUGH MICROSPORE CULTURE

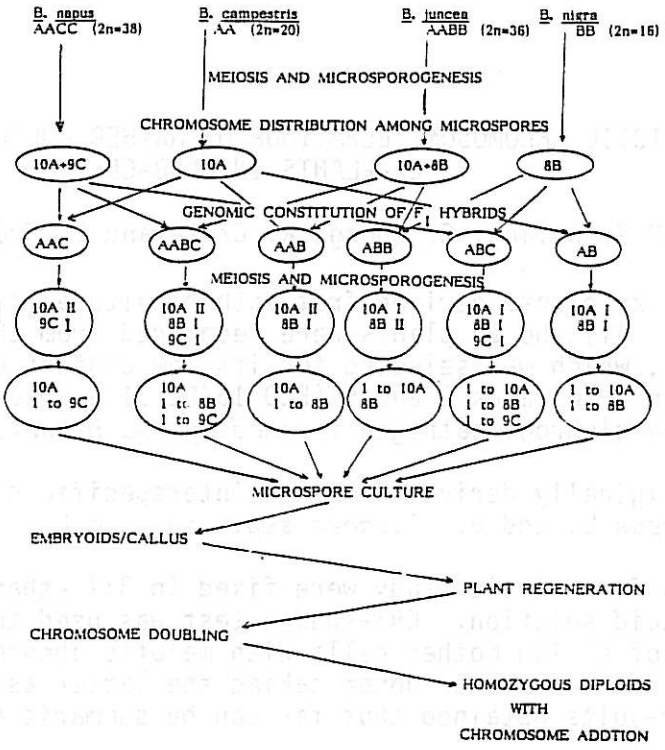

FIGURE - 1

A DWARF MUTANT OF RADISH, RAPHANUS SATIVUS L., OBTAINED BY GAMMA RAYS IRRADIATION

U. C. Mehta and N. DAYAL

The presence of dwarf mutants are not uncommon in crop plants including crucifers (Williams, 1985). They are of considerable importance in plant breeding. However, there is no report about the presence of dwarf mutants in radish. The present study deals with the morphological and cytological study on a γ rays (100 KR) induced dwarf mutant of radish. This mutant was screened in M2 generation plants of an European variety of radish, Doppel Bock. The mutant plant showed normal root but dwarf habit, slow and stunted growth, fewer branches and delayed flowering in comparison to the control. It also had smaller flowers and fruits. The cytological study showed that it had significantly a higher mean chromocentre frequency, increased meiotic abnormality, higher pollen sterility (50%) and very low seed set.

This mutant may be utilized in the germplasm improvement and further cytogenetical studies of radish, a valuable root crop in India.

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MEIOTIC CHROMOSOME BEHAVIOUR OF ANTHER-CULTURE-DERIVED 2x-PLANTS IN CABBAGE

M.S. Chiang, C. Chong, R. Crête and S. Fréchette

Twenty nine 2x-plants derived from anther culture (A_1) were used in this study. All the 29 plants were recovered from the anther donor plant, B-21, which was selected for its low content of glucosinolates and resistance to races 2 and 6 (ECD 16/02/31 and 16/02/30, respectively) of the clubroot pathogen *Plasmodiophora brassicae* Wor.

B-21 was originally derived from the interspecific cross between *Brassica napus* L. and *B. oleracea* ssp. *capitata* L.

Flower buds for meiotic study were fixed in 3:1 ethanol (95%):ferric propionic acid solution. Chi-square test was used to compare the percentage of pollen mother cells with meiotic abnormalities between A_1 and that of the anther donor taking the latter as the expected value. The results obtained thus far can be summarized in the following tables.

Table 1. First meiotic division

Population	Stickiness	X^2	Laggards	X^2	Bridges	X^2
A_1	+	**	+	*	+	**
B-21	-		-		-	

Table 2. Second meiotic division

Population	Laggards	X^2	Bridges	X^2	Micronuclei	X^2
A_1	+	ns	+	**	+	**
B-21	-		-		-	

+ and - denote respectively high and low percentage of PMC with abnormality.

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EFFECT OF COLCHICINE ON CHROMOCENTRES IN ROOT TIP MERISTEM OF RADISH, *RAPHANUS SATIVUS* L.

Iqbal Ahmad and N. Dayal

Colchicine has been widely used in inducing polyploidy and mutation in several crop plants including radish (Tokumasu, 1961). However, the effect of this important alkaloid on the heterochromatin fraction of the genome has not been studied so far. Here we report the study on the effect of colchicine on the number and distribution of chromocentres representing pericentric constitutive heterochromatin, in root tip meristem of radish.

Only one varietal population of radish, Japanese White, was used in the present study. 100 seeds were treated with different concentrations of aquous colchicine solution (0.1%, 0.2% and 0.3%) for different durations (6, 12, 24 and 48 hr). Some seeds were left untreated (control). These seeds were germinated on moistened filter papers in Petri dishes in identical conditions at 25°C. Methods for cytological analysis was the same as described earlier (Dayal, 1975). Scoring was made in 50 cells in each item.

Colchicine affected both the number and the distribution of chromocentres at different concentrations and durations of treatment. Mean chromocentre frequency decreased significantly with increasing concentration and duration of the treatment. The distribution pattern of chromocentres in the interphase nuclei of root meristematic cells was also noticeably affected. The most severe effect was noted in 0.3% concentration at 12, 24 and 48 hr. treatment. At these concentrations and durations even the meristematic cells were greatly affected and chromocentres were not at all observable.

Reduction of mean chromocentre frequency and variation in the distribution pattern of chromocentres on treatment with an anticancer drug, vincristine, and rays have earlier been reported (Panicker and Dayal, 1985; Mehta and Dayal, 1986). Similar effect is noted here on treatment with colchicine.

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Seed Mutagenesis of *Brassica campestris* for Generation of Cytoplasmic Mutants

T. Walters, M.R. Moynihan, M.A. Mutschler and E.D. Earle

Summary. Seed mutagenesis using nitroso-methyl-urea (NMU) can generate cytoplasmic mutants. We report here experiments using NMU to induce heritable mutations in *Brassica campestris*. The proportion of variegated plants with near-normal male and female fertility was greatest when seeds were presoaked for 3h in water before a 5h mutagenesis with 25 µg/ml NMU. This level is much lower than optimal levels for *Antirrhinum* and *Lycopersicon* seeds (Hagemann 1982). Variegated progeny were obtained from some variegated plants.

Materials and Methods. Seed of rapid cycling *B. campestris* (CrGC #66) was mutagenized at 625 µg/ml, 188 µg/ml, and 125 µg/ml NMU. Later experiments with 12.5 µg/ml, 25 µg/ml and 50 µg/ml NMU used a rape seed cultivar of *B. campestris* (ATR 5 Candle, a kind gift of W.D. Beversdorf); these plants were more vigorous and produced more seed than the rapid-cycling material. Seeds were presoaked 0-5.5 hours in water before mutagenesis. For each experiment, a fresh NMU stock solution was made up in a buffer (0.5 M citrate, 0.5 M monobasic sodium phosphate, pH 6.0) and diluted to the appropriate concentration (12.5 - 625 µg/ml) in the same buffer. Seeds were shaken for 5 h on a gyrotory shaker (50 rpm) in petri dishes containing this solution, after which they were rinsed in water 5 times, and planted in speedling trays. Pollen viability was evaluated using Alexander's stain (Alexander, 1969), and a condensed Barrett-Horsfall (1945) scale was used to evaluate the variegated proportion of the leaf area .

Results and Discussion. Plants from seed presoaked 2h and mutagenized at 625 µg/ml NMU had a high frequency of variegation, but had gross morphological abnormalities including root and leaf malformation and slow growth. Failure of these plants to flower and low fertility made these plants undesirable for genetic analysis. Seed mutagenized with 188 µg/ml NMU produced plants with a near-normal vegetative morphology. However, one third of the plants had floral abnormalities, including bud abortion and flowers of reduced size or twisted shape. 100 of 126 plants produced anthers, but 48 of these plants had no stainable pollen, and only 14 of the plants produced more than 20% stainable pollen. When pollinated with normal pollen, only 18 of 77 plants set one or more seeds, indicating considerable female sterility. Plants and flowers of seed mutagenized with 125 µg/ml NMU were more normal in appearaance and had an average pollen stainability of 70%, but still had reduced seed set. The effect

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of NMU on pollen production may be transitory; M2 plants generally had a higher percentage of stainable pollen than their M1 parent.

Since low male and female fertility limited the usefulness of plants from seed mutagenized with 188 and 125 µg NMU/ml, mutagenesis was carried out at still lower levels of NMU using ATR5 Candle seed presoaked for 3 h. The most obvious trait of plants from seed mutagenized at these levels was leaf variegation. Mutagenesis with 25 µg/ml NMU resulted in plants with a wide range of variegation: some showed variegation on more than 88% of the leaf surface, others appeared unaffected, and 2/3 of the plants lay between these extremes. Unmutagenized control plants showed little or no speckling or variegation. Pollen viability was comparable to that of control plants, even in most of the highly variegated plants. Seed set on open-pollinated mutagenized plants was comparable to that on the controls except for plants exhibiting more than 50% variegation. Plants from seed mutagenized with 50 µg/ml NMU were shorter and less vigorous than controls: many of these were highly variegated but set few seeds. Those mutagenized at 12.5 µg/ml NMU exhibited very little variegation.

Hagemann (1982) presoaked *Antirrhinum* and *Lycopersicon* seeds in water for 16h before mutagenesis. In our work with ATR5 Candle, presoaking for 1 to 4.5h before mutagenesis with 25 µg/ml NMU enhanced frequency and degree of variegation.

Six seeds were sown from each of 19 variegated M1 (ATR5 Candle) plants. Six of these progeny groups included at least 1 variegated plant. Other progeny of mutagenized plants exhibited a uniform light green color, at least at the seedling stage We are currently performing reciprocal crosses on the progeny to see if any of the variegation is maternally inherited. Generation of variegated plants in such small populations suggests that NMU seed mutagenesis may be an efficient method of generating cytoplasmic mutants in *B. campestris*.

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COMPARATIVE STUDY ON THE ISOZYMES OF BRASSICA CAMPESTRIS, B. OLERACEA, AND B. NAPUS

M.J. Truco and P. Arús

Additional evidence on the origin of B. napus as an amphydiploid species between B. campestris and B. oleracea has been provided by comparative studies of the electrophoretic banding patterns of proteins and isozymes of these 3 species (Vaughan and Denford, 1968; Coulthart and Denford, 1982; Quirós et al., 1985). In a previous paper (Arús 1984), the regions of activity of 6 isozyme genes of B. oleracea were studied in a sample of plants of 4 cultivars of B. napus. Fixed heterozygosity was observed in 5 genes, and the remaining locus was apparently homozygous. At least one of the alleles of B. oleracea was present in all the loci analyzed. Allozymes different than those of B. oleracea were found in 3 of the 6 isozyme genes. It was suggested that these allozymes were likely to be specific of B. campestris.

The inheritance of the isozyme variation of B. campestris has been studied by Truco (1986). The enzymes of regions PGM-1, PGI-2, and LAP-1 are encoded by 3 polymorphic genes (with 3 alleles each); regions PGM-2 and ADH-2 are apparently monomorphic. Co-migration between allozymes of B. oleracea and B. campestris at these 5 loci occurs only in PGM-1 (alleles 4 and b, respectively). At least 4 isozymes are present in glutamate-oxalacetate transaminase (GOT) of B. campestris. GOT is a dimeric enzyme, and 2 of these 4 isozymes are able to form active intergenic heterodimers. All GOT enzymes band in a relatively narrow zone of the anode of the gel, and overlapping between regions of activity is frequent. GOT has also a complex interpretation in B. oleracea, and we only know the genetics of Got-3 because the enzymes encoded by this gene band in a zone of the gel (GOT-3) that can be analyzed separately from the rest. With the present information it is still unclear the pattern of homology between the GOT isozymes of these 2 species.

In this report, we have studied the isozymic variation of B. napus compared with controls of known phenotype of B. oleracea and B. campestris. Methods, cultivars (20 plants per cultivar), and regions of activity analyzed were the same as in Arús (1984). Results are summarized in Table 1.

All the allozymes found in B. napus corresponded with known allozymes of the 2 diploid parental species. The most common pattern in PGM-1, PGM-2, PGI-2, and LAP-1 was the presence of two allozymes, one of B. campestris and the other of B. oleracea, as expected considering that B. napus is their amphydiploid derivative. Some plants of cultivar "Wesno" had only alleles of B. oleracea in PGI-2. This observation can be explained if there is a high frequency of a null allele in the other duplicated gene.

Two allozymes of ADH-2, a of B. campestris and 2 of B. oleracea, migrate very closely. The single band observed at this region in B. napus is located in a similar position than allozymes a and 2. We have not been able to determine whether the phenotype of the amphydiploid was a heterozygote for these 2 alleles , or a homozygote for either of

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them. Preliminaryly, we have assigned the phenotype a/2 to all the plants studied, but further analysis will be necessary in order to clarify this point.

The banding pattern of GOT-3 in B. napus appears as fixed heterozygote for alleles 1 and 2 of B. oleracea. When comparing the zymograms of the 2 diploid species at this region, we observed that B. campestris had 2 bands: a slow band located approximately at the same position that the hybrid enzyme between the monomers encoded by alleles 1 and 2, and a fast band co-migrating with the homodimeric product of allele 1. The three-banded phenotype observed in B. napus could be formed by the fast band of B. campestris, allozyme 2 of B. oleracea, and their intergenic hybrid enzyme in an intermediate position (this band would overlap with the slow band of B. campestris). Evidence favoring this hypothesis will be provided if it can be demonstrated that the faster electromorph of B. campestris is a homodimer which is active in the same subcellular compartment as Got-3 of B. oleracea.

Table 1. Electrophoretic phenotypes of B. napus. Phenotype notation: alleles of B. oleracea have been labelled with numbers, and alleles of B. campestris with letters. The number of plants of each phenotype in the variable regions has been indicated in parenthesis. The phenotypes of ADH-2 and GOT-3 are tentative (see text).

	Region of activity					
Cultivar	PGM-1	PGM-2	ADH-2	PGI-2	LAP-1	GOT-3
Dong Hae	2/b	a/3 (2) a/3/4 (4) a/4 (14)	a/2	b/2 (1) b/2/3 (3) b/3 (16)	b/2	a/2
Midas	2/b	a/3	a/2	b/2 (10) b/2/3 (3) b/3 (7)	b/2	a/2
Wesno	2/b	a/3	a/2	b/3 (5) 2 (1) 2/3 (1) 3 (12) 3/4 (1)	b/2	a/2
Siberian kale	2/b	a/3	a/2	1/b	a/2 (1) b/2 (19)	a/2

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A NEW SYSTEM ON SYMBOLIZING THE GENES CONTROLLING THE SYNTHESIS OF ERUCIC ACID IN *BRASSICA*

Ding-fu Liu and Hou-li Liu

Since Stefansson et al (1961) isolated the mutant plants with seed oil free from erucic acid in Liho, originated from a German forage rapeseed cultivar (*Brassica napus*), the inheritance of the content of erucic acid was reported by many authors and the mutants with seed oil without erucic acid in other species of genus *Brassica*, such as *B. campestris*, *B. juncea* and *B. olercea*, were found. A lot of studies demonstrated that there is a locus controlling the synthesis of erucic acid in the seed oil in every genome of *Brassica*, i.e. two loci in the secondary species and one locus in the primary species.

In the recent two decades, several genes controlling the different levels of erucic acid were reported and lots of evidences were given that there are a series of multiple allels in each locus. But these genes or allels were given different symbols by the authors. So the symbols of genes controlling erucic acid content in the literature were very confused, and they neither reflected what genome the genes belong to nor the multiple allelism. We suggest, therefore, following rules for symbolizing the genes according to the relationship among *Brassica* species.

Firstly, the genes controlling the synthesis of erucic acid, regardless of its effects, are symbolized as E (the first letter of erucic acid), the mutant genes governing zero erucic acid content (<1% erucic acid) as e. Thus EE is a genotype which can synthesize erucic acid, and ee the genotype of the mutant with seed oil free from erucic acid.

Secondly, the genome which the gene locates in is represented by subscripts A, B, C, etc. Thus E_A is a gene that can synthesize erucic acid in genome A (the genome of or from *B. campestris*). So the genetical structures of *B. campestris*, *B. nigra*, *B. oleracea*, *B. juncea*, *B. napus* and *B. carinata* with erucic acid in their seed oils are E_AE_A, E_BE_B, E_CE_C, $E_AE_AE_BE_B$, $E_AE_AE_CE_C$ and $E_BE_BE_CE_C$, respectively.

Finally, the multiple alleles on the same locus will be distinguished from each other by superscripts 1, 2, 3, etc. The order is determined by the reported time. Thus E_A^1 is the gene which was found earliest in genome A. The allele responsible for zero erucic acid in any genome will not be given a superscript even though there are two or more alleles from different origin in a genome, because they all can not synthesize erucic acid. So $E_A^1e_A$ is the progeny of $E_A^1E_A^1 \times e_Ae_A$.

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Following above rules, the symbol of any gene consists of three characters (two Roman letters and one Arablic numeral) except the genes that does not synthesize erucic acid (only two letters in this case). So we can call these rules as "three-character rule". According to this rule, the new and old symbols, effects and origin of some genes involved in the synthesis of erucic acid reported in the literature were tabulated in Table 1.

TABLE 1 THE SYMBOLS, EFFECTS AND ORIGIN OF SOME GENES CONTROLLING ERUCIC ACID CONTENTS

New symbols	Old symbols	Effects (%)	In species
e_A	e, e_1, or e_2, E^o	<1	cam, nap, jun
e_B	E^o	<1	jun
e_C	e_2, or e_1	<1	nap, oler
E^1_A	E_1, or E_2	10	nap
E^2_A	E^b	15	cam
E^3_A	E^c	30	cam
E^1_C	E_2, or E_1, E^a	10	nap
E^2_C	E^d	3.5	nap

Lee et al (1974), Zhou and Liu (1987), and Guan and Wang (1986) reported that the genes in some Japanese and Chinese cultivars (B. napus) can synthesize more than 10% erucic acid. And Kondra and Stefansson (1965) found the genes less than 10% in Canadian spring rapeseed. The effects of the genes mentioned above were all the average levels of each alleles, but it was not demonstrated whether they are true multiple alleles on all the two loci or only on one, or whether these differences were caused by some unknown factors. So we do not give them new symbols.

Kirk and Hurlstone (1983) reported two genes controlling 12% and 20% erucic acid in B. juncea (AABB) respectively, but they did not point out what genomes these genes belong to. Therefore, we can not symbolize the two genes following the new rule. But it is certain that there is a gene e_B in genome B, because at least three zero-erucic mutants from different origin were reported up to now. One is Zem 1 and Zem 2 developed by Kirk and Oram (1981), the others were found by Anand and Robbelen (1984) and Olsson (1984) in Afghan and Pakistan accessions, respectively. The former is yellow-seeded, and the later black-seeded.

(19 references omitted)

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

Y.S. Chauhan and K. Kumar

Normally the seeds of commercial varieties of Indian mustard are blackish-brown in colour. However, yellow-seeded types have been evolved through irradiation (Nayar, 1968). These have higher oil content and lower hull, fibre contents than their brown-seeded counterparts (Stringam *et al*.1973). Despite the importance of seed coat colour in mustard few studies have been conducted on its inheritance and the present investigation was, therefore, under taken.

MATERIALS AND METHODS

Six crosses were made among 5 blackish-brown seed coloured strains and cultivars of mustard viz. Varuna, T 6342, Sekhar, PR 34 and RW 75-123-2 and three yellow-seeded types viz. TM 9, K 1 and YSRL 1 during 1982-83 and the F_1's were raised in winter 1983-84. All the parents, F_1's and F_2 populations were grown during the winter season of 1984-85. Each F_2 plant was studied separately for seed colour. The segregation for seed coat colour was observed at the time of harvesting.

RESULTS AND DISCUSSION

It was observed that in F_1 generation all six crosses had blackish-brown seed coat colour, indicating that this character is dominant over yellow. The F_2 population of all six crosses showed a segregation pattern of 15 blackish-brown: 1 yellow (Table -1) indicating control of seed coat colour by duplicate genes R_1, R_2. Thus the presence of one or both dominant genes produces blackish-brown seeds while yellow seed coat results when both genes are recessive. The gene symbols R_1 and R_2 are those proposed by Sun (1945). A similar finding was reported by Vera *et al*(1979) but Nayar and George (1970) observed monogenic inheritance. The yellow seed character can be used effectively for breeding varieties of mustard with high oil content and low hull and fibre content in the meal cake.

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Table 1. Segregation of seed coat colour in F_2 populations of mustard.

Cross		Blackish-brown	Yellow	Total	Ratio	Chi-square	P
TM-9 x Varuna	Obs.	147.00	13	160	15:1	0.960	.50-.30
	Expt.	150.00	10				
T-6342 x K 1	Obs.	68.00	4	72	15:1	0.059	.90-.80
	Expt.	67.50	4.50				
Sekhar x K 1	Obs.	84.00	6	90	15:1	0.026	.90-.80
	Expt.	84.37	5.63				
Varuna x YSRL-1	Obs,	53.00	5	58	15:1	0.556	.50-.30
	Expt.	54.37	3.63				
PR-34 x YSRL-1	Obs.	135.00	6	141	15:1	0.957	.50-.30
	Expt,	132.19	8.81				
TM-9*RW-75	Obs.	45.00	3	48	15:1	0.000	
-123-2	Expt.	45.00	3				

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24

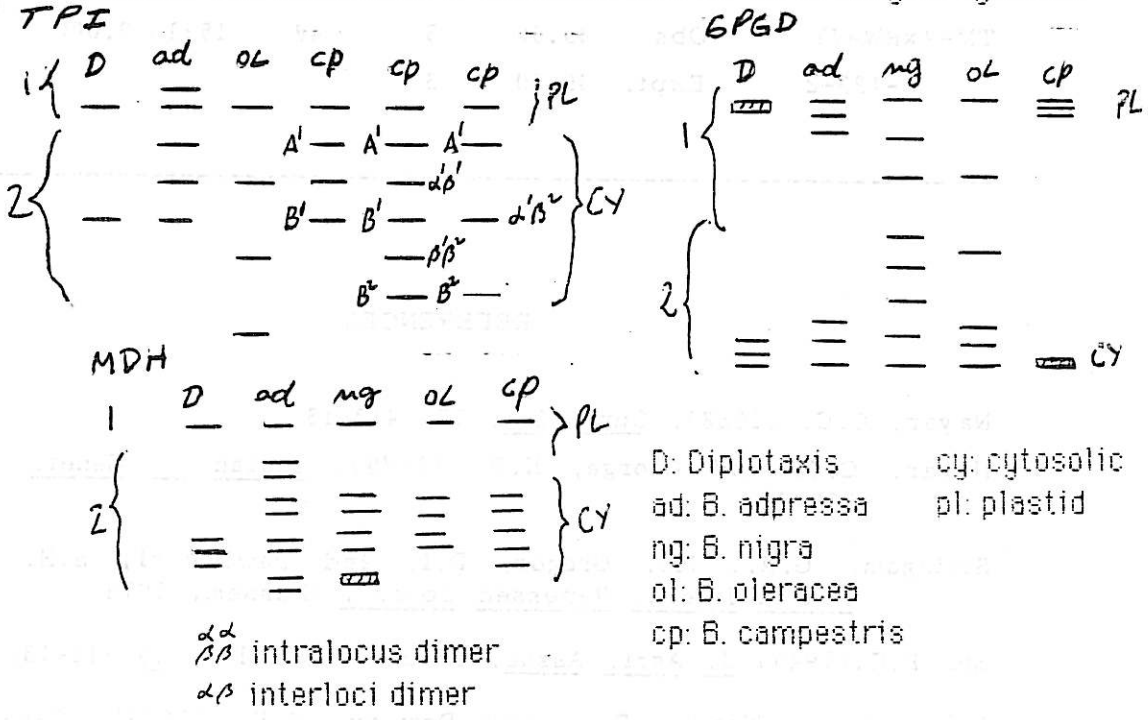
DUPLICATED ISOZYME LOCI AND CELLULAR COMPARTMENTATION OF THEIR PRODUCTS IN BRASSICA

Carlos F. Quiros

Duplicated loci were detected in various *Brassica* diploid species for several isozyme loci by progeny testing and by analysis of chromosome addition lines. Cellular compartamentation of isozymes was determined by electrophoresis of pollen leachates and leaf tissue following the technique of Weeden and Gottlieb (Plant Phys. 66:400,1980). The enzymes triose phosphate isomerase, (TPI), phosphogluconate dehydrogenase (6PGD) and malate dehydrogenase (MDH) have both plastid and cytosolic isozymes coded by a series of duplicated loci. The loci expressing in the gel zone proximal to the anode coded plastid isozymes, while those expressing proximal to the origen coded for cytosolic isozymes

Duplicated loci were not only present in the diploid species of higher chromosome number such as *B. campestris* and *B. oleracea* but also in those with lower chromosome number such as *B. nigra*, *B. adpressa* and *Diplotaxis erucoides*. Furthermore, several plants of *B. adpressa* displayed MDH zymograms of higher complexity than those observed in species with higher chromosome number. Inheritance studies are underway in order to determine whether this complexity is due to the presence of additional duplications. The presence of duplications in *Brassica* supports the hypothesis stating that *Brassica* species are secondary polyploids derived from an unkown species of x=6 by chromosomal rearrangements and aneuploidy.

Schematic representation of duplicate loci for three enzyme systems:



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MONOGENIC DOMINANT WHITE FLOWER (PETAL) IN RESYNTHESIZED *BRASSICA NAPUS*

B. Y. Chen and R. Jönsson

Cross was made between yellow flowered turnip rape (*B. campestris*) cv. Sv38301 (♀) and pure white flowered *B. oleracea* ssp. *alboglabra* line No4003 (♂) for resynthesizing *B. napus*. The resynthesized *B. napus* line No7076 is pale white flowered, thereby indicating that the pure white flower of No4003 is interspecifically epistatic, but not complete, over the yellow flower of Sv38301.

When the resynthesized pale white flowered *B. napus* line No7076 was crossed with the yellow flowered *B. napus* cultivars or breeding lines i.e. SvTopas, SvGlobal and Sv28053, all the F_1 hybrids were pale white flowered. Pale white flower thus is dominant over yellow. The segregation of flower color in F_2 and F_2' (backcross) progenies of all the cross combinations fit well with a monogenic model (See Table 1.)

There was a slight flower color variation amongst the pale white flowered plants of the F_2 and F_2' (backcross) progenies, though they were grouped together. However, this slight variation of flower color was also found to the same extent amongst the pale white flowered plants of No7076, which is supposed to be a pale white flowered homozygote. Therefore, it was not possible to separate the pale white flowered homozygote from the pale white flowered heterzygote in the F_2 and F_2' (backcross) progenies. But the pale white flowered plants were apparently distinct from those yellow flowered.

Since it is known that the dominant pale white flower gene is located in the C genome of the resynthesized *B. napus* line No7076, it is proposed to be symbolized by W_c. The recessive counterpart is accordingly symbolized by w_c. The letter "c" at the right down angle of "W" indicates the location of the gene in C genome.

It is noteworthy that this newly introduced pale white flowered character in *B. napus* can find a good use in marking the chromosome and establishing linkage group with other qualitatively inherited characters, due to its monogenic nature and visual ease.

Table 1. Segregation of flower (petal) color in F_2 and F_2' (backcross) progenies of combinations between one resynthesized pale white flowered *B. napus* line and three yellow flowered *B. napus* cultivars or breeding lines

Cross combination (♀ x ♂)	Segregation of flower color			X^2	P
	Observed plants		Expected ratio		
	Pale white	Yellow			
No7076 x SvTopas	123	46	3 : 1	.44	.70-.50
(No7076xSvTopas)xSvTopas	6	8	1 : 1	.29	.70-.50
SvGlobal x No7076	126	29	3 : 1	3.27	.10-.05
No7076 x Sv28053	40	14	3 : 1	.03	.90-.80
Sv28053 x No7076	102	29	3 : 1	.57	.50-.30

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SPECIES IDENTIFICATION OF CULTIVATED BRASSICAS WITH ISOZYME ELECTROPHORESIS

P. Arús, J.J. Baladrón and A. Ordás

The study of chromosome numbers has been one of the most efficient methods for species allocation in cultivated brassicas. Cytogenetic determinations are highly informative given that most of the brassica crops have different chromosome numbers (exceptions are Brassica oleracea and Raphanus sativus both with 2n = 18). On the other hand, morphological characterization, particularly when dealing with cultivated forms not studied previously, may be misleading because of the tremendously high level of intraspecific variation of the cultivated species, and the presence of similar cultivated types in different species.

Isozyme electrophoresis can be used as an alternative or complementary technique to cytogenetic and morphological studies in species identification. The basis of this application is that, in general, populations of the same species are isozymically very similar (including populations of different subspecies or morphologically dissimilar cultivated types), whereas differences between populations of two species are much more important (Crawford, 1983). The available information on isozymes in brassicas, particularly in the six species of U's triangle, is in agreement with these observations (Coulthart and Denford, 1982; Arús, 1984; Quirós et al., 1985; Truco and Arús, 1987).

Seed of 45 landraces of Galicia (Northwestern Spain) brassicas (Ordás and Baladrón, 1985) were germinated, and leaf extracts of 5 plants per accession were submitted to starch gel electrophoresis and stained for 3 enzymes (phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and leucine aminopeptidase (LAP)). Four regions of activity (PGI-2, PGM-1, PGM-2 and LAP-1), whose variation is controlled by 4 genes in B. oleracea, were studied (see Arús and Orton (1983) for methods and genetic interpretation).

Comparisons between the zymograms of problem samples and controls of the 6 species of U's triangle allowed us to determine that 30 accessions were of B. oleracea, 5 of B. campestris, and 10 of B. napus. These results were in full agreement with the cytological analyses made previously by Baladrón and Ordás (1987).

Although leaf extracts were used in this study, the regions of activity analyzed are also active in seeds. Hence, electrophoresis can be started as soon as seed samples are available, and results for any given sample can be obtained within the following 24 h.

In all cases, each of the 4 regions of activity analyzed was sufficient for clear identification of the species. It seems, however, advisable to study two or more regions of activity, since

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it would allow to double-check with a minimal additional expenditure of time, work and expense (the enzymes used can be stained in different slices of the same gel). Given that PGM has two test regions of activity, it seems a reasonable choice if only one enzyme system has to be used.

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POSSIBILITY OF FROST RESISTANCE TESTING OF BRASSICA NAPUS WITH THE HELP OF DELAYED LUMINESCENCE INTENSITY

A. Brzóstowicz, B. Barcikowska

There have been obtained results of low temperature influence in the range of 273K to 253K on the delayed luminescence intensity /IDL/ in the seconds scope for the leaves of three rapeseed /Brassica napus L./ varieties with different frost resistance. Low temperature had influence on delayed luminescence of the photosynthetic apparatus of the investigated plants. It may be stated, that the differentiation in IDL /T/ among varieties Siberian, Quinta and Bishop, characterizing themselves with different frost resistance, can be the premise for comparing various rapeseed /B.napus L./ forms in their sensitivity on low temperature conditions.

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RECURRENT SELECTION FOR OIL IN-ZERO ERUCIC WINTER RAPE (BRASSICA NAPUS L.)

WERNER SCHWEIGER and EICKE RUDLOFF

The introgression of the zero-erucic trait into winter rape (Brassica napus L.) results in a strong decrease of the oil content. To increase the low oil content is therefore an important aim of zero-erucic breeding. One possible means is the phenotypical recurrent selection as proposed by GRAMI and STEFANSSON (1977) for rape and carried out in Sinapis alba by OLSSON (1960, 1983). In 1976 we began a selection programme to study the possible success in winter rape. This paper reports the first results.

Material and Methods

Two breeding stocks named BNW 9 and BNW 11 were involved. A cycle of selection consisted of two steps. In the first step the selection was carried out. For that some 800 plants were bagged at 2-3 inflorescences at the beginning of flowering. On the seeds from open pollination the oil content was determined by NMR method and the five percent highest oil plants were chosen to form a population in the second step.

For that the "bagging" seeds of the respective plants were used to seed a polycross-like design where the plants were harvested in bulk after open pollination. To guarantee the zero level of the population some 100 single seeds were analyzed for erucic acid content. Half-seed technique for selection of zero-erucic plants was used when any seed exceeded 2,0 percent erucic acid. In BNW 9 the same procedure was used to select for low oil content, too.

In 1985/86 we conducted a field trial which involved the both basic populations (pop 0) and the populations of all the selection cycles carried out so far. In BNW 9 + (high oil) and BNW 9 - (low oil) we tested 4 cycles (pop 1...pop 4) and in BNW 11 5 cycles (pop 1...pop 5). But in the latter the pop 3 couldn't been tested for it's seed yield was to low. We seeded a latin square design with 10 m^2 plot area and 4 replications and analyzed the harvested seed for oil content (NMR-method) and protein content (KJELDAHL method).

Results

The examined populations and the year of production are shown in table 1. The last column contains the erucic acid content of the basic seed for the trial. The relatively high erucic content of pop 2 from BNW 11 is probably caused by a contamination of the polycross area with seeds of high erucic level. Therefore the half-seed technique was nessecary in this case.

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The response of oil and protein content on the selection is shown in table 2. In BNW 9 we see a distinct reaction of oil content in both directions of selection. Selection on high oil content resulted in a mean increase of 0,6 percent per cycle, whereas selection on low oil content decreased it for 0,5 percent per cycle in average. Already after the second selection cycle we observed significant differences from pop 0 in both directions. In BNW 11 this trend was not so well defined. This was particularely due to the above mentioned contamination in cycle 2 which may have caused the strong increase up to 45,5 percent oil in pop 2. But the difference between pop 5 and pop 0 was 2.0 percent and significant, too. The protein content responded on the selection on oil content in a different way. Whereas the selection on high oil in BNW 9 resulted in a continuous decrease in protein content from 24,5 percent in pop 0 down to 23,2 percent in pop 4, in BNW 11 it remained relatively constant and ranged from 24,3 to 24,9 percent. In both BNW 9 and BNW 11 we observed a strong and significant correlation between oil and protein content which was $r = -0,80$ and $-0,75$, resp. The selection on low oil in BNW 9 had not the expected effect on the protein content. There is no directed trend in the course of the selection cycles. The correlation between oil and protein is very weak and not significant ($r = -0,34$). The reason for this is still unknown. Possibly there are differences between types with high and low oil content in the relation in that the conversion of assimilates to carbohydrates, oil and protein occures. That's a very important question for the breeding of high protein types and needs further investigation.

Regarding the oil content this first results indicate that the phenotypical recurrent selection is an useful means in changing the oil content of zero-erucic winter rape.

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Table 1: Description of the examined populations in field trial 1985/86

number of population		year of production	erucic acid cont.[1] in basic seed (%)
BNW 9	pop 0	1975	0,3/7,2
BNW 9 +	pop 1	1977	0,2/0,3
	pop 2	1979	0,2/0,3
	pop 3	1981	1,8/2,8
	pop 4	1983	0,3/3,2
BNW 9 -	pop 1	1977	0,5/0,3
	pop 2	1980	0,2/0,2
	pop 3	1982	0,3/0,4
	pop 4	1984	0,5/0,4
BNW 11	pop 0	1976	0,3/2,0
	pop 1	1978 [2]	0,2/0,1
	pop 2	1981 [2]	4,0/10,8
	pop 4	1984 [2]	0,2/0,2
	pop 5	1985	0,3/03

[1] analytical results of two 10-seeds samples

[2] creating of population in the glasshouse

Table 2: Response of oil and protein content on selection on oil content in zero-erucic winter rape stocks

number of population		oil content (%)	protein content (%)
BNW 9	pop 0	43,0	24,5
BNW 9 +	pop 1	43,6	24,3
	pop 2	44,2	23,9
	pop 3	45,4	23,6
	pop 4	45,4	23,2
BNW 9 -	pop 1	42,7	24,9
	pop 2	41,7	25,4
	pop 3	41,3	25,1
	pop 4	41,1	24,8
BNW 11	pop 0	43,0	24,6
	pop 1	42,9	24,9
	pop 2	45,5	24,3
	pop 4	44,5	24,6
	pop 5	45,0	24,5

STUDIES OF CORRELATED CHARACTERS OF FLAVOUR QUALITY AND HEREDITARY LAWS IN CHINESE HEAD CABBAGES

ZHAO YI PING TAN QI MENG WEI YU TANG

Seven inbreds of the different flavour quality and F1, F2,B1P1 and B1P2 population made by crossing P1 with P2 of the best and the worst ones of them - a total of eleven lines - were used to study correlated characters of flavour quality and hereditary laws in Chinese Head Cabbages (Brassica campestris L. ssp. pekinensis (lour) Olsson).The sugar (mg/g dry w.) - dietary fibre (mg/g dry w.)ratio(sugar/fibre), and soluble protein (mg/g dry w.) in alcohol and water (protein) show significant correlated relationship with the flavour quality.Soluble solids (refractometer), soluble pectin (mg/g dry w.), pro-pectin (mg/g dry w.), chlorophyll ($\mu g/cm^2$), vitamin C (mg/100g fresh w.), various ratios, except the sugar-dietary fibre ratio, have no significant relation to the quality.

Formulation Y=0.7354+0.012286 X (protein mg/g dry w.)+ 3.4432 X (sugar mg/g dry w.) / X (fibre mg/g dry w.) could be used to approximately estimate the quantitative appraisal of the flavour quality. Formulations Y=2.3342+4.023 X (sugar mg/g dry w.) / X(fibre mg/g dry w.) or Y=-1.7+0.04435 X (sugar mg/g dry w.) could also be used, but the accuracy is lower, especially last one.

The broad heritability of sugar was 64%, dietary fibre and protein was around 50%. Half of the phenotype variations in dietary fibre and protein among plants is caused by genetypic differences. The genotypic influence of sugar is lightly larger than that of the environmental factor. The broad and narrow heritability of the three characters is very close. The lowest gene couple number of the fibre could be three, and of the protein and sugar may be one. Simple correlation between the three characters shows significant level at 1%. The coefficient of simple correlation of protein with sugar was 0.5864**, of protein with fibre was -0.5135**, of sugar with fibre was -0.7348**. The parent of lower quality is partially dominant. The quality has light negative heterosis.

Furthermore, in this experiment we studied mechanisms of action on quality characters and breeding methods of the quality. Also, we discussed the concepts of flavour and nutritional quality.

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BRUSSELS SPROUT BREEDING - PROGRESS REPORT
INSTITUTE OF HORTICULTURAL RESEARCH (WELLESBOURNE)

B.M. Smith and C.P. Werner

The current project was initiated in 1980 to investigate the potential for producing vigorous, high yielding inbred lines as varieties to replace F_1 hybrids. From 1980-84 a series of biometrical genetical experiments was carried out to investigate the causes of heterosis, which has been so successfully utilised by plant breeders in this crop. Simultaneously single seed descent inbreeding (SSD) and anther culture (AC) programmes were started with the intention of producing several thousand inbred lines by both SSD and AC.

The biometrical experiments indicated that for all measured characters heterosis was due to unidirectionally dominant genes dispersed between the parents and that overdominance was not involved (Smith, 1985). Therefore there was no genetical reason why high yielding inbred lines should not be obtainable. Predictions were also produced from these trials of the likely performance of random SSD and AC inbred lines. Although there were some differences between the predictions from different trials (Rogers, Kearsey & Smith, 1987; Werner, Smith & Kearsey, 1986) they indicated that high yielding, high quality lines, comparable with F_1's would be obtained only at very low frequency.

In 1985 and 1986 over 2600 F_5 and F_6 SSD and 300 AC lines were grown in preliminary screening trials. Most of the lines were represented by unreplicated single row (12 plants) plots. F_1 hybrid and inbred parent controls were included, occupying approximately 1 control row to every 20 experimental rows. Due to the very large areas involved a systematic control (a conventional inbred of relatively good performance) was included at 1 row in every 40 experimental + control rows.

For harvesting purposes the trials were divided into two parts:

1. A predetermined random sample of SSD and AC lines was recorded to test the accuracy of predictions obtained earlier (in 1985 this random sample constituted a much greater proportion of the trial than in 1986).
2. The remaining lines (ie. majority) were inspected and only those better than the breeder's subjective standard were recorded. To harvest and record all the lines would have been impractical, and many were agronomically worthless.

Detailed analyses of the results for 1985 have appeared elsewhere (Werner, Smith & Kearsey, 1986). Here we present a simple diagramatic summary of the results obtained in 1985 and 1986 for two characters, marketable weight of sprouts and sprout quality. It is apparent that a number of SSD and AC lines were comparable with the F_1 controls for quality in both 1985 and 1986. For marketable weight the results from 1985 and 1986 differ markedly; in 1985 no SSD and AC lines were comparable with the F_1 controls whereas in 1986 the results were more encouraging.

In 1987 and 1988 the best lines obtained from the screening trials will be grown in fully replicated trials, designed to minimise intergenotypic competition, which was unavoidable in the design of the

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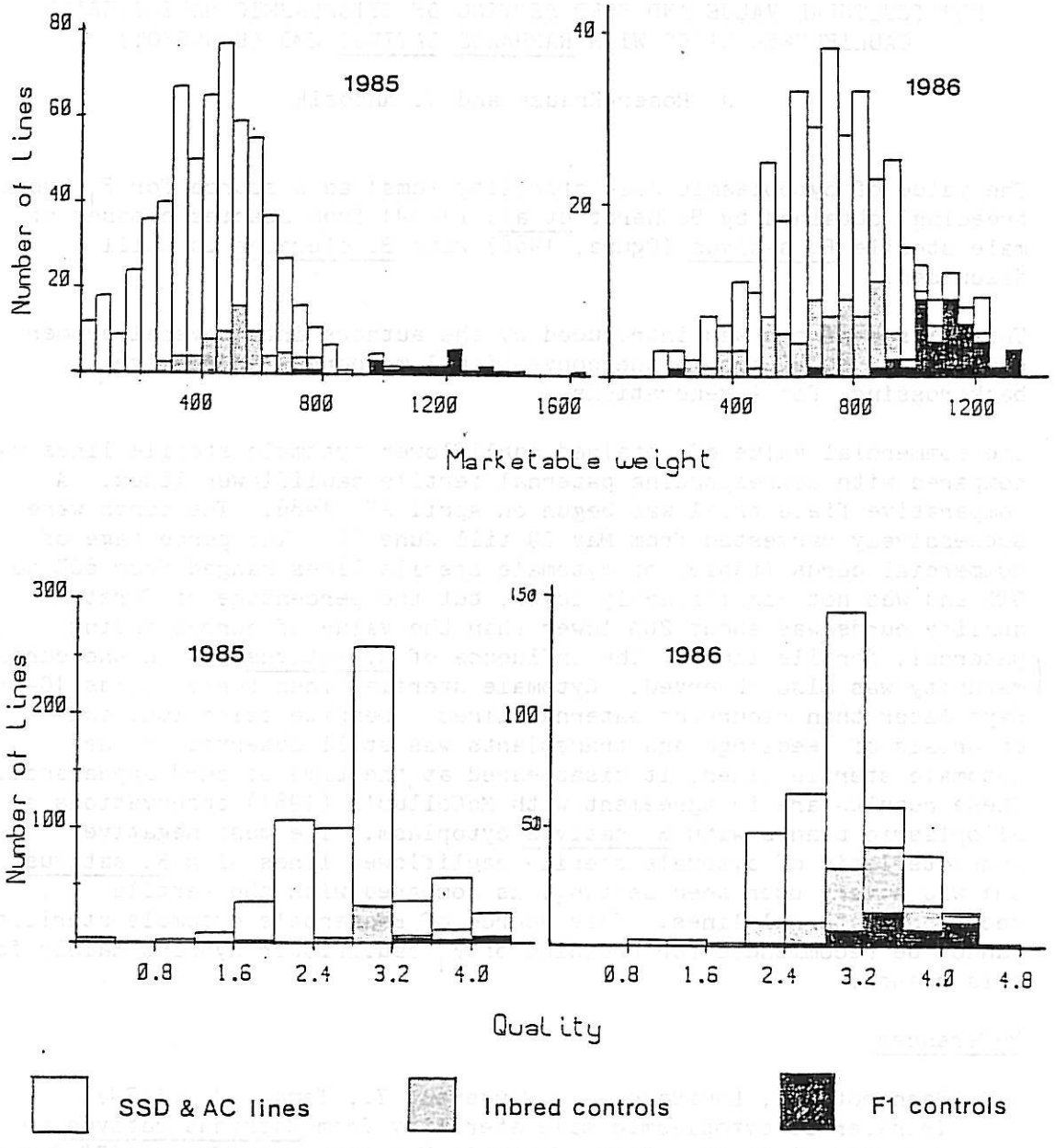

screening trial. This will then allow direct comparisons of the best individual SSD and AC lines with the control F_1's.

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HORTICULTURAL VALUE AND SEED SETTING OF CYTOPLASMIC MALE STERILE CAULIFLOWER LINES WITH RAPHANUS SATIVUS CMS (BANNEROT)

J. Hoser-Krauze and J. Antosik

The value of cytoplasmic male sterility (cms) as a source for F_1 hybrid breeding, obtained by Bannerot et al. (1974) from species crosses of male sterile R. sativus (Ogura, 1968) with B. oleracea is still discussed.

This source of cms was introduced by the authors into several summer cauliflower cultivars in the conventional manner by successive backcrossing, for 4 generations.

The commercial value of obtained cauliflower cytomale sterile lines was compared with corresponding paternal fertile cauliflower lines. A comparative field trial was begun on April 28, 1986. The curds were successively harvested from May 29 till June 18. The percentage of commercial curds (table) of cytomale sterile lines ranged from 60% to 94% and was not significantly lower, but the percentage of first quality curds was about 20% lower than the value of corresponding paternal, fertile lines. The influence of R. sativus cms on the curds maturity was also observed. Cytomale sterile lines formed curds 10-14 days later than recurrent paternal lines. Despite selection, the chlorosis of seedlings and transplants was still observed in the cytomale sterile lines, it disappeared at the time of curd appearance. These results are in agreement with McCollum's (1981) observations of alloplasmic plants with R. sativus cytoplasm. The most negative characteristic of cytomale sterile cauliflower lines with R. sativus cms was a very poor seed setting, as compared with the fertile recurrent paternal lines. This source of Bannerot's cytomale sterility cannot be recommended for breeding of F_1 cauliflower hybrids mainly for this reason.

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Table - Earliness and horticultural value of cytomale sterile cauliflower lines with R. sativus cms (1986)

Line no.	Days to curd maturity[1]	Percentage of marketable crop	First quality of curd %	Average number of seeds per pod after pollination by	
				hand	bees
RK - 1[2] 401[3]	54	60.0	53.3	0.83	0.13
	45	73.8	52.1		2.30
RK - 2 527[3]	73	90.0	70.0	0.24	0.33
	57	96.4	89.5		9.20
RK - 3 644[3]	67	94.1	64.7	0.95	0.26
	55	90.0	82.2		10.10
RK - 4 645[3]	70	63.4	30.0	0.49	0.21
	57	91.1	62.1		8.20

[1] From planting date, April 27
[2] RK - cytomale sterile lines
[3] Paternal fertile line

BRASSICA NAPUS: A POTENTIAL NEW OILSEED CROP FOR BANGLADESH

M.W.Zaman and K.P.Biswas

Among the oleiferous Brassicas, B. napus has the highest seed and oil productivity (Zaman,1987). As it is a species adapted to the temperate regions, its spring type is either unable to flower or flowers too late in the shortday winter (rabi) season, in the subtropics. B. napus (AACC) is an amphidiploid (U, 1935) between B. campestris (AA) and B. oleracea (CC) and shares one genome with the other allotetraploids, B. juncea (AABB) and B. carinata (BBCC). While B. napus lacks ecotypes adapted to the subtropics, the other four species are well represented in this climatic zone. In an attempt to increase vegetable oil production in Bangladesh adaptation of B. napus by species introgression was considered a realistic approach. With this aim the A genome of carefully selected early representatives of B. campestris and B. juncea and C genome of B. oleracea var. alboglabra and B. carinata were used for introgression with the analogous genome of B. napus, cv. Olga from Sweden. B. campestris, B. juncea and the late maturing B. oleracea var. alboglabra and B. carinata seem to be equally effective for introgressing appropriate earliness for Bangladesh (Zaman, 1987).

Materials and methods

The selection for early B. napus was carried out alternately growing the segregating population at Bang. Agril. Res. Inst., Joydebpur, Bangladesh in winter season and at W. Weibullsholm Pl. Breed. Inst. Landskrona, Sweden in summer season. A large number of lines from different crosses were selected with full fertility. Multilocational yield trials were conducted with some advanced B. napus lines (F7-F9) having recommended variety of B. campestris, Sonali as check. Sonali gives high yeild with better management in fertile soil.

Results and discussion

Introgressed B. napus lines switched over earlier to the generative stage but took longer time in pod filling stage than the cv. Sonali. B. napus lines derived from napus x juncea, napus x oleracea and napus x carinata gave higher yield than Sonali. The lines from napus x carinata is most promising in performance as well as disease reaction but needs to improve its oil content (Table 1). Better photosynthetic ability in B.napus may be responsible for its higher yield (Tsunoda, 1980). Another possibility is that the short vegetative stage in B. napus increased availability of more residual moisture which prevailed in the beginning of the season for generative stage and thereby increased yield.

SSO (Oilseeds Research Project), B.A.R.I., Joydebur, BANGLADESH.

While a serious constrain is prevailing for development of new varieties of B. campestris due to limited variability in the species concern, B. napus looks to be a new alternative. The earliness and photoinsensitivity gene(s) introduced in these materials can help to push rape seed cultivation to further north where growing period is limited by short summer and at the subtropical regions where shortday length is a limiting factor. Interspecific crosses specially with B. oleracea and B. carinata which earlier had been relegated can be exploited profitably in widening the gene pool in Brassica.

Table 1. Earliness and yield potential showing the prospect of B. napus as a crop in Bangladesh.

Treatments and origin	Difference in days to flowering	Difference in days to maturity	Relative yield*	Oil content
Sonali (campestris, check)	0	0	100	45.0
nap 1 (napus x campestris)	-10	+ 2	97	46.0
nap 2 (napus x juncea)	- 9	+ 4	107	45.0
nap 3 (napus x oleracea)	-11	+ 5	103	46.1
nap 4 (napus x oleracea)	-10	+ 3	110	46.0
nap 10 (napus x oleracea)	-11	+ 3	102	46.1
nap 12 (napus x oleracea)	-10	+ 4	110	46.0
nap 13 (napus x carinata)	- 3	+10	136	41.4

*Average of six trials. Sonali: Days to flowering = 37, days to maturity = 93 and yield = 873 kg/ha.

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A Preliminary Study on Cold Hardiness in Non-Heading Chinese Cabbage.

I. An Improvement on Method Determining Lethal Temperatures and Its Verification.

Zhu Yuelin, Cao Shouchun and Liu Zuqi

21 cultivars and 5 F1 hybrids of non-heading Chinese cabbage (Brassica campestris L.ssp.chinensis (L.) Makino) were used as experimental materials in this study. A formula, by which cell membrane injury percentages (MIP) of plant tissues treated with a series of sub-zero temperatures can be calculated, was presented: MIP=100(Rt-C)/(K-C).The relationship of treated temperatures and cell membrane injury percentages was studied. An improvement on method determining lethal temperatures of plant tissues was made.The usefulness of this improved method was further verified by recovery test of treated tissues and exploring the relations of cold hardiness levels among excised leaf tissues,calli and intact plants.Following results were obtained.

1.The relationship between treated temperatures and cell membrane injury percentages.

Among 26 cultivars used in this experiment,Guangdong Sijiucaixi,which was frozen to death in mid-December 1985 can not overwinter in Nanjing area.Leaf samples of the other 25 cultivars acclimated under natural conditions were collected and treated with a series of sub-zero temperatures.After measuring the percentages of electrolyte leakage,cell membrane injury percentages were further calculated according to the above-mentioned formula.The relationship of cell membrane injury percentages to treated temperatures can be described statistically with the logistic equation:$y=k/(1+a\exp(bx))$.

2. The relation of inflexion-point temperature of the logistic equation and recovery ability of treated tissues.

After the regression of cell membrane injury percentages to treated temperatures was made with the logistic equation,the temperature at which the inflexion-point of the equation occurs was further estimated and used as the lethal tempera-

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ture of plant tissues.The relation of inflexion-point temperature and recovery ability of treated tissues was investigated with the following method.Leaf tissues treated with a series of low temperatures were divided into two parts,one was used immediately for measuring the percentage of electrolyte leakage,and the other was used after recovery for 24 hr at room temperature in the dark.After recovery,percentages of electrolyte leakage before the occurence of the inflexion-point temperature decreased,while those after the inflexion-point temperature increased somewhat.This result clearly demonstrates that the inflexion-point temperature is the critical temperature of cell membrane injury and a good estimation of lethal temperature of plant tissues.

3.The relation of inflexion-point temperatures and indices of frost injury of field plants.

The frost injury scales of field plants which suffered from a severe freeze were surveyed.Indices of frost injury were further calculated,which were used as an measurement of cold hardiness levels of field plants.Inflexion-point temperatures of excised leaf tissues from 25 cultivars acclimated under natural conditions were determined after a controlled freezing test.Result of statistical analysis shows that the coefficient of correlation between inflexion-point temperatures and indices of frost injury of field plants is significant at 0.01 level ($r=0.8467$).Thus the inflexion-point temperatures of excised leaf tissues are consistent with cold hardiness levels of field plants.

4.Relation of cold hardiness levels between calli and excised leaf tissues.

Calli were induced from leaf tissues of 17 cultivars.After acclimating in the growth chamber the inflexion-point temperatures of calli were determined with a controlled freezing test.The coefficient of correlation of inflexion-point temperatures between calli and excised leaf tissues is statistically significant at 0.01 level ($r=0.9163$).The coefficient of correlation between inflexion-point temperatures of calli and indices of frost injury of field plants is also statistically significant at 0.01 level ($r=0.7362$).Therefore the method,we presented here,for the determination of lethal temperatures is suitable not only for excised leaf tissues but also for calli.

A Preliminary Study on Cold Hardiness in Non-Heading Chinese Cabbage.
II. Determination of Cold Hardiness and Its Correlated Characters.

Zhu Yuelin and Cao Shouchun

21 cultivars and 5 F1 hybrids of non-heading Chinese cabbage (Brassica campestris L. ssp. chinensis (L.) Makino) were used as experimental materials in this research. An experiment with randomized block design of two replicates was conducted from the autumn of 1985 to the spring of 1986. Cold hardiness levels of experimental materials were determined both under natural and controlled conditions. Some physiological or biochemical characters of leaves were also measured. The main purposes of this study are to explore suitable techniques for the determination of cold hardiness and its correlated characters, by which some practical and theoritical guidance will be provided for the introduction, cultivation and breeding of non-heading Chinese cabbage.

1. Cold hardiness levels of non-heading Chinese cabbage are highly related to geographical distributions and developmental characteristics of genotypes. In general, genotypes from North China are hardier than those from South China. Late-bolting genotypes are hardier than early-bolting ones. Among different types of non-heading Chinese cabbage, the Tsai-Tai (var. Tsai Tai Hort.) type is the tenderest while the Tai-Tsai (var. Tai Tsai Hort.) type the hardiest. A wide variation in cold hardiness levels is observed among different types of Pu Tung Pe Tsai (var. communis Tsen et Lee). The above-mentioned relationship of cold hardiness to geographical distributions and developmental characteristics of genotypes has important instructive functions for the production of non-heading Chinese cabbage. When introducing a cultivar from other region, it is of primary importance to consider its habitate conditions and developmental characteristics, and after a cultivation trial, by which a comprehensive understanding of the cultivar can be reached, the final decision can be made whether the cultivar can be popularized or not. When releasing a cultivar

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after a breeding procedure,its adaptability in a given region can be forecasted by analysing the ecological factors of the region.In a breeding program for resistance to low temperatures,special attention should be paid to the selection of parental materials.

2.Significant differences in capability of repairing for frost injury were observed among cultivars.Special superiority in this respect was observed in F1 hybrids.

The estimation of inflexion-point temperature as described in our previous paper,is a direct measurement of resistance of cell membrane to low temperatures,but can not measure the capability of repairing for frost injury,which is an important factor influcing growth and yield of non-heading Chinese cabbage.The comprehensive understanding of cold hardiness in non-heading Chinese cabbage,therefore,will be reached by combining the controlled freezing test with field observations.

3.A significant correlation was observed between cold hardiness levels and contents of soluble sugars in acclimated leaves.Other physiological or biochemical factors measured in this experiment,such as water contents,cell sap concentrations, proline contents,soluble protein contents,starch contents, chlorophyll contents and viability (measured by TTC reduction method) of leaves are not related with cold hardiness levels of non-heading Chinese cabbage.

CANONICAL CORRELATION ANALYSES BETWEEN CHARACTERS IN *BRASSICA NAPUS*

Yongming Zhou and Houli Liu

There is interrelationship in different degrees between characters of crops. It is very important to understand the interralationship for the decision of efficient breeding strategies. Only was the simple correlation between single characters analysed in most of previous studies on the relationship between characters in rapeseed, *Brassica napus*. There was litlle information about the interrelationship between groups of various characters in this crop. To fill the gap, canonical correlation analyses which can evaluate the correlation between two groups of characters as a whole have been conducted in present study to investigate the interrelationship between different sets of characters in *Brassica napus*.

MATERIALS AND METHODS

Thirty genotypes used in this investigation were taken from main areas of rapeseed production in China. Eleven of them are the conventional cultivars with high content of erucic acid in seed oil and high content of glucosinolates in meal, and the others are the single or double-low strains developed in China since 1978. The experiment was designed according to complete random block with three replications. The size of each plot with three rows was 1.66 X .66 m, and no space row between plots. Ten plants were randomly chosen from each plot and the following traits were examinated: plant height (PH), first primary branch height (BH), primary branch number (BN), main raceme length(MRL), siliquae on main raceme (SMR), siliqua-setting density (SD), siliqua length (SL), seeds per siliqua (SS), 1000-seed weight (SW), siliquae per plant (SP), seed yield per plant (SYP), oil content in seed (OC). The following traits were measured at one plot as a unit: date to 30% plant flowering per plot (DF), the contents of six main fatty acids in seed oil, i.e. palmitic, oleic, linoleic, linolenic, eicosenoic and erucic acids, and total content of glucosinolates (TG). Canonical correlation analyses were done on the base of matrix of overall means.

RESULTS AND DISCUSSION

The characters examinated were divided into three sets, i.e. plant morphological character group (PMC), consisting of PH, BH, BN, MRL, SMR, SD, SL and DF; yield character group (YC), covering SS, SW, SP as well as SYP; and quality character group (QC), including TG, OC and the contents of six main fatty acids. The canonical correlation analyses

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between the groups were conducted, and only the canonical variate pairs which are significant at probability .05 (Bartlett's test) will be discussed here.

The two canonical variables are needed to express the dependency between PMC and YC, which indicates that close correlation between these groups of traits exists. The correlation coefficients of the two canonical variable pairs are .9614 and .8125 with probabilities .001 and .01, respectively. As we know, the loadings of a canonical variable on each character estimate the contribution to the canonical variable by that character. The loadings of canonical variable for PMC on PH (.6819), SMR (.5876) and BH (-.5304) are larger than the loadings on the other characters, which shows that these characters are the main factors determining the high correlation between the conanical variabl pair. In the linear compound of YC, the coefficient of SP (1.1199) is strikingly higher than the others. the results reveal that higher plant, lower primary branch height and more siliquae on main raceme may result in more siliquae per plant.

The explanations similar to the first canonical variable pair for PMC and YC are given to the second one. The canonical variable for YC places more weights on PH, MRL SMR and DF and the another variable places more weights on SP and SYP. The analyses for the two canonical variable pairs may give us the suggestion that breeders should pay more attention to those traits which have closer relationship with seed yield and yield components.

One correlation coefficient(.9103) between the canonical variable pair for PMC and QC is highly siginicant (P=.01). The correlation between the two canonical variables is mainly determined by PH, BH, MRL, and BN in PMC and the contents of erucic and oleic acids in QC.It is well known that there is strong correlation between the contents of these two fatty acids, so it could be considered that the change in the content of erucic acid will affect the other plant morphological traits.

The closer relationship between QC and YC has been discovered from the correlation coefficient (.8055) of canonical variable pair for the two sets of traits. Like the case in the correlation between QC and PMC, much larger loadings of canonical variable for QC on the contents of erucic and oleic acids are observed. The canonical variable for YC puts the largest weight on SW, and the loading on SS also sizable, which indicates that the alternation of SS and SW may result from the change of the content of fatty acids, especially erucic acid. As a result, seed yield will be influenced because SW and SS are two of three yield components. This is consistant with obsorvations from the practice of breeding for quality in rapeseed.

EXTENT OF OUTCROSSING IN INDIAN MUSTARD (*BRASSICA JUNCEA* L. CZERN & COSS.)

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

The knowledge of natural selfing and outcrossing is important for deciding appropriate breeding procedures and maintaining the genetic purity of the varieties. In *Brassica juncea*, outcrossing as high as 14 per cent (Howard *et al*. 1915) and 7.6 to 18.1 per cent (Labana and Banga, 1984) has been reported. Different genetic markers have been used to determine the extent of outcrossing. Olsson (1960) used recessive flower colour and Rudloff and Schweiger (1984) used erucic acid as a marker for the estimation of percentage of cross-pollination in rape.

In the present study, yellow seed coat colour was used as a genetic marker which is governed by two recessive genes (Chauhan and Kumar, 1987). Extent of outcrossing was studied in 4 yellow seeded strains of Indian Mustard. These strains were allowed to open pollinate during 1985-86 and were surrounded by brown seeded types. Subsequently, progenies of open pollinated yellow seeded plants were grown during 1986-87 and frequency of brown seeded plants was recorded.

A perusal of the Table 1 indicates that outcrossing ranged from 11.94 per cent (NDYSR-1) to 24.00 per cent (NDYSR-2) with an average of 19.34 per cent.

The extent of outcrossing in the present study is quite high. Thus breeding approaches employed in Indian mustard needs to be reviewed for yield improvement.

Table 1. Extent of out crossing in Indian mustard.

Strain	Yellow seeded	Brown seeded	Total	Per cent outcrossing
1. NDYSR-1	140	19	159	11.94
2. NDYSR-2	133	42	175	24.00
3. NDYSR-3	77	21	98	21.42
4. NDYSR-4	84	21	105	20.00

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M. H. Dickson and M. Kyle

In 1972 , Pearson (2) reported on the development of cytosterile B. oleracea using B. nigra cytoplasm. He indicated the petaloid anther form was stable, while the vestigial anther form was unstable and that there were problems with lack of nectaries, poor seed set and floral abnormalities. Dickson (1) in 1975 released 3 broccoli lines with fair seed set, following vigorous field selection.

Since 1975 much of the effort made on developing cytosteriles in B. oleracea has involved raphanosteriles. However, they all suffer from low temperature chlorosis and only fair seed production. Recently there have been reports of the chlorosis being overcome by protoplast fusion to replace the radish chloroplasts.

In 1984 we crossed two of the cytosterile broccoli lines 1102A and 1106A released in 1975 with B. nigra cytoplasm to broccoli cauliflower. We also crossed a cytosterile cabbage with 'Bonanza' as maintainer (obtained from Dr. Pearson) with five different hybrid cabbages. The F2 seed was produced and the broccoli and caulifower populations grown in the field in New York State with a total of about 6000 plants. All the plants were scored for horticultural characters, then for sterility, and subsequently for seed set. Three bee hives were placed in the field to ensure availability of pollinators.

The broccoli maintainers, NY1102B and NY1106B, were crossed to broccoli and cauliflower. F2 selections were made and test crossed onto male sterile selections for the presence of the appropriate ms gene.

We also evaluated the nigra sterile cabbage lines available from Dr. Pearson for seed set,We used the Bonanza A and B selections to make crosses with 5 cabbage lines. The F2 seed of the Bonanza x cabbage cultivar crosses were planted in California near San Juan Bautista, overwintered, and scored for sterility and seed set.

In the cabbage crosses, 75% of the plants produced no seed, but a few produced excellent seed crops. Generally, the broccoli and cauliflower F2s produced more plants which set seed well than did the cabbage. This may have been due to the fact that while the broccoli parental lines 1102A and 1106A had been field screened in a previous season for seed set, the Bonanza cabbage line had not, and its seed setting ability was much less than for the broccoli lines.

In two subsequent season of field testing, as well as with hand pollinations in the greenhouse the sterile broccoli selections have generally set well. Hybrid combinations with the broccoli steriles have looked attractive. We are optimistic the nigra cytosteriles can find a useful place in B. oleracea hybrid production.

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SOME INVESTIGATIONS ON POLIMA CYTOPLASMIC MALE STERILITY IN BRASSICA NAPUS L.

Fu Ting-dong, Yang Guang-sheng and Yang Shao-niu

In the spring of 1972, we found nineteen CMS plants in field material of cv. Polima. The present study has been conducted on lines derived from this CMS material.

In 1980, we began to select single and double low Polima CMS lines. We have now obtained suitable combinations of CMS, maintainer and restorer lines. Tests on specific cross combining ability and hybrid yield capacity are under way.

1. Breeding of maintainers: According to the results of different test crosses, Polima CMS can be divided into three types, as shown in Table 1. The first type is a low-temperature CMS, the second type

Table 1. Influence of maintainers on Polima CMS sensitivity to temperature

Cytoplasm	Maintainers	Sensitivity of male-sterility to temperature
Polima	Bronowski	At low temperature, the sterility is better*.
"	Al-x	At high temperature, the sterility is better*.
"	Bao-3	The sterility is stable at both high and low temperatures.

*The low temperature represented the range of 10-15^{o}C; the high temperature represented >20^{o}C.

is a high-temperature CMS, but the third type is a stable CMS at both high and low temperatures. Therefore, we think that the sensitivity to temperature of Polima CMS depends on the maintainers. This means that it is possible to breed stable Polima CMS lines.

2. Breeding of restorers: A large number of test crosses was previously carried out for finding restorers. We can now restrict test crosses to a narrow range of materials and easily find restorers.

As seen from Table 2, we have found restorers of Polima CMS from either European or Asian B. napus cultivars. Also, there exist restorer genes in B. juncea and B. campestris.

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Table 2. Test crosses of Polima CMS with various cultivars of B. napus, B. juncea and B. campestris

Source of cultivars	B. napus			B. juncea			B. campestris		
	No	No of restorers	%	No	No of restorers	%	No	No of restorers	%
European	14	9	64.3	1	1	100			
Asian*	16	3	12.5	8	5	62.5	9	3	33.3

*Including the breeding lines descended from crosses involving European cultivars

In 1984-1986, we crossed a Polima CMS with 56 single low lines derived from crosses between double high restorers and single low or double low cultivars. As shown in Table 3, it is easy to breed single low restorers of Polima CMS line.

Table 3. Test crosses of a Polima CMS line with 56 single low testers

Restorers		Maintainers		Partial restorers		Total
No	%	No	%	No	%	
31	55.3	18	32.1	7	12.5	56

3. The differences between Polima CMS and Napus CMS: As seen from Table 4, Huaye and 1811 are the restorers of Polima CMS, but the maintainers of Napus CMS; Canada-3 and Xin-4 are the maintainers of Polima CMS, but the restorers of Napus CMS; Xiangai-B and 5021B are maintainers of both Polima CMS and Napus CMS. It is thus concluded that the Polima CMS and Napus CMS are apparently different.

Table 4. Performance of male fertility of the F_1 from the crosses of Polima CMS and Napus CMS* with different testers

CMS types	Testers					
	Huaye	1811	Canada-3	Xin-4	Xiangai-B	5021B
Polima	F	F	S	S	S	S
Napus	S	S	F	F	S	S

*Napus CMS included both SCMS and TCMS.

Acknowledgements: Many thanks are expressed to Professor Liu Hou-li for his supervision of the present work.

RESULTS OF BREEDING OF SUMMER CAULIFLOWER HOMOZYGOUS SELF-INCOMPATIBLE LINES

J. Hoser-Krauze

Cultivars belonging to the group of summer cauliflowers generally cultivated in the temperate climate are self-compatible (Nieuwhof, 1974; Hoser-Krauze, 1979). On the contrary numerous Indian cultivars are self-incompatible. The highest percentage (62) of self-incompatible plants was found in the Indian cv. Pusa Katki (Hoser-Krauze, 1979). Because of the dominance of premature formation of poor quality curds in temperate climate (Hoser-Krauze, Gabryl, 1978) cv. Pusa Katki cannot be directly used as a maternal component of F_1 hybrids. Therefore the homozygous self-incompatible lines of this cultivar were used as a source of S-alleles to be introduced into summer cultivars, by successive backcrossing in three or four generations.

The horticultural value of successive backcrossed hybrids, F_2 of BC_3 and BC_4 was compared to that of paternal lines of three summer cvs in the field trial 1985.

The results obtained proved that homozygous self-incompatible lines of cv. Pusa Katki were a good source of self-incompatibility. The negative characters such as premature formation of flat, small and loose curds (class 3,4) were eliminated in BC_1, BC_2 hybrids (fig. 1). The time of maturity and commercial crop of curds in BC_3, BC_4 was as good as in the corresponding recurrent paternal lines. These qualities were not changed in F_2 generation of BC_3 and BC_4 (fig. 2,3).

The inheritance of self-incompatibility and self-compatibility in BC hybrids and their F_2 appeared to conform to the theory put forward in the previous publication (Hoser-Krauze, 1981).

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The comporation of herticultural value of successive backrossed hybrids, their F_2 and recurrent paternal cauliflower lines.

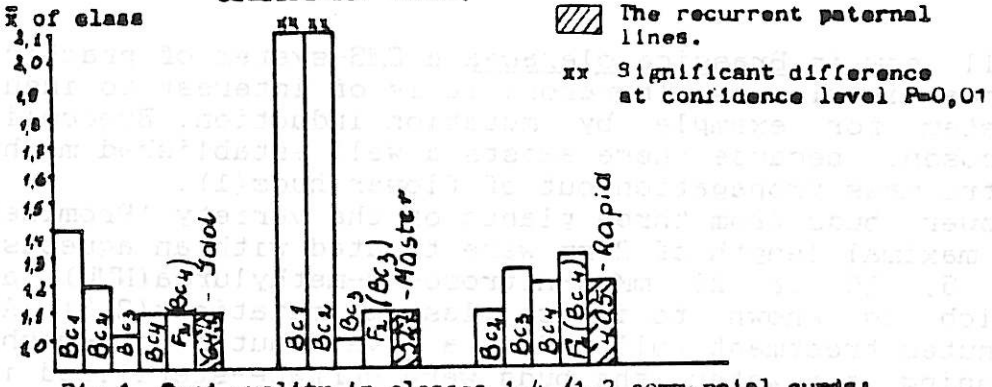

Fig.1. Curd quality in classes 1-4 /1,2 commercial curds; 3,4 not commercial curds.

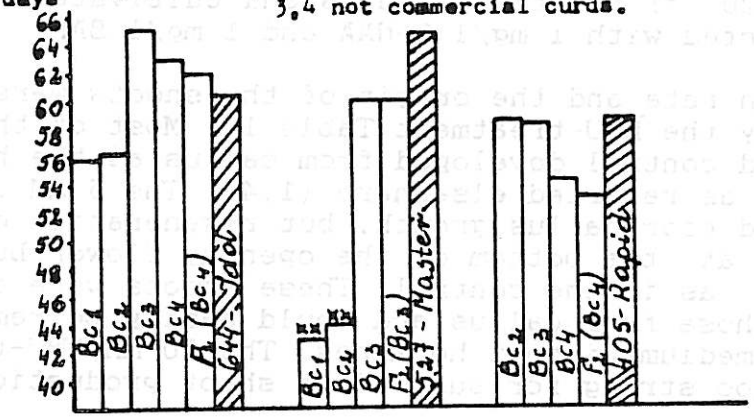

Fig.2. Earliness /days from planting date April 30 to curd maturity/.

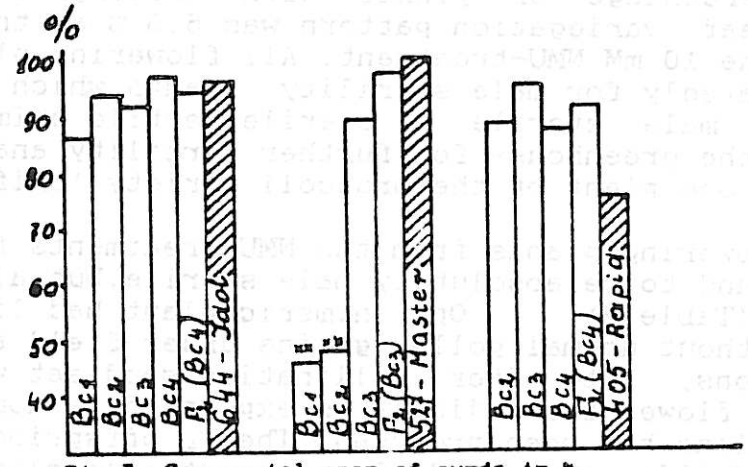

Fig.3. Commercial crop of curds in %.

MALE STERILE BROCCOLI (BRASSICA OLERACEA VAR. ITALICA) INDUCED BY IN VITRO MUTAGENESIS

F Dunemann and J Grunewaldt

Till now in Brassica oleracea a CMS-system of practical importance is missing. Therefore it is of interest to induce such a system for example by mutation induction. Broccoli has been choosen, because there exists a well established method for in vitro mass propagation out of flower buds(1).
Flower buds from three plants of the variety 'Prominence' with a maximal length of 2 mm were treated with an aqueous solution of 5, 10 or 20 mM N-nitroso- N-methylurea(NMU), a chemical which is known to induce plastome mutations(2,3). After a 45 minutes treatment followed by a five minutes post-washing under running tap water, the buds were surface-sterilized in Sodium-hypochlorite (20 %) for five minutes and cultivated on an MS-medium supplemented with 1 mg/l 2-NAA and 1 mg/l BAP.

The regeneration rate and the origin of the shoots were strongly influenced by the NMU-treatment(Table 1). Most of the shoots in the untreated control developed from callus at the basal end of the pedicel, as reported elsewhere (1,4). The 5 mM and 10 mM variants showed poor callus growth, but regeneration of plantlets occurred at the bottom of the opening flower bud in the same dimension as in the control. These shoots were of better quality than those from callus and could easily be removed and rooted on MS-medium without hormones. The 20 mM NMU-treatment obviously was too strong for sufficient shoot production.

After ten weeks of in vitro culture about 560 M_1-plants could be transferred to the greenhouse and later planted in the field. The percentage of plants with white/green or light green/green leaf variegation pattern was 6,3 % at the 5mM and 10,7 % at the 10 mM NMU-treatment. All flowering plants were recorded repeatedly for male sterility. Plants which seemed to be completely male sterile or sterile/fertile chimeras were replanted in the greenhouse for further fertility analysis and crossings with one plant of the broccoli variety 'Skiff'.

Out of 339 flowering plants from the NMU-treatments finally 15 plants were found to be absolutely male sterile,but also partly female sterile(Table 2). One chimeric plant had little white anthers without normal pollen grains under field and greenhouse conditions, but after pollination seed set was nearly normal. Using flower bud-pedicels as explant(5) a homogeneous, male sterile clone has been produced. The F_1 offspring from the cross with 'Skiff' contained 50 % male sterile plants, which could be the result of a dominant allele for male sterility in the M_1-plant used. Besides sterility some other mutants were found, for example different flower and leaf types, white flower colour and a waxless plant. Some of the mutations seems to be dominant ones, because we found a 1:1-segregation in the progeny of the M_1-plant.

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Table 1: Influence of NMU concentration on number and origin of regenerated shoots

Treatment	number of explants	number of shoots originating from pedicel callus abs.	rel.(%)	flower bottom abs.	rel.(%)	total
control	120	1117[a]	83,3	223[a]	16,7	1340[a]
5 mM	400	130	36,6	225	63,4	355
10 mM	400	39	12,9	264	87,1	303
20 mM	400	1	8,3	11	91,7	12

a) projection on 400 explants

Table 2: Influence of NMU concentration on number of M_1-plants and degree of male sterility

Degree of male sterility		NMU concentration 5 mM	10 mM	20 mM
absolute MS		6	9	–
normal	FF	–	1	–
partial	FF	3	3	–
	FS	3	5	–
partial MS[a]		11	22	1
normal	FF	–	1	–
partial	FF	6	10	1
	FS	5	11	–
partial MS[b]		55	72	4
normal MF		103	54	2

a) very low pollen production only under greenhouse conditions
b) low pollen production in the field

MS=male sterility,MF=male fertility
FS=female sterility,FF=female fertility

TESTING INBRED LINES OF RADISH (RAPHANUS SATIVUS L.) AS MAINTAINERS FOR MALE STERILE LINES

M. Nieuwhof

At the IVT research is carried out on male sterility (ms) in radish. Use was made of ms radish material of Ogura (Ogura, 1968). The Japanese material received consisted of an incompletely ms line (A-line) and its maintainer (B-line). By test crosses between ms A-plants and male fertile (mf) B-plants we produced a completely ms line, which remained completely ms after mass propagation. Ms plants of this line were used to test inbred lines of early radish types for the prospects of using them as B-lines.

Material and methods

Mf plants, which were selected from early radish lines for further propagation by selfing, were placed in isolation cages, together with 1 or 2 ms plants. The ms plants were pollinated with pollen of the mf plants by means of flies. The progenies of the ms plants were checked for ms, mostly about 50 plants per progeny.

Results

The percentages of ms plants of the progenies of ms plants pollinated by the same mf plant did not differ significantly. Therefore, when 2 progenies per test cross were present, the average percentages of ms plants of both progenies are given.

Of most lines 1 plant was tested as maintainer. Table 1 shows that 18 of these 33 test crosses (55%) were completely ms.

As the lines of Table 1 were selfed for only 1 or 2 generations, between plants of a line genetic differences may still occur. To check this, from a number of lines 3 or 4 mf plants were chosen for test crosses. It appears from Table 2 that indeed between plants of such lines still differences were found for their abilities as maintainer.

Tables 2 and 3 show that the results of test crosses with mf plants of successive generations of the same line are mostly identical. In a few cases the test crosses with mf plants of an intermediate generation were incompletely ms, while those of an earlier and later generation were completely ms (Table 3, Robijn 1 and 3). The results with Robijn 1 and 3 also demonstrate that plants of I5's may still differ for their genetic constitution for ms.

Discussion

The percentages of ms plants of the incompletely ms test crosses differed largely, which indicates that ms in the radish lines tested is not determined by one recessive factor as was found by Ogura (1968). The small percentages of mf plants in test crosses of later generations, while test crosses of earlier generations were completely ms, may indicate that not only recessive genes with an additive gene action are involved.

From the test crosses it appeared that in most early radish varieties maintainers can be found. When producing B-lines it is recommended to test each generation for the occurrence of segregating mf plants.

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Table 1. Results of test crosses between mf plants from inbred lines and ms plants.

Origin line	Number of lines tested	Number of completely ms F1's	Percentages of ms plants of incompletely ms F1's
Revosa	5	4	11
Robijn	13	5	0, 51, 51, 77, 86, 87, 93, 99
Rota	3	3	
Saxa	4	1	0, 0, 46
Triplo	8	5	54, 68, 95

Table 2. Results of test crosses between mf plants from inbred lines and ms plants.

Origin line	Mf line	Plant (I1)	% of ms plants	%'s of ms plants in test crosses of next generation (I2)
Katra	1	1	100	
		2	100	
		3.	100	
Revosa	1	1	100	
		2	100	100, 100, 96
		3	4	0, 0, 0
Saxa	1	1	100	
		2	0	0, 0, 0
		3	2	0, 0, 0
		4	54	
	2	1	100	
		2	100	
		3	100	
		4	100	

Table 3. Results of test crosses in successive generations (percentages of ms plants)

Origin line	Mf line	I1	I2	I3	I4	I5 [1]
Robijn	1		87	100		83, 100, 100
	2		0			0, 0
	3			92	100	65, 100
	4		100	100	100	
	5		86	82		
	6		51	25		
Triplo	1	68	100	100		
	2-6	100	100	100		
	7	54	29	91		

[1]) Of the I5 generation 2 or 3 test crosses per line were made, and of the other generations 1 test cross per line.

MECHANISM OF MALE STERILITY IN BRASSICA JUNCEA. VII.

CYTOLOGICAL INVESTIGATIONS OF RESTORER MICROSPOROCYTES

S P Angadi, I J Anand and Anita Gogia

Anand et al (1985) identified pollen fertility restoring genes for CMS B. juncea in B. nigra and B. campestris. In order to transfer the restorer genes, the restorer plants were separately crossed to B. juncea and through recurrent back crossing, plants similar to B. juncea in gross morphological features but having the restorer genes from B. nigra (RN) and B. campestries (RC) were obtained. Highly promising RC and RN plants were intercrossed to obtain RC X RN combinations that were found to produce higher amount of fertile pollen than the component plants.

Meiotic studies were taken up in the pollen mother cells to determine the chromosome number and their pairing behaviour, in the above mentioned newly constituted restorer plants and their crosses with CMS B. juncea.

Restorers from B. campestris source - RC's

Pollen mother cell meiosis in eight RC plants chosen for the study showed more or less similar chromosomal behaviour. They were found to have the full chromosomal complement of B. juncea. However, some abnormalities were also observed. The most common was the occurrence of univalents numbering up to two. Percentage of cells having univalents varied from 3 to 10. Occasionally a quadrivalent and a trivalent were also noticed. It was also observed that the same cell had a quadrivalent and two univalents. Anaphase-I had normal distribution of chromosomes; where no abnormalities were observed. However, some of the cells showed laggards, which probably arose due to the occurrence of univalents at metaphase.

Second division was normal in 90 per cent of the cells. However,10 per cent of the cells had irregularities which resulted in sterile pollen grains.

Restorers from B. nigra source - RN's

The reconstituted B. juncea from restorer B. nigra also had disturbed pairing to some extent which expressed in the form of two univalents (in 6 to 12 per cent of the cells). Anaphase-I cells showed laggards similar to the ones observed in RC. The pollen fertility ranged from 82 to 90 per cent.

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RC X RN

The fertility restoring B. juncea derived from B. nigra and B. campestris sources was reconstituted to bring the two genomes and thereby the restorer genes present in them together. Meiotic studies in selected plants of these stocks revealed that, apart from regular chromosomal pairing in majority of the cells, there was a disruption in chromosome pairing in the form of two univalents. However, number of cells showing univalents was low as compared to RC and RN. Number of cells at Anaphase-I having laggards was also quite low. Second division was quite normal and only few cells had irregular meiosis, which was reflected in higher pollen fertility levels ranging from 83 to 93 per cent.

When the three reconstituted restorers (i.e. RCs, RNs and RC X RNs) were crossed with CMS B. juncea, chromosome pairing behaviour in the progenies revealed some amount of abnormality in the form of two univalents. Anaphase- I and the second division too followed the same course as that of the above mentioned cases. Pollen fertility in these hybrids was lower compared to the respective restorers.

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MECHANISM OF MALE STERILITY IN BRASSICA JUNCEA. VIII. HISTOLOGICAL BARRIERS TO POLLEN FERTILITY RESTORATION

S P Angadi, I J Anand and Anita Gogia

In the CMS B. juncea (Anand and Rawat, 1978) faulty stamen differentiation resulted in stigmoid, petaloid and rudimentary structures. In the crosses of CMS with restorers, stamen differentiation was normalised to different degrees. Histological studies in partially restored B. juncea (Mishra and Anand, 1985) showed one pollen sac in each anther instead of the normal two. Further selection of full fertiles based on the well developed anthers have yielded restorers with normal anthers and 91 - 98 % fertile pollen. Histology of these floral buds was taken up to see the pollen sac development and the differences in the anthers of partially and fully restored plants with regard to tapetal behaviour, and pollen development.

Development of ovary was normal in almost all the cases showing that male sterile cytoplasm had not affected female fertility. Shape, size and number of anther locules differed for partial and fully fertile and partially and fully restored plants. Partially restored plants showed drastic changes as compared to the fully restored ones that were nearer to normal. Tapetum was single layered, parietal and showed normal differentiation at early stages; thus indicating that hinderance in tapetal differentiation is not the cause for failure of full fertility restoration.

Degree of tapetal degeneration differed within a floral bud and also between locules of the same anther.

Differential degree of tapetal breakdown appeared to affect pollen development. In the restorers with high levels of pollen fertility tapetum had almost completely disintegrated and pollen grains were well developed. Similar situation was observed in fully restored plants. In some crosses, inspite of the complete degeneration of tapetum pollen development was poor suggesting to other causes like meiotic abnormalities.

Differences in anther locule development in fertile restorers are natural as they have ms cytoplasm back ground. Restorer genes are not fully effective in negating the faulty stamen differentiation either due to their incomplete dominance or presence of modifiers. Even when the anther locule development was completely normalised differential behaviour of tapetum seemed to influence pollen development.

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In conclusion, it may be said that the restorer genes might be acting by way of suppressing the recessive genes responsible for faulty stamen differentiation. Further the degree of normalisation might be depending on the accumulation of favourable modifiers. Differential degrees of tapetal degeneration affected the pollen grain development.

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ACTIVITIES OF THE CRUCIFER GENETICS COOPERATIVE 1987

P H Williams

Membership in the Crucifer Genetics Cooperative exceeds 1000, representing 43 countries. As of October 1987 there were 451 suscribing members and 23 sustaining members. During 1987, 794 seed requests were filled. Over 140 seed stocks of Brassica, Raphanus and other crucifer species are available. The CrGC has added the Koornneef collection of Arabidopsis thaliana mutants and chromosome markers to its collection and these will be available during 1988. Seed stocks are available for $3.00 U.S. per packet payable to the CrGC. Packets contain prorated amounts ranging from 10 to 100 seeds. Subscribing members are entitled to five 'free' packets of seed and receive the Resource Book, a member list and annual additions of information documents.

On October 11-14 the CrGC sponsored the Crucifer Improvement Cooperative (CrIC) meetings at the University of Wisconsin-Madison. Under the CrIC the following organizations convened: 1) NCR-100 Committee on Seedborne Diseases of Crucifers; 2) The Crucifer Genetics Workshop IV, 1987; 3) The Crucifer Improvement Committee; 4) The Crucifer Crop Advisory Committee of the U.S. National Plant Germplasm system. The meetings were attended by 150 persons from 14 countries, 16 discussion sessions were held and 47 posters presented. A working group on incompatibility was organized. Proceedings of the workshop including poster abstracts and discussion session summaries is available from the CrGC for $10.00 U.S. The Crucifer Genetics Workshop will meet again in the spring of 1989 in California. Organizer of the 1989 meeting will be Daniel Cohen, MacCabee Seed Co., P.O. Box 401, Davis, CA 95616, U.S.A.

VARIATIONS IN POLLINATION REQUIREMENTS OF BRASSICA NAPUS L(RAPESEED) CULTIVARS REFLECT DENSITY OF INSECT POLLINATORS IN THE REGIONS WHERE EACH CULTIVAR WAS BRED AND/OR CULTIVATED

H Namai and R Ohsawa

It is well known that plant species and cultivars vary in the pollination requirements, especially in the necessity of some pollinators even in autogamous plants. Brassica napus L.(rapeseed) is generally considered to be self-compatible and autogamous plant. However, a few researchers have reported the inter- and intravarietal variations in self-fertility (Olsson 1960, Namai 1983) and different results on the pollination requirements, namely role of pollinators such as insects and/or wind for enhancing seed set percentage of rapeseed cultivars (Jenkinson and Glynne-Jones 1953, Persson 1956, Olsson and Pearsson 1958, Koutensky 1959, Free and Nuttall 1968, Eisikowitch 1981, Williams 1978, 1984, Williams et al 1986).

In this paper, we describe clear correlation of environmental conditions concerning visiting frequency of insect pollinators with self-fertility and automatic self-pollination ability in rapeseed cultivars. The extent of self-fertility and automatic self-pollination ability of each rapeseed cultivar should reflect the quality and quantity of insect pollinators in the regions where each cultivar was bred and/or cultivated.

We tested six rapeseed cultivars in terms of self-fertility, automatic self-pollination ability and pollination requirements (Table 1). As shown in Table 2, three Japanese cultivars were used namely Taisetsu Natane and Aomori 1 which were bred in the regions where frequent insect pollinators visited, and Karafuto which was bred in severe cold Saghalien early in the 20th century. Two cultivars, Bronowski and Lembkes, were European cultivars, and the remaining one was cv. Tower from Canada where fairly few wild insect pollinators and a few honeybees visited in the extensive fields. As shown in Table 1, the following four pollination methods were performed in order to reveal the extent of (1) self-fertility, (2) automatic self-pollination ability and pollination requirements, namely effect of (3) wind and (4) both wind and insect pollinators in the six cultivars:

(1) Artificial self-pollination onto blooming flowers; pollinated and observed 14 flowers × 10 plants × 3 days per cultivar
(2) Mechanical automatic self-pollination by the flower itself(autogamy), in an isolation cage in a netted plastic house from which pollinators such as wind and insects were excluded; observed 15 flowers in main inflorescence × 12 potted plants per cultivar
(3) Self- and cross-pollination by wind in isolation cages without insect pollinators in field; planted 50 plants in the cage and observed 10 flowers in main inflorescence × 20 plants per cultivar
(4) Self- and cross-pollination by wind and artificially reared insect pollinators in isolation cages in field; planted 50 plants in the cage, released 20 Shimahanaabu(Eristalis cerealis) and observed 10 flowers in main inflorescence × 20 plants per cultivar

As shown in Table 2, two Japanese cultivars namely, Aomori 1 and Taisetsu Natane had the lowest self-fertility with about 10% seed set, whereas cv. Karafuto with 58.2% showed the highest self-fertility among all the cultivars in the pollination method (1). The other cultivars with about 30-35% seed set were intermediate in extent of self-fertility. Seed set percentage in the pollination method (2) viz. extent of automatic self-pollination ability varied

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from 2.2 to 12.7%. Taisetsu Natane and Aomori 1 also had the lowest seed set percentage which was about 2%, whereas Karafuto was similar to two European cultivars being 8-9%. Canadian cv. Tower was 12.7% and the highest of all.

Seed set percentage in the pollination method (3) that corresponds to wind pollination efficacy varied from 15.1 to 51.3%. Wind pollination efficacy was found definitely in all cultivars tested, especially in Karafuto and Tower which had the highest seed set percentage of about 50%. Seed set percentage in the pollination method (4) that corresponds to wind and insect pollination efficacy varied from 32.9 to 63.4%. In four cultivars, Taisetsu Natane, Aomori 1, Bronowski and Lembkes, insect pollination efficacy was found definitely. Therefore, insect pollinators should be the absolute pollination requirement for the four cultivars which were bred in the regions where a lot of insect pollinators visited. On the other hand, in two cultivars, Karafuto and Tower, seed set percentage by wind pollination bore comparison with that by wind and insect pollination. Therefore, wind is the sufficient pollination requirement in the two cultivars which were bred in the regions where fairly few insect pollinators visited.

According to these data and Ohsawa and Namai (in press), it is evident that B. napus is an incomplete autogamous species and the pollination requirements of rapeseed cultivars reflect the quality and quantity of insect pollinators in the regions where they were bred and/or cultivated. Consequently, the different data obtained in previous studies by other authors should not be considered as contradictory as they apparently are, but rather should be regarded as a consequence of individual pollination requirement of each cultivar being dependent upon the breeding environment, especially the quality and quantity of insect pollinators. The same phenomena should be found generally in many incomplete autogamous species.

Table 1 The details of pollination methods

Pollination methods	Way of pollination				Kinds of pollen deposited	
	Selfing	Autogamy	Wind	Insect	Selfed	Crossed
(1) Artificial	○				○	
(2) Automatic		○			○	
(3) Wind			○		○	○
(4) Wind and Insect			○	○	○	○

Table 2 Variations in seed set percentage of typical rapeseed cultivars in different pollination methods

Cultivars	% selfed seed set		% selfed and crossed seed set	
	(1)	(2)	(3)	(4)
Taisetsu Natane (Jpn)	8.7 c	2.2 c	21.7 c	32.9 d
Aomori 1 (Jpn)	12.0 c	2.5 c	15.1 d	38.4 d
Karafuto (Jpn)	58.2 a	8.0 b	51.3 a	59.4 a
Bronowski (Poland)	27.3 b	7.9 b	35.4 b	63.4 a
Lembkes (Germany)	36.5 b	9.1 b	22.5 c	51.6 b
Tower (Canada)	33.9 b	12.7 a	50.9 a	43.3 c

Means followed by the same letter in each column do not differ at 5% level probability according to Duncan's multiple range test.

STUDIES ON POLLEN-PISTIL INTERACTION BETWEEN BRASSICA NAPUS AND ITS RELATIVE SPECIES AND GENUS

Meng Jingling and Liu Houli

When Brassica napus was pollinated with B. oleracea or Sinapis alba, the prefertilization barriers acted mainly at the stigma surface (Kerhoas et al., 1983; Meng Jinling, 1987). To determine whether interspecific incompatibility performed as the pollen-stigma interaction exists universally in crosses between B. napus and other basic types of Brassica genus or related genera and to give a basis for research into the genetics of interspecific incompatibility, hybridizations were carried out using B. napus as maternal parent with B. oleracea, B. campestris, B. nigra, B. juncea, B. carinata and Crambe abyssinica; the status of pollen grains bound to and germinating on stigmas and the growth of pollen tubes in pistil were also investigated using aniline blue fluorescence (Martin, 1959).

The ability of pollen grains to bind to the stigmas varied with species depending on their genomic relation with B. napus. In contrast with B. napus selfings, where about 100 pollen grains were bound on each stigma 2 hours after pollination, there were 10-50 pollen grains on each B. napus stigma following hybridization with B. campestris, B. juncea, B. oleracea and B. carinata, and only a few pollen grains of B. nigra whose genome is completely different from that of B. napus. In addition pollen grains of C. abyssinica were seldom observed on B. napus stigmas, even after 24 hours of pollination.

Once bound on the stigma, pollen grains of different species among Brassica commonly were able to germinate. However, when pollen tubes contacted the stigma papillae, a strong callose reaction, accompanied by a cessation of growth of pollen tubes, took place at the top of papillose cells reaching a peak 18 hours after pollination. The tubes were usually short, less than the diameter of pollen grains, and sometimes twisted on the stigma surface. Often the top of the tubes expanded with a heavy callose deposition. Such abnormalities occurred most seriously in the cross of B. napus x B. nigra and the frequency of pollen tubes of B. nigra penetrating the stigma of B. napus was the lowest among the 5 interspecific cross combination tested. On the other hand, even though C. abyssinica pollen tubes were unable to induce any callose reaction with the papillar cells of B. napus, they failed to penetrate the stigma papillae.

The numbers of pollen tubes entering the style in all the interspecific crosses were usually less than one-tenth those of self pollinated B. napus. It was observed that the callose diffused radiatively from callose plugs to the directions of two ends of pollen tubes in which it deposited with bands in the interspecific pollinations.

The indexes of pollinating compatibility (IPC) were calculated and a pattern analysis (quantification method III) was conducted based on the information from the quantity of bound pollen, germinated pollen, and pollen tubes entering stigmas and styles. The IPCs were 3.20, 1.04,

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1.04, 0.92, 0.61, 0.25 and 0.02 when the pollinators were B. napus, B. campestris, B. juncea, B. oleracea, B. carinata, B. nigra and C. abyssinica respectively. The pattern analysis showed that the sequence of pollinating compatibility with B. napus was napus > campestris, juncea, oleracea > carinata > nigra and C. abyssinica; just coinciding with the result of IPC analysis.

It was found that female parent genotypes significantly influenced the pollen-pistil interaction in interspecific hybridizations when 6 B. napus cultivars, differing in genetic basis, were used as maternal parents with B. oleracea. The self-line 184, a self incompatible line of B. napus with Asian lineage, bound more germinated pollen on its stigmas and allowed more pollen tubes to grow through its stigmas and styles. The IPC was 1.55 when line 184 was used as female parent, which was the highest in six pollination combinations. In contrast, Marnoo, the Australian cultivar, had the lowest compatibility with B. oleracea with an IPC of 0.36.

It might be suggested that the callose rejection which occurred in the stigma papillae was one of the main obstructions to interspecific hybridizations among the Brassica genus. It is also possible that there was a gene or gene system controlling the pollen-stigma reaction of interspecific hybridizations, similar to the S-gene in sporophytic self-incompatible plants. However, it seems that the two gene systems may be independent of each other since the self-incompatible line 184 had a higher interspecific compatibility than the other (self-compatible) cultivars.

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A new method for collection of high quality Brassica pollen.

DE Evans, SG Dungey and I Grey.

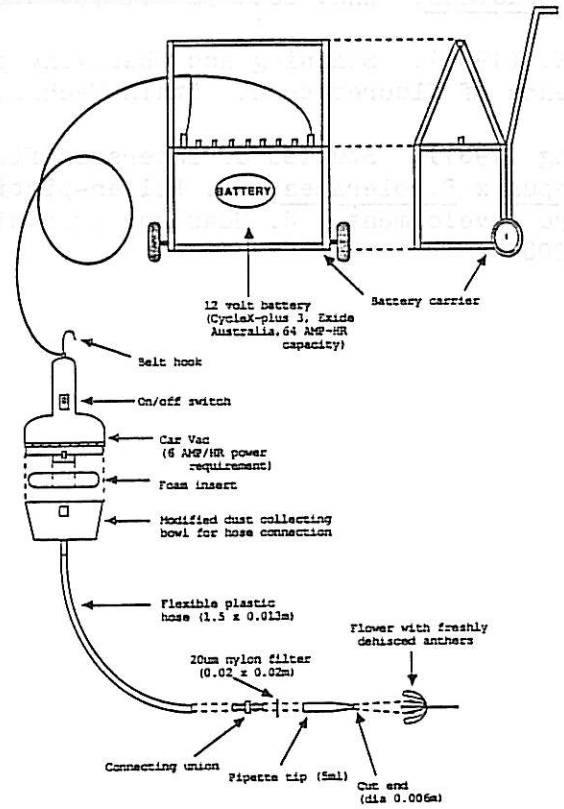
In order to conduct chemical analyses on rapeseed (B.napus L.) pollen we required large quantities (gram) of high quality pollen. The most effective method of pollen collection in both the field and glasshouse was to use a modified 12 volt car vacuum cleaner (model 9510 "Car Vac",Black and Decker Australia, PTY LTD). The alternative techniques of pollen collection tested were; brushing dehisced anthers with a fine brush, and allowing detached anthers to dehisce, followed by separation of pollen from anthers by sieving though a 0.75 mm brass sieve.

In the field 1.5 to greater than 3.0g per day of pollen was collected by the modified "Car Vac" depending on weather conditions and insect competion. Maximum pollen yields were favored by dry,fine weather and no pollinating insects. In contrast 0.5 to 1.0g per day could be collected with the alternative methods.

The "Car Vac" can be readily modified by equipment available in most workshops (Fig 1).

Pollen was collected by placing the collecter tip over the dehisced anthers (6) of a flower while holding its base and brushing the anthers in a circular motion with the inside of the tip to separate the pollen from the anthers. The pollen collects against the nylon filter by the suction from the "Car Vac". The collector tip and nylon filter are replaced or cleared when approximately 50 - 70mg of pollen has been collected.

FIG 1 Modifications to convert a Black and Decker 9510 "Car Vac" into a pollen collector



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SOMATIC EMBRYOGENESIS IN HYPOCOTYL PROTOPLAST CULTURE OF RAPESEED (BRASSICA NAPUS L.)

P.B. KIRTI and G. ROBBELEN

Somatic embryogenesis is of considerable importance as it combines efficient cloning with genetic modification (Sharp et al., 1980). It was observed in mesophyll protoplast culture of an androgenic line in rapeseed by Li and Kohlenbach (1982). In the present communication, somatic embryogenesis in hypocotyl protoplast culture of varieties Ceres and Duplo of rapeseed is reported.

Culture conditions until the stage of microcallus are reported by Kirti in a communication in this newsletter. Somatic embryogenesis was tried by first spreading the microcalli of about 1 mm diameter on a modified MS medium (Murashige and Skoog, 1962) containing 3.0 mg/l 2,4 D and 10% coconut water (Gibco) solidified with 0.4% agarose (Type 1, low EEO, Sigma). After about two weeks of culture, calli of 2 mm diameter were plated on another modified MS medium with 10% coconut water, 3.0 mg/l BAP and 0.1 mg/l GA_3 solidified with agarose. Green and well developed embryoids were successively transferred on MS medium lacking growth regulators until shoots appeared on the proliferating embryoids. Shoots were maintained on the same medium until intact plantlets were obtained. Embryoids could be observed initially as green nodular structures on creamy calli, which developed rapidly. In the variety Ceres, 3.8% of the calli produced embryoids whereas in Duplo as many as 15.8% of the calli showed embryogenesis. Embryoids were bipolar right from induction. As they grew rapidly, a transfer on to a medium devoid of growth regulators was quite essential; the continued maintenance on media containing growth regulators sometimes resulted in their proliferation into callus again. On the whole, 10 to 12 shoots could be regenerated from each bipolar embryo.

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MICROPROPAGATION OF CHINESE KALE (BRASSICA ALBOGLABRA BAILEY)

K.W. WONG AND C.S. LOH

The Chinese kale (Brassica alboglabra) is an economically important vegetable in Asia. However this plant is susceptible to many pathogens including Peronospora parasitica, Plasmodiophora brassicae, Corticium solani, Erwinia carotova and Alternaria spp. To meet crop improvement objectives, a predictable high frequency of plant regeneration would be useful, first, as a tool for large-scale clonal propagation of novel, disease-resistant variants selected in the field and, secondly, the methodology developed could be used for Ti and Ri-plasmid mediated transformation via co-cultivation with Agrobacterium tumefaciens and A. rhizogenes respectively. For this purpose, a detailed examination of shoot organogenesis in seedling and mature plant explants of B. alboglabra var. "Ripple leaf" is conducted.

The sources of explants were hypocotyls and cotyledons of seedlings less than two weeks old. Nodes, internodes and shoot apices were taken from 10-12 week old plants. The explants were excised from seedlings and plants grown in vitro. The segments were cultured on Murashige-Skoog basal medium (Murashige and Skoog, 1962) supplemented with various concentrations of Kinetin, BA and NAA.

The results of our experiments (Wong and Loh, 1987) have demonstrated that:

1. A high frequency (60-100%) of shoot regeneration was obtained from hypocotyl explants, nodal stem segments, internodal segments and shoot apices cultured on the basal medium.

2. The auxin NAA had an inhibitory effect on the initiation of shoots from hypocotyl explants at 1-10 mgl-1). However, the number of shoots per explant was increased by two-fold or more at increasing concentrations of kinetin or BA (1-10 mgl-1). Combinations of NAA/Kinetin or NAA/BA showed promotion of shoot induction at low auxin/cytokinin ratio (0.1/5.0 mgl-1).

3. The frequency of shoot regeneration in cotyledon explants cultured on the basal medium was low (10%). This frequency was improved with the addition of the

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following concentrations of growth regulators: BA (1 mgl-1), NAA/Kinetin (1.0/1.0 mgl-1) and NAA/BA (1.0/1/0 mgl-1).

4. Regeneration from nodal segments showed on enhancement in the number of shoots per explant at BA concentrations of 0.1-10 mgl-1 and NAA/BA concentrations of 0.1/1.0 mgl-1.

5. The addition of BA alone or in conjunction with NAA did not improve the frequency of internodal segment explants forming shoots or the number of shoots per explant when compared to those cultured on the basal medium.

6. Nearly 100% of the shoot tips cultured on the basal medium elongated to form well-developed plantlets. Multiple shoot formation only occurred on media supplemented with BA or NAA/BA. The highest number of shoots per explant (41.80 ± 6.50) was observed on media with BA (1 mgl-1).

7. The regenerated shoots could be detached and rooted on the basal medium. The plantlets could be successfully transplanted in soil.

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AGAROSE PLATING TO IMPROVE MICROCALLUS FORMATION IN HYPOCOTYL PROTOPLAST CULTURE OF RAPESEED (BRASSICA NAPUS L.)

P.B. KIRTI and G. ROBBELEN

Continuous liquid culture of hypocotyl protoplasts in rapeseed was not successful because of the release of brown phenolics into the culture medium by the growing cell colonies. To overcome this problem, embedding of cell colonies in agarose was tried and the observations are reported here. Hypocotyl protoplasts were prepared according to the schedule of Glimelius (1984). Protoplasts were plated at a density of 2-2.5 x 10^4 per ml in a modified 8P medium of Kao and Michayluk (1975) with 7% mannitol as osmotic stabiliser, 1.0 mg/l 2,4 D, 0.1 mg/l NAA and 0.4 mg/l BAP. Cultures were incubated in dark at 25C. On the seventh day of culture, when colonies reached 8-16 celled stage, they were diluted 1:1 with the same medium having 5% mannitol and 0.6% 'Sea Plaque' agarose giving a final concentration of 0.3% of the latter. Then cultures were exposed to incandescent light of 2500 lux. On the tenth day, agarose blocks were transferred to Petri dishes of 10 cm diameter. To each dish, 20 ml of the same medium with 3% mannitol was added. Cultures were incubated in light until microcalli were obtained. In these experiments, continuous liquid culture has resulted in colony browning and death. Embedding in agarose of freshly prepared protoplasts, three and five day old cell colonies was not successful. Plating of seven day old colonies showed excellent proliferation into microcalli in three to four weeks.

In the materials studied, viz Ceres, Lirakotta, Lirabon, Doral, Duplo and Andor, the plating efficiency ranged from 46% in Doral to 60% in Ceres and Duplo. In continuous liquid culture, only Ceres hypocotyl protoplasts reached the stage of microcallus stage, which, however, could not proliferate on the solid medium. Embedding seven day old cell colonies resulted in very good microcallus formation ranging from 3.0% in Lirakotta, Lirabon and Doral to 5.0% in Ceres (in relation to the original number of protoplasts plated).

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In vitro embryogenesis in callus of hypocotyl explants of Brassica juncea L. Gern & Coss

P.B. Kirti, Seema Dargan and V.L. Chopra

Somatic embryos are structures with bipolar organization produced on somatic tissues like callus. In vitro embryogenesis offers great potential for crop improvement by coupling of genetic manipulation with efficient cloning (Sharp et al., 1980). Successful embryo development from callus of mustard, Brassica juncea, is of considerable importance in realizing the advantage of in vitro manipulation. In this communication, we report the induction of somatic embryogenesis and plant regeneration in Brassica juncea c.v. RLM 198.

Hypocotyls from 5-7 day old seedlings maintained in sterile culture under 16/8 hour photoperiod provided the source of explants. Modified MS medium (Murashige and Skoog, 1962) with 2% sucrose, 0.25 mg/l 2,4-D, 0.5 mg/l NAA and 0.5 mg/l BAP-riboside was used for induction of callus and embryos. Embryos appeared in four week old cultures. About 35% of calli showed two or more embryos. The embryos were easily separated and showed proliferation on MS medium devoid of growth regulators. When the proliferated embryos were transferred onto MS medium with 0.2 mg/l BAP, secondary embryos were produced which germinated to produce multiple shoots. As many as 20 shoots were produced by a single proliferated embryo. The system is being utilized for in vitro selection for disease resistance and salt tolerance.

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ANTHER CULTURE OF x BRASSICORAPHANUS

Lee, Soo-Seong · Yoon, Yeo-Jung

A total of 466 F_1 plants between heading Chinese cabbage, Brassica campestris ssp. pekinensis and large rooted radish, Raphanus sativus was obtained by in vitro culture of hybrid ovules of 9 to 12 days old[3]. Eleven plants out of them were chosen randomly and used for anther culture with the purpose of acquiring inbred lines in the earlier generation. Their chromosome constitution was not confirmed, but several tens of flowers on the main stem were self-pollinated to identify the seed bearing ability. Accordingly flower buds for anther culture were taken from branch stalks. The media and procedures for anther culture were the same as described in the previous report[1]. Only the period of heating at 35°C prior to maintenance at 25°C was diversified from 1 to 4 days. Anthers were cultured from February to May of 1987.

The results obtained are summarized in Table. Seeds and embryogenic anthers were produced from 5 and 6 plants, respectively. Some plants produced embryogenic anthers as well as self-pollinated seeds, while others produced only seeds or embryogenic anthers. This result means the seed bearing ability is not coincident with the ability of microspore embryogenesis. A total of 20 out of 4,800 cultured anthers produced embryoids. Since most of the embryogenic anthers produced multiple embryos, 84 microspore-derived plants could be obtained. This number is equivalent to 1.75 % of the inoculated anthers. Microspore embryogenesis was influenced by heating period. Generally the number of embryogenic anthers and embryoids per embryogenic anther increased as heating period reached up to 3 days. This result is not identical to the records obtained in heading Chinese cabbage, in which one day heating was much better than 2 days[2]. No embryoid was induced from 4 days heating. Apparently the process of embryogenesis was very similar to that of heading Chinese cabbage and the developed plants were intergeneric hybrids in the morphological aspect. Therefore it is believed that all the obtained plants originated from microspores of x Brassicoraphanus plants.

Excellent achievements have been recorded on anther culture of heading Chinese cabbage in our lab during last 3 years. However no microspore-derived plant has been obtained from radish anther culture conducted with the same procedure as applied for Chinese cabbage. It seems that the embryogenic ability of Brassica microspore functions dominantly to that of Raphanus in x Brassicoraphanus. The anther-derived plants will be investigated for their chromosome constitution and seed fertility, then uniformity and genetic stability in pedigree generations.

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Table. Results of anther culture of x Brassicoraphanus plantmaterials

Code of plant-materials	No. of seeds obtained[y]	No. of cultured(A) and embryogenic(B) anthers by the heating period(day)									
		1		2		3		4		Total	
		A	B	A	B	A	B	A	B	A	B
115 c	30	90	3(4)[z]	120	0	90	5(29)	90	0	390	8(33)
169 c	1	90	0	120	0	90	0	90	0	390	0
156 c	13	150	1(2)	90	1(1)	150	0	90	0	480	2(3)
106 c	0	60	0	60	0	-	-	-	-	120	0
161	5	120	0	90	0	120	1(2)	90	0	420	1(2)
179	13	90	0	90	0	60	0	-	-	240	0
240	0	120	0	-	-	-	-	120	0	740	0
873	0	150	0	150	3(10)	150	1(31)	-	-	450	4(41)
849	0	150	0	150	0	150	0	-	-	450	0
713	0	300	0	360	0	240	2(0)	-	-	900	2(0)
700	0	210	1(10)	210	1(5)	300	1(10)	-	-	720	3(5)
Total	62	1,530	5(6)	1,440	5(16)	1,350	10(62)	480	0	4,800	20(84)

y/ Seeds were harvested from several tens of flowers self-pollinated with the fine brush at the blooming stage.

z/ The numeral in parenthesis presents the total number of developed plants.

Tissue Culture Studies in Brassica carinata A.Br.

S.B. Narasimhulu and V.L. Chopra

Anther Culture

About 150 anthers of uninucleate microspore stage of B. carinata, accession no. 3 were cultured on MS medium. Thirty growth regulator combinations involving 2,4-D (0.1 to 0.4 mg/l) NAA, IAA, IBA, KN and BAP in concentrations varying from 0.1 to 2 mg/l were tested for their effectiveness to form pollen plants. Other variables tested included supplementation with coconut water, continuous light, complete darkness, light and dark cycles of 16 and 8 hrs respectively and sucrose levels varying from 2 to 10%. The important observation was the formation of one to three shoots per anther (probably from anther filaments) in medium supplemented with IAA (1 and 2 mg/l) + KN (0.1, 0.2, 0.5 and 1 mg/l) at a sucrose level of 2%. The frequency of response ranged from 2.5 to 7.2%. Callus formation was obtained only in 2,4-D supplemented medium.

Somaclonal Variation

R_1 regenerants: Three plants among 487 regenerants of cotyledon derived callus had white flowers, compared to yellow flowers of donor plant contributing the explant.

R_2 regenerants: Among the progeny of regenerants, variation has been observed for qualitative traits like plant height, leaf texture and pigmentation ranging from intense purple all through to light specks of purple on stem and leaf petioles. The most important variant recovered is a pollen sterile plant which failed to set any seed when selfed but formed a few seeds in influorescences which were not bagged. The pollen of the plant is small and irregular with about 2% grains stainable with acetocaramine. The male sterile plant is characterised by more primary, secondary and tertiary branches. The number of pod clusters are more compared to segregating male fertile plants. The number of pods on this plant, when left for open pollination, equal that on a fertile plant. However, the number of seeds per siliqua is low : in the range 1-3. The male sterility seems to be genetic and not cytoplasmic as all plants which originated from a single regenerant are not sterile; if it was cytoplasmic, the entire progeny should have been male sterile.

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A simple method of generating somatic embryos from mesophyll protoplasts of Brassica juncea (L.) Czern & Coss

P.B. Kirti and V.L. Chopra

Li and Kohlenbach (1982) reported somatic embryogenesis from mesophyll protoplasts of an androgenic line of rapeseed. Chatterjee et al. (1985) reported the occurrence of shoot regeneration and the formation of somatic embryos from callus in Brassica juncea. Jourdan and Earle (1985) and Klimaszewaska and Keller (1986) have shown, more recently, somatic embryogenesis in B. oleracea and B. nigra respectively. In this communication, we report a simple method for direct somatic embryogenesis in the mesophyll protoplast culture of B. juncea.

Seeds of B. juncea var. RLM 198 were surface sterilized and germinated aseptically. Shoots were maintained on half strength MS (Murashige and Skoog, 1962) medium with 1% sucrose. Leaves from such cultures, after incubation in dark at 25°C for 24 hours, were the source for mesophyll protoplasts. Enzyme solution used for protoplast isolation consisted of CPW salts, 2% cellulase R-10 (Onozuka), 1% Macerozyme R-10 (Onozuka), 0.4M sucrose and 300 mg/l L-Ascorbic acid. Leaf segments were incubated in the enzyme solution in dark at 25°C overnight. After purification, 2.5 ml of protoplast suspension was plated at a density of Ca 10^5 per ml per 5 cm petridish. Culture medium used was the modified V47 medium (Binding, 1974) with 1% sucrose, 1% glucose, 7% mannitol, 1.0 mg/l 2,4-D, 0.1 mg/l NAA and 0.4 mg/l BAP. After one week of culture, cell colonies were diluted with the same medium having 2% sucrose (without glucose) and 5% mannitol, and exposed to light (Ca 2500 lux). After two weeks of culture, cell colonies were plated on MS medium with 2% sucrose, 1.0 mg/l NAA, 1.0 mg/l BAP solidified with 0.25% agarose (Sigma, Type I, low EEO). Green, well formed embryoids started appearing within 25 days of culture without the intervening callus phase. This procedure provides a very useful system for studies of basic and applied interest.

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MICROSPORE CULTURE OF SUMMER TURNIP RAPE

Jolanta Ziemborska and Jane De Pauw

The objective of this experiment was to evaluate microspore culture as a means of producing haploid and doubled haploid plants of Brassica campestris L. These in turn would lead to homozygous self-incompatible lines of summer turnip rape. B. campestris is a recalcitrant species to any manipulations in vitro and responds very poorly to anther culture. Our short term aim was to identify B. campestris genotypes which are responsive to the microspore culture techniques developed and routinely used for B. napus (Chuong and Beversdorf, 1985).

The microspores were suspended in a modified Lichter medium (Lichter, 1982) as described by Swanson et al, (1987). Eighty four plants belonging to 14 different cultivars, breeding lines and natural populations of B. campestris were used in this study. Since the initial results of our microspore cultures were poor, several factors and modifications of the techniques were examined. These included:

- different developmental stages of flower buds as based on a relative length of petals and anthers;
- isolation of microspores by whole bud maceration versus manual excision of anthers;
- high temperature treatment of microspore culture in the first 1 - 2 days of culture;
- replenishment of culture medium after 2 - 3 days of culture;
- increased level of sucrose in the initial culture medium.

The highest embryo yields were obtained when microspores were isolated from buds with petals about 3/4 length of anthers, concentration of sucrose in the initial hormone-free medium was increased to 20-25% for the first 2 - 3 days and then diluted down to 13%, and cultures were incubated for one week at 30°C and then transfered

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to 25°C. There were no significant differences between the whole bud maceration technique and excision of anthers.

Twenty plants belonging to 12 different cultivars and natural populations responded to microspore culture and produced 260 embryos. The results indicate a greater variability in embryogenic response within cultivars than between cultivars. Microscopic observations show that the majority of microspores underwent a first division induction but further divisions were stopped. Embryo yields of the responsive cultures were very low in comparison with B. napus.

Initially, when using the plant regeneration procedure developed for B. napus (Keller and Armstrong, (1978), the regeneration of B. campestris was poor. The efficiency of regeneration was improved considerably when benzyladenine was replaced by kinetin at concentration of 3 mg/l in the shoot inducing medium. The capacity of plant regeneration seems to be under genetic control, since some genotypes could be regenerated fairly easily and the others did not respond to any shoot inducing subcultures. Until now, 40 microspore culture-derived plants have been regenerated and have flowered. Thirty two plants had small sterile flowers indicating that 80% of the regenerants were haploid.

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PROTOPLAST ISOLATION AND CALLUS FORMATION FROM A CELL SUSPENSION OF RAPID-CYCLING *BRASSICA CAMPESTRIS* L.

M.C. CHRISTEY, Z. LENTINI and E.D. EARLE.

Previously we reported on the induction and maintenance of a rapidly growing *Brassica campestris* cell suspension (Lentini et al,1986). Friable callus to initiate this suspension was obtained from hypocotyl explants from a self-compatible rapid-cycling line of *B. campestris* (CrGC#66). This suspension has now been maintained by weekly subculture (1:4 dilution) for 36 months, but is non-regenerable. Growth analysis, by measuring the settled cell pellet in side arm flasks, indicates that the suspension exhibits exponential growth for 6 days with a doubling time of 1.5 days.

Further work with this suspension has included the isolation and culture of protoplasts. Protoplasts were isolated either from white friable callus obtained from cells 7 days after plating onto Gelrite-solidified M medium (Lentini et al, 1986) or from green friable plated callus which had been maintained on M medium for 3 years. By using callus 5-10 days after plating or subculturing, protoplast yields of $3\text{-}5x10^5$ protoplasts/gmFW were reproducibly obtained using an enzyme solution containing 0.4% CELF Cellulase (Cooper Biomedical) and 0.02% Pectolyase Y-23 (Seishin Pharmaceutical) in 0.2M mannitol and 80mM CaCl, pH5.6. Incubation was performed at 25C, 45rpm for 18 hours. Protoplasts were cultured at a density of $5x10^4$ protoplasts/ml in 500µl modified 8p medium (Glimelius, pers. comm.) in 24 well plates and cultured in the dark at 25C. Every second day, between days 8-18 of culture, 125µl of 8p medium containing 0.1M sucrose as the only osmoticum were added to each well. On day 20 excess liquid was removed and colonies were plated onto agar-solidified M medium for proliferation and cultured at 25C, 16 hour photoperiod (100 $\mu Em^{-2}s^{-1}$). Callus was subsequently maintained on M medium.

Using the white friable callus as a protoplast source gave division frequencies of 5-10% with an overall plating efficiency of 0.02%. This protoplast-derived callus consistently responds well to protoplast culture with increased division frequencies (20%) and plating efficiencies (0.06%) obtained. It grows vigorously and has remained white and friable through several subcultures (7 months of culture). On transfer of the callus to M medium containing reduced sucrose (1.5% or 0.5 %), some regions of greening have been observed but the callus becomes hard and slow growing and cannot be maintained. Alternatively, using the green friable callus as a protoplast source has resulted in consistently low division frequencies (<5%) with cell browning a problem and only rare colonies on plating.

To determine the reproducibility of suspension initiation further attempts were made to obtain soft friable callus from fresh hypocotyl explants. These experiments have been successful and have shown that considerable variability exists within the population with regard to the formation of friable callus of the type necessary for the successful induction of cell suspensions. CrGC#66 seeds were surface

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sterilized and germinated on hormone-free Linsmaier-Skoog (LS) medium (Linsmaier and Skoog, 1965) at 25C in the dark for 5 days. Hypocotyl explants (1-2cm) from 38 different seedlings were placed horizontally on LS medium containing 5mg/l each of NAA and BA and cultured in the light (100 $\mu Em^{-2}s^{-1}$,16 hr photoperiod, 25C). In all cases explants were arranged so that their position and orientation on the original plant was known. This was to determine whether any polarity existed with respect to callus production, as we have clearly observed with regard to shoot organogenesis from *B. oleracea* peduncle explants.

Of the 294 explants cultured, 78% produced callus over the entire explant but only 52% of these produced soft friable callus, the others producing firm callus. The remaining explants (22%) only produced small amounts of callus from their cut ends. Much variation was observed between individual hypocotyls in terms of the type and amount of callus produced. Of the hypocotyls cultured from 38 seeds, 8 showed little response, only producing callus from the cut ends of explants, while 13 produced unsuitable firm callus. Hypocotyl explants from 8 seeds produced green friable callus while the remaining 9 produced a mixture of both callus types. Some polarity was observed with respect to callus production as generally the uppermost explants produced more callus. After 4 weeks soft friable green callus was subcultured onto M medium and is currently being maintained on this medium. We hope to initiate suspensions with callus derived from individual hypocotyls. As the apical meristem of each seedling was cultured separately on LS medium we intend to obtain seed from highly responsive plants in an effort to increase the tissue culture responsiveness of this material.

Now that we have clearly established the growth characteristics of our *B. campestris* suspension and the protoplast isolation and culture conditions, we are attempting to develop lines with markers which could be useful in protoplast fusion experiments. *In vitro* selection for resistance to the amino acid analogues, aminoethyl-cysteine and 5-methyl-tryptophan is underway. We are also using *Agrobacterium tumefaciens*-mediated transformation to introduce antibiotic markers for fusion experiments. The initiation of suspensions derived from individual hypocotyls will be useful to confirm earlier results that have shown that the mitochondrial genome of the original plant material has undergone change in culture (J.D. Palmer, pers. comm.).

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PRODUCTION OF ATRAZINE-RESISTANT BRASSICA OLERACEA FROM SOMATIC HYBRIDIZATION BETWEEN B. OLERACEA AND ATRAZINE-RESISTANT B. NAPUS

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As part of a breeding program designed to modify cytoplasmic traits in Brassica vegetables through protoplast manipulations, we have been fusing protoplasts from different breeding lines which carry unique cytoplasms. Most of our work so far has dealt with the traits of cytoplasmic male sterility, conditioned by mitochondria (designated mt^{cms} for sterile and mt^{F} for fertile) , and with atrazine resistance, conditioned by chloroplasts (designated cp^{atR} for resistant and cp^{atS} for susceptible). Fusion of leaf protoplasts from B. oleracea ssp botrytis (cauliflower inbred #7642A, carrying the Ogura male sterile cytoplasm: mt^{cms}, cp^{atS}; Dickson, 1985; 2n = 18) with hypocotyl protoplasts of B. napus ssp oleifera (cv 'Tower', carrying the atrazine resistant cytoplasm derived from B. campestris: mt^{F}, cp^{atR}; Beversdorf et al., 1980; 2n = 38) resulted in the production of six atrazine-resistant cauliflower 'cybrid' plants (2n = 18) and three atrazine-resistant somatic hybrids (2n = 56). The fusion, culture and in vitro selection procedures have been previously described by Jourdan et al. (1986). The mitochondrial and chloroplast genomes of plants regenerated after fusion were analysed by DNA restriction and hybridization with cloned, organelle-specific probes. All regenerated plants contained mitochondria and chloroplasts that were identical to those of the B. napus fusion partner (i.e., mt^{F} and cp^{atR}). In contrast to the results of fusions between B. oleracea (mt^{cms}, cp^{atS}) and B. campestris (mt^{F}, cp^{atR}) (Jourdan et al., 1986), we found no evidence for intergenomic recombination between the mitochondrial genomes in any of the plants. While the cybrids exhibited the typical cauliflower morphology, the somatic hybrids resembled the B. napus parent in growth habit. All plants were male fertile and self compatible.

Seeds obtained from self-pollination of one atrazine resistant cauliflower plant were germinated in the greenhouse and transplanted to the field after 40 days for evaluations of resistance to the herbicide. As a control, selfed seed from a single plant of cauliflower inbred 7642B, the maintainer for line 7642A, was also included in the field evaluation. The resistant seedlings were less vigorous than the alloplasmic, atrazine-susceptible check (selfed progeny of one plant from cauliflower inbred 7642B, Dickson, 1985). Four-week-old seedlings of the resistant line had, on average, two leaves with one emerging, whereas the seedlings of the

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susceptible line had four leaves with one emerging by that time. This reduced vigor is typical of plants carrying the atrazine-resistant cytoplasm. In replicated trials, resistant seedlings showed no damage from atrazine which had been previously applied to the soil at rates of 0, 0.56, 1.12, 2.24 and 4.48 kg/ha. The susceptible check showed extensive injury at 0.56 kg/ha, and was killed at all higher rates. We found no segregation for the resistance trait in the selfed progeny of the atrazine-resistant cybrid.

The results of this experiment demonstrate that it is possible to obtain complete cytoplasm replacement in B. oleracea via protoplast fusion in about one year. A similar replacement by conventional backcrosses may take anywhere from three to five years.

The availability of herbicide-resistant B. oleracea may prove useful in rotation schemes with, for example, sweet corn in areas where atrazine carryover is a serious problem. However, we believe that resistant crops should be used judiciously and, under no circumstance, should they promote the indiscriminate use of herbicides. Such practices are foolhardy since they would exacerbate and already acute problem with atrazine-resistant weeds.

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A DWARF VARIANT AMONG *IN VITRO* REGENERATED PLANTS OF INDIAN MUSTARD (*BRASSICA JUNCEA* L.)

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Of late, many *in vitro* techniques have been used to complement conventional plant breeeding in different crop species. Somaclonal variation is one of them, which takes the advantage of naturally occuring genetic variation in *in vitro* regenerated plants and thus provides an increased genetic base for breeding new value-added varieties. The technique till to date has successfully been used to generate agriculturally useful variation in many different crop species (Larkin and Scowcroft, 1981; Evans et al., 1984).

The regeneration system developed by Jain et al. (1986), which involves the regeneration of whole plants from cotyledon explant calli on Murashige and Skoog's (1962) basal medium supplemented with kinetin (2.0 mg l^{-1}) and indole-3-acetic acid (0.2 mg l^{-1}), was used to generate a plant population (R_0 generation) for studying somaclonal variation in *Brassica juncea* cv. Prakash. The self fertilized progeny of R_0 plants (somaclones) are referred to as R_1 generation. A subsequent cycle leads to R_2 generation. Data were recorded on yield, plant height, siliqua number, number of primary branches, 1000 seed weight and oil content. Each time the plants were harvested individually and data were recorded for every individual plant. Standard cultivation practices were followed. The oil content of the seed was estimated by the Nuclear Magnetic Resonance (NMR) technique using a MKIIIA Newport Analyser (Gupta et al., 1985). While the detailed account of this study will be published elsewhere (Jain et al.), the present communication specifically deals with the inheritance and field evaluation of a dwarf somaclone of Indian mustard.

As compared to the plant populations grown from the seeds, a wide range of variation was observed among the progeny of *in vitro* regenerated plants. In R_1 generation, one of the somaclones (Sc-10) showed invariably dwarf characteristics. This line showed a plant height of 153 ± 9 cm as compared to 214 ± 14 cm in the parent 'Prakash' (table 1). While the maturity period of the parent and Sc-10 was almost same, the R_1 plants of dwarf somaclone flowered one week

Table 1. Data on yield, yield components and oil content in parent and R_1 progeny of dwarf somaclone (Sc-10) in *Brassica juncea* cv. Prakash

Material	Plant height (cm)	No. of primary branches	Siliqua no.	Yield (g)	1000 seed wt. (g)	oil content (%)
Parent	214 ± 14	11 ± 2	1167 ± 424	35 ± 8	2.4 ± 0.4	40 ± 2
Sc-10	153 ± 9	9 ± 3	1146 ± 178	38 ± 9	3.5 ± 0.2	39 ± 4

earlier. This variant also had higher 1000 seed weight as compared to the control. As the seeds harvested from Sc-10 showed very poor germination, the subsequent R_1 generation was too small to comment upon the genetic nature of the variant.

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As it is clear from the table 2, the dwarf somaclone bred true for its dwarf character. Rather Sc 10-1 and -2 displayed even less average plant heights. The yield and oil content of this particular somaclone was statistically at par with the parent.

Table 2. Data on yield, yield components and oil content in parent and R_2 progenies of three selected R_1 plants of dwarf somaclone of Indian mustard.

Trait	R_2 generation			
	Parent 'Prakash'	Dwarf somaclone (Sc-10) Sc 10-1	Sc 10-2	Sc 10-3
Plant height (cm)	199 ± 10	139 ± 11 (155)	136 ± 28 (160)	159 ± 6 (158)
Primary branches (no.)	8.2 ± 1.3	6.2 ± 1.1	5.8 ± 0.9	7.0 ± 1.1
Siliqua no.	614 ± 160	474 ± 176	492 ± 106	602 ± 111
Yield/plant (g)	23 ± 7	28 ± 9	26 ± 6	29 ± 6
1000 seed wt. (g)	4.1 ± 0.5	4.6 ± 0.4	4.5 ± 0.3	4.6 ± 0.5
Oil content (%)	42 ± 2	43 ± 2	43 ± 2	41 ± 2

Values in brackets gives the quantitative value of the corresponding trait in R_1 generation

Our experiment on somaclonal variation in *B.juncea* cv. Prakash resulted in the recovery of a stable dwarf somaclonal variant, which was at par with the donar parent with regard to other agronomically important traits. Bigger seed size and higher seed weight of this somaclone could be attributed to the increased reproductive phase as a result of early flowering. Such dwarf genotypes are very valuable to improve the standability of the crop. Similar somaclonal variants have also been observed in rice (Schaeffer et al., 1984). Further experiments are being done to define the genetic nature of this dwarf variant.

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EFFECT OF SUBLETHAL CONCENTRATIONS OF INSECTICIDES ON PROTEIN CONTENT OF MUSTARD LEAF AND INFLORESCENCE

G.C.Sachan and N.L.M.Tripathi

Insecticidal treatments for the control of mustard aphid affects the protein content of the Brassica juncea seeds (Arora etal, 1969). In the present study sublethal doses of insecticides to mustard aphids were applied on mustard plant and effect of these on protein content was studied.

The experiment was laid in split plot design with 3 replications. Insecticides were allotted to the main plots and their sublethal concentrations to sub plots. Mustard, variety PR 15, was sown in 2.5m x 2.5m plots with plants to plant and row to row spacings of 10 and 45 cm respectively. Normal agronomical practices were followed for raising the crop. Insecticidal application was done on 33 days old crop and samples were collected when there was 50% flowering. There was no attack of mustard aphid. The leaves and inflorescence were air dried and 0.2 g sample was ground and nitrogen content was analysed by modified microkjeldahl method (Jackson, 1973) and protein content was computed.

Perusal of Table 1 indicates that insecticidal treatments adversely affected the protein content of the leaf. This reduction in protein content was more marked at higher concentration as compared to lower concentrations. Similarly protein content also decreased with insecticidal application. This reduction was more pronounced at higher concentrations.

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Table 1. Effect of sublethal concentration of insecticides on protein content of mustard leaf (I) and inflorescence (II)

Insecticides	Regression equation		Concentrations LC_{50}	LC_{40}	LC_{30}	LC_{20}	LC_{10}	Control
Cypermethrin	Y=3.3244+0.2923x	(I)	2.70 c	2.52 cd	2.52 cd	3.42 d	3.62 b	4.06 a
		(II)	3.12 cd	2.93 d	3.14 cd	3.75 a	3.40 bc	3.66 ab
Decamethrin	Y=3.7784+0.2236x	(I)	3.72 b	3.91 b	3.75 b	3.91 b	4.34 a	4.27 a
		(II)	3.44 d	3.61 cd	3.66 bc	3.78 bc	3.92 ab	3.97 a
Dimethoate	Y=2.9626+0.3178x	(I)	3.24 c	3.58 b	4.12 a	3.98 a	3.96 a	4.18 a
		(II)	2.98 d	3.15 cd	3.44 bc	3.59 b	4.00 a	4.19 a
Fenvalerate	Y=3.2284+0.2943x	(I)	2.88 c	2.96 c	3.53 b	3.76 b	3.77 b	4.10 a
		(II)	2.74 c	3.35 b	3.77 a	3.83 a	3.77 a	4.03 a
Methyl-o-demeton	Y=2.9561+0.3404x	(I)	3.23 c	4.26 a	3.31 c	3.74 b	4.01 a	2.12 a
		(II)	2.81 e	2.95 de	3.21 cd	3.40 bc	3.52 b	3.87 a
Permethrin	Y=3.1649+0.2912x	(I)	2.73 c	2.80 c	3.38 b	3.54 b	3.90 a	3.96 a
		(II)	2.95 d	2.89 d	3.36 c	3.54 bc	3.68 ab	3.97 a
Phosphamidon	Y=3.2456+0.2678x	(I)	2.99 c	3.02 c	3.64 b	3.83 c	4.20 a	4.16 a
		(II)	2.97 d	3.24 cd	3.27 c	3.66 b	4.12 a	4.17 a
Quinalphos	Y=3.5902+0.2710x	(I)	3.51 c	2.87 d	3.78 b	4.04 a	4.11 a	4.10 a
		(II)	3.18 c	3.80 b	3.77 b	4.14 a	4.17 a	4.30 a

x=Log concentration x 10^{10} in case of cypermethrin and decamethrin and x 10^{8} in others. Sublethal concentrations for spraying were computed from the respective regression equation. Means followed by a common letter in a row are not significantly different.

HONEY DEW EXCRETION BY LIPAPHIS ERYSIMI AS A CRITERION FOR ASSESSING SUSCEPTIBILITY IN BRASSICAS

G.C.Sachan and S.K.Sachan

For this study Sinapis alba, varieties Varuna and Porbiraya of susceptible group, RLM 198, RH 7361 and T 6342 of moderately resistant and RW 2-2, RW 15-6 and B 85 of resistant groups were selected. Leaves and inflorescence of 50 days old plants were selected and collected in plastic containers to avoid loss of water.

Plastic petridishes of 10 cm diameter were used. The leaves/inflorescence of the respective variety was attached to the roof of the cover lid with adhesive tape, while the stalk which protruted through the hole of the side wall was inserted inside a homeopathic vial containing water. The filter paper treated with 0.2% bromocresol green dye in ethanol and air dried were placed in the bottom dish. Five 3 day-old nymphs, starved for 4 hours, were released on the leaf/inflorescence. Each treatment was replicated 4 times. The excreted honey dew fell on the bromocresol green treated filter discs. Insects were allowed to feed and excrete honey dew for 24 hours. The area of the blue spot which developed was measured.

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Perusal of Table 1 indicates that amount of honey dew excreted due to feeding on leaf and infloreseence of different varieties differed significantly. Significantly larger amount of honey dew was excreted by aphid when fed on leaf and inflorescence of S.alba followed by Varuna and Porbiraya of susceptible group. Significantly least amount of honey dew was recorded on Rw 15-6 followed by RW 2-2 and B 85 of resistant group on both leaf and inflorescence. Mean of leaf and inflorescence both, the amount of honey dew excreted on different varieties also differed significantly.

From this study it is clear that quantity of honey dew excreted can be used as a criterion for screening large number of Brassica germplasms quickly for their susceptibility to mustard aphid.

Table 1. Amount of honey dew excreted by L.erysimi on different varieties of mustard

Varieties		Amount of honey dew (mm^2) Leaf	Inflorescence	Mean
RW 2-2	(R)	23.25	25.25	24.25
RW 15-6	(R)	21.67	23.00	22.33
B 85	(R)	23.25	26.42	24.83
RLM 198	(MR)	26.75	29.50	28.12
RH 7361	(MR)	30.16	31.92	31.00
T 6342	(MR)	28.00	30.08	39.04
Varuna	(S)	41.00	45.16	43.08
Porbiraya	(S)	38.17	43.25	40.71
S.alba	(S)	55.17	64.08	59.62

CD at 5%	2.00	Test plant
	0.94	Stage
	2.84	Test plant x stage

R = Resistant
MR= Moderately Resistant
S = Susceptible

Varietal Resistance in Cabbage against Mustard Aphid (Lipaphis erysimi Kalt.)

O.P. Lal, H.S. Gill and Ram Singh

Mustard aphid, Lipaphis erysimi Kalt., is a serious pest of cruciferous crops in India and causes heavy losses every year. Cabbage (Brassica oleracea Linn. var. capitata Linn.) is also attacked by this pest. Merely presence of few aphids on a cabbage affects its market value very adversely. The aphid is a soft bodied, yellowish green or greenish and measures about 2 to 2.5 mm in length. The chemical control measures pose problems of toxic residues, environmental pollution and upset the natural balance affecting parasites, predators, pollinators, etc. An experiment was, therefore, conducted during 1985-1986 on field resistance of cabbage varieties against this pest.

Fifty two varieties and crosses were grown in a replicated field trial at the experimental farm, Indian Agricultural Research Institute in New Delhi. There was a light infestation of aphids in December. However, the population increased slowly and a peak population was found during the period between end of February to beginning of March, 1986. During this period all the varieties and crosses of cabbage were found infested with the aphid. It has been reported by Lal (1969), Teotia and Lal (1970) and Verma et al. (1981) that a gradation system should be followed for screening the varieties against mustard aphid, L. erysimi and cabbage aphid, Brevicoryne brassicae Linn. to classify them into different categories of resistance or susceptibility. So, depending upon the population of aphids per plant, the grades were given as 0 = Nil, I = 1-50, II =51-100, III = 101-200, IV = 201-500, V = 501-1000 and over 1000. A variety with grade 0 on an average could be considered as immune, grade I and II as highly resistant, grade III as moderately resistant, grade IV as susceptible, and grade V and VI as highly susceptible.

None of the varieties was found immune or highly resistant against L. erysimi. However, the varieties Red Rock Mammoth, Red Drum Head, Glory, Early Queen, Express Mail, Eclipse Drum Head, Rainy Princess, All Season and Red Pickling were less infested and graded as moderately resistant varieties. Some of the plants of Early Queen, Glory, Eclipse Drum Head, All season and Red Drum Head even exhibited high resistance but the average grade of the varieties was that of moderately resistant. However, Early Queen, Glory and Eclipse Drum Head formed very good compact heads of good size and shape and also possessed less number of outer leaves. The aphids on these varieties were mostly confined on outer leaves and, therefore, from market point of view did not pose much problem as outer leaves may be easily removed before marketing. The varieties which were badly damaged by aphids included Golden Acre, Pusa Drum Head, September, Spitzkool, Baby Head, Selection-8 and Little Leagre and graded as highly susceptible. Some plants of Golden Acre were so heavily attacked that no head was formed and the plants were completely killed by the aphids. The other varieties, on an average were graded as susceptible against this pest.

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FIELD AND GLASSHOUSE STUDIES ON COMPONENTS OF RESISTANCE TO ROOT FLY ATTACK IN SWEDES

A.N.E. Birch

Turnip root fly (Delia floralis) and cabbage root fly (D. radicum) are two of the most important insect pests of swedes and other brassica crops. Control using insecticides is often uneconomic or unreliable, due to the pests prolonged attack periods, variable peaks of activity and lack of insecticide persistence in soils favouring enhanced microbial degradation. Breeding for dual resistance to both root flies is therefore likely to be the most effective and economic control method.

Field and glasshouse experiments have been undertaken to investigate components of resistance to root flies in selected swede cultivars including SCRI cvs Angus and Melfort (Birch 1985, 1987). Two years' field data indicates that ovipositional antixenosis (female adult fly non-preference) is the major component of resistance in cvs Angus and Melfort. Up to eight times as many D. floralis eggs were laid on the susceptible control cv. Doon Major, compared to the most resistant cv., Angus. Although the oviposition preferences of D. radicum were more variable, up to 34 times as many eggs were laid on the most susceptible cv., Sator Otofte, compared to cv. Angus.

These experiments also indicated that root antibiosis, operating against larval feeding and development, is a second but less important component of resistance against D. floralis. Reduced development of D. floralis pupae on roots of resistant cultivars in the field was confirmed in glasshouse tests using standardised egg inoculations so that oviposition preference effects were removed. Between 1.3 and 2.0 times as many D. floralis pupae developed on cv. Doon Major compared to cvs Angus and Melfort. In both field and glasshouse experiments larval feeding on resistant cultivars was restricted to surface tissue only, compared to variable but deeper tunneling when feeding on susceptible cultivars.

Although increased dry matter content is significantly correlated with root resistance to larval feeding, this association does not seem to be due to increased tissue hardness in high dry matter cultivars. It seems probable that differences exist between cultivars in the chemical content of outer root tissues, which influence larval feeding and development. Studies are now being made, in collaboration with chemists, to identify the plant chemicals influencing root fly oviposition and larval feeding. Through an understanding of the mechanisms of resistance it is hoped to devise rapid bioassays or chemical screens which will identify genotypes with dual resistance to both D. floralis and D. radicum.

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TOWARDS GENETIC ANALYSIS OF CLUBROOT RESISTANCE IN BRASSICA OLERACEA.

R.E.Voorrips.

In the vegetable and fodder crops of B.oleracea, clubroot (caused by Plasmodiophora brassicae) is an important disease. Although some partial resistance exists in these crops, mainly in white cabbage and kale, the genetics of these resistances have not yet been determined. It is suggested that resistance depends on several, predominantly recessive genes (Crute et al., 1980). In combination with a large variation for pathogenicity within and between pathogen populations and a large environmental influence on the expression of resistance, this means that genetic analysis and resistance breeding are difficult.

At the IVT a new project has been started to elucidate the genetics of clubroot resistance in B.oleracea. In short, the problems mentioned above will be approached as follows :
The variation between and within pathogen populations will be investigated and controlled by the use of single-spore isolates. In this way, a better reproducibility of resistance tests will be achieved, and fysio-specific resistance will be recognized more easily.
The environmental variation will be reduced by using greenhouse tests for resistance.
An effort will be made to identify several components of resistance, such as resistance in various phases of the infection process, and to investigate their genetic regulation. For this work, use will be made of anther or microspore culture techniques to obtain homozygous genotypes. This approach is expected to make the genetic background of resistance more accessible to analysis and interpretation.

The results of this project will be used to create higher levels of resistance than those currently available, by combining genes for several resistance components.

Many partially resistant genotypes have been collected already. However, our collection is probably still incomplete. Therefore, any resistant B.oleracea genotype that readers may be willing to contribute to our project will be greatly appreciated.

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Comparsion of Inoculation techniques for Testing **Brassica** Seedling Resistance against **Plasmodiophora brassicae Wor.**

M. Schoeller and J. Grunewaldt

A prerequisite for the production of genotypes with resistance against a pathogen is an efficient system of inoculation and infection. Resistance breeding is possible, if a technique for producing reproductable results is available. For resistance breeding to clubroot a lot of methods for inoculation are used. The question is, wether the different techniques achieve comparable results.

We tested and compared seven inoculation methods under glasshouse conditions. For inoculum extraction and growth conditions after inoculation the method of BUCZACKI et al.(1975) was used. In Experiment I the spore suspension contained 10^7 spores/ml. The host was the universally susceptible trap crop Brassica campestris var. pekinensis cv. Granaat. Seven weeks after inoculation each plant was scored visually for clubroot infection on a 0 to 3 scale. The percentage of diseased plants and the disease index (DI) (WILLIAMSON, McRITCHIE, 1981) was calculated. Per variation 35 plants were examined.

In Experiment I the following inoculation methods were applied:
Method 1: Slurry method (TOXOPEUS, JANSSEN, 1975): 400 ml of the spore suspension were mixed under 6000 ml soil and the seeds were sown in the slurry.
Method 2: Sluury method, modified: After the mixture of spore suspension and soil, seven days old seedlings were planted in the slurry.
Method 3: Root dip method (JOHNSTON, 1968): The roots of seven days old seedlings were dipped in the spore suspension for two hours.
Method 4: Root dip method (RAA, 1971): The roots of the host plantlets were dipped in the spore suspension for three minutes.
Method 5: Seed dipping method (LEWIS, BROKENSHIRE, 1978): Before sowing the seeds were dipped in the spore suspension for six hours.
Method 6: Injection method (THESING, GRUNEWALDT, 1985): Seven days old seedlings were planted in the non infected soil. Close to each plantlet a deepening, in which two ml of the spore suspension was given, was made. So the roots of the plantlets are contaminated with the suspension.

Only with the methods 2,3 and 6 100 % of the tested plants are infected. Table 1 shows the results of Experiment I. These methods, which may attack all test seedlings were further examined in Experiment II. With decreasing spore concentrations in the suspension differences between the inoculation methods were better expressed. Three spore suspensions with 10^5, 10^6 and 10^7 spores/ml were used. The results are shown in Table 2.

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Table 1: Effect of different inoculation techniques on the infection of 'Granaat' against clubroot.

Method	% diseased plants	DI
1: slurry method - seeds sown in the slurry	90,3	64,5
2: slurry method - seedlings planted in the slurry	100,0	83,8
3: root dip method - inoculation time 2 hours	100,0	71,7
4: root dip method - inoculation time 3 minutes	94,0	51,5
5: seed dipping method	12,1	10,1
6: injection method	100,0	96,9

Table 2: Examination of inoculation techniques by using different spore concentrations in the suspension.

Method	Spore-concentration	% diseased plants	DI
2: slurry method - seedlings planted in the slurry	10^7	100,0	83,3
	10^6	93,9	64,6
	10^5	69,7	38,4
3: root dip method - inoculation time 2 hours	10^7	100,0	68,7
	10^6	84,4	37,5
	10^5	21,9	7,2
6: injection method	10^7	100,0	85,3
	10^6	100,0	77,1
	10^5	100,0	75,5

By using a suspension with a lower spore concentration than 10^7 spore/ml only the injection method showed 100% diseased plants. With the slurry method and the root dip method not all tested plants may be attacked with the spore suspensions containing 10^6 and 10^5 spores per ml, so that these inoculation techniques are less efficient for the testing seedling resistance against clubroot than the injection method.

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INFLUENCE OF PLANTS EXTRACTS ON PATOGENICITY OF *Plasmodiophora brassicae*

I Djatnika

Soil fungicides have not given satisfactory control of *P. brassicae* (Dixon, 1986), or the value of these crops does not usually justify their use (Dobson *et al.*, 1983), Consideration is now moving towards improving methods of biological control.

Extract from garlic-leaf or bulb has been shown to have fungicidal and innibitory action against several fungal species (Singh *et al.*, 1979). Extract from Zingiberaceae-rhizome, in Java - Indonesia, has been used against several human diseases.

In this work, some extracts from several plants have been evaluated for clubroot control on chinese cabbage.

Material and Methods

Extracts from carrot-leaf, garlic-leaf and bulb, *Tagetes erecta*-leaf, and Zingiberaceae-rhizome (including: *Kaempferia galanga*, *Zingiber officinale*, *Alpinia galanga* and *Curcuma domestica*) were tested on the patogenicity of *P. brassicae* in greenhouse condition.

Each bag of soil inoculated with suspension of *P. brassicae* (10^6 cell of spores/g soil) were put in a greenhouse. A day after inoculation, the plant extracts were applicated to the soil. Fiveteen days later, 10 plants of chinese cabbage (15 days old) were planted each in a plastic bag. Each treatment nas 5 replication. Plants were examined for clubroot symptoms after 6 weeks.

Results

Treatments with extracts of carrot-leaf, *T. erecta*-leaf, garlic-leaf, Zingiberaceae-rhizome were not effective to control clubroot (Table 1, 2, 3, and 4).

Table 3 show that garlic-leaf extract did not significantly affect clubroot severity, but garlic-bulb extract suppressed clubroot disease index, while the % of diseased plant was not suppressed. The higher the concentration of garlic-bulb extract were, the lower the disease index was. Wheight of chinese cabbage-leaf tent to decreased with the increasing of garlic-bulb concentration, except for the highest concentration, that is 4 g (control), 3.1 g (50000 ppm), 2.0 g (70000 ppm of extract), and 2.7 g (90000 ppm of extract).

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Table 1. Influence of Carrot-Leaf Extract on Clubroot Severity

Extract (ppm)	Disease index (%)	% of diseased plant
0 (tap water)	100 a	100 a
6 000	82.2 a	100 a
12 000	83.6 a	100 a

Value with the same letters do not differ significantly from one another ($P = 0.05$).

Table 2. Influence of Tagetes-Leaf Extract on Clubroot Severity

Extract (ppm)	Disease index (%)	% of diseased plant
0 (tap water)	100 a	100 a
25 000	87.4 a	100 a
50 000	84.1 a	100 a
75 000	100 a	100 a

Table 3. Influence of Garlic Extract on Clubroot Severity

Extract (ppm)	Disease index (%)	% of diseased plant
0 (tap water)	96.7 a	100 a
Leaf of garlic:		
50 000	100 a	100 a
70 000	88.3 a	100 a
90 000	100 a	100 a
Bulb of garlic:		
50 000	76.2 b	100 a
70 000	64.5 b	100 a
90 000	63.4 b	100 a

Table 4. Influence of Zingiberaceae-Rhizome Extract on Clubroot Severity

Extract (ppm)	Disease index (%)	% of diseased plant
Tap water (Control)	100	100
Kaemferia galanga	100	100
Zingiber officinale	100	100
Alpinia galanga	100	100
Curcuma domestica	100	100

BLACKLEG RESISTANCE IN WEEDY CRUCIFERS

P. A. Salisbury

Wild species are known to be an important source of genes for disease resistance for cultivated species. An Australia-wide collection of weedy crucifer species was therefore screened for resistance to blackleg (Leptosphaeria maculans), both in the field and the glasshouse. Lines of 18 weedy species from the genera Brassica, Camelina, Capsella, Carrichtera, Diplotaxis, Hirschfeldia, Myagrum, Raphanus, Rapistrum, Sinapis and Sisymbrium were evaluated. Cultivated Brassica and Sinapis species were included as controls.

Field. 272 lines were sown in a blackleg nursery. All of the weedy species were highly resistant to blackleg. In comparison with the large number of leaf lesions found on the cultivated species, a small number of leaf lesions were evident only on some populations of Raphanus raphanistrum. No leaf lesions were observed on the other weedy species. Likewise, no stem cankering was observed in any weedy species. In contrast, the susceptible B. napus cultivar Tower, was badly cankered. Near maturity, 20 plants of each line were evaluated for degree of internal infection on a 0 (no infection) - 5 (complete infection - cankered) scale. Most weedy species were completely free of disease. A small amount of internal infection was found in some species, particularly Brassica tournefortii and Raphanus raphanistrum. In contrast, virtually every Tower control plant was badly infected, with control row scores averaging over 4.

Glasshouse. The resistance of the weedy species was confirmed by a glasshouse test, in which seedlings of 58 lines were inoculated with a pycnidiospore suspension, and lesion size was scored on a 0-14 scale four weeks later (Table 1). The B. napus cultivar Niklas was used as the susceptible control.

Table 1. Blackleg scores from glasshouse trial

Line	Score
B. napus Niklas	11.2
B. juncea Stoke	4.0
All weedy species	<4.0, mostly <2.5
e.g., Brrassica tournefortii	3.3
Diplotaxis tenuifolia	1.0
Hirschfeldia incana	0.5

The weedy crucifer species thus appear to be a very good source of genes for resistance to blackleg.

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Development of an *in vitro* screen in Brussels sprout for resistance to *Alternaria brassicae*

J. Williams & D.A.C. Pink

Genotypic variation in reaction to infection by *A. brassicae* was found by Pink *et al* (1987) in inbred lines of Brussels sprout assessed in the field in 1985 and 1986. Disease severity in field material was scored on a 1-5 scale: 1 indicating slight infection and 5 indicating severe infection. In 1986 work began to explore this variation further by developing an *in vitro* system that could be used to screen large numbers of such lines for disease resistance.

Meristem cultures of field material with scores 1-5 were initiated to provide a basis for this approach. The initial aim was to screen these meristems with a toxic culture filtrate isolated from *A. brassicae* which could be used as a marker of disease resistance.

The first priority was to isolate a toxic filtrate. There is evidence that *A. brassicae* (Degenhardt, 1977) and *A. brassicicola* (MacDonald & Ingram, 1986) produce toxic principles. Filtrates from the fungus grown in several different media including Czapek Dok, Potato-Dextrose Broth and V8 juice media were tested for toxic activity on leaf discs and detached leaves from 5 week old Brussels sprout seedlings (cv. Tornado). Toxic activity was found only in culture filtrates from V8 media. Severe chlorosis and necrosis of detached leaves was visible within 5 days, whilst treatment with uninoculated media produced no symptoms.

Detached leaves from 4 lines (2 with field scores of 5 and 2 with scores of 1) were treated with *A. brassicae*/V8 culture filtrate. Little difference was found between the two pairs of lines except that the most resistant pair exhibited slightly less chlorosis. Another experiment is in progress treating representative meristem lines (isolated from field material in groups scoring 1-5) with V8 culture filtrate.

It may prove necessary to purify the toxic principles in order to detect any differential sensitivity between genotypes. We are using gel filtration as a first step to this.

The overall aim of this project is to identify sources of resistance to *A. brassicae* in horticultural brassicas. One approach is to generate novel resistance *in vitro* through somaclonal variation. To this end we are currently investigating the use of callus and protoplast cultures.

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VARIATION AMONG INBRED BRUSSELS SPROUT LINES FOR REACTION TO INFECTION BY *ALTERNARIA BRASSICAE*

D.A.C. Pink, B.M. Smith, C.P. Werner, J. Williams

During the 1985 field assessment of Brussels sprout inbred lines produced at Wellesbourne by single seed descent (SSD) or anther culture (AC) (Smith & Werner, 1988) the plants were naturally infected by *Alternaria brassicae*, one of the causal agents of dark leaf spot. The opportunity was taken to score mature field grown plants for their reaction to this pathogen. No immune plants were found and the plants were scored on a subjective 1-5 scale, plants with a score of 5 being severely infected. Analysis of the scores for the systematic control, inbred GA1, indicated that disease levels were fairly uniform throughout the trial. The majority of lines were represented by only a single observation row of 12 plants (Smith & Werner, 1988). The central 2 plants of each of these rows were scored and although there appeared to be variation for *A. brassicae* symptoms statistical analysis was not possible.

A subset of 27 inbred lines was included in a replicated trial. This included lines derived by SSD from 4 different starting populations and lines derived by anther culture from the F_1 hybrid cvs Gower and Nym and the double cross Nym x Pinnacle. The experiment was designed as a single randomised block and each line was represented by two individually randomised single rows of 12 plants. Control F_1 hybrid cultivars and their inbred parents were also included. All but the end plants of each row were scored for *A. brassicae* symptoms.

Analysis of the data showed homogeneity of the within plot variation for all the different types of material, ie. SSD = AC = F_1 cvs = inbred parents. There was also homogeneity of a significant between-plots item taken over all the types of material. Highly significant genotypic differences were detected between lines for the parental inbreds, the SSD lines and the AC lines. There were, however, no differences between SSD versus AC lines or between groups of lines derived from different starting material.

A second experiment was carried out in 1986. This involved 24 of the SSD and AC lines grown in the 1985 replicated trial plus commercial F_1 hybrid and inbred parent controls. The experiment was arranged as three blocks and each line was represented in each block by a row of 12 plants. The plants were scored twice for *A. brassicae* symptoms on 23 October and 12 November. Analysis of the data showed similar results to the previous year: highly significant genotypic differences between the lines but no differences between the SSD versus AC lines or starting material from which they were derived. There was a significant ($p < 0.01$) correlation between the line means in the two years, r_{33df} = 0.59 and 0.62 for the first and second 1986 scoring dates respectively. The correlation between the two scoring dates within 1986 was 0.82.

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The results clearly showed genotypic variation for susceptibility to A. brassicae within the SSD and AC lines. This material is being used as the basis to develop an in vitro method to screen for resistance to A. brassicae (Williams & Pink, 1988).

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EFFECT OF INSECTICIDAL SPRAYS ON THE VISITS OF INDIAN HONEY BEE, APIS CERANA INDICA FAB. ON RADISH CROP

R.R.S.Rathore, M.L.K.Reddy and G.C.Sachan

Insecticides are useful for the control of Lipaphis erysimi at the time of flowering. These could affectthe foraging behaviour of bees. With this view in mind the effect of some insecticides namely permethrin 0.015%, cypermethrin 0.01%, fenvalerate 0.033%, phosphamidon 0.025%, quinalphos 0.025%, methyl-0-demeton 0.025%, monocrotophos 0.05%, dimethoate 0.03% and endosulfan 0.035% which are commonly used for the control of mustard aphid on radish crop, on foraging behaviour was studied. The mean number of bees hovering over the crop canopy and the number of bees settling on the flowers during a period of one hour between 10 and 11 A.M. were recorded daily.

There was significant reduction on the number of bees hovering over plant canopy in phosphamidon, methyl-0-demeton and monocrotophos treated crop up to 13 days after the application. Quinalphos, dimethoate and permethrin exhibited their effect up to 10, 11 and 8 days, respectively. Fenvalerate and cypermethrin affected the bee activity up to 9 days. Endosulfan which proved to be safest insecticide against bees, lost its effect on 6 th day of application.

Almost similar results were found with regard to bees landing on sprayed flowers.

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POST-INFECTIONAL VARIATIONS IN THE AMINO ACID CONTENT IN RADISH BY ALBUGO CANDIDA (GMELIN) KUNTZE

B. B. LAL and N. DAYAL

Amino acids are known to play a significant role in conferring either resistance or susceptibility to crop plants (1,2). Enhancement in amino acid metabolism occurs in the vicinity of the infected region (3). Infected tissues exhibit vigorous utilization of amino acids resulting into appreciable decrease in their amount during pathogenesis (4). The present study deals with a comparative analysis of amino acids in a local variety of radish 'Pusa Desi' before and after infection by 'white rust' pathogen Albugo candida (Gmelin) Kuntze.

Amino acid analyses were made by paper chromatographic technique described earlier (5). For this, healthy inflorescence (control), healthy tissue from the vicinity of hypertrophied inflorescence (intermediate tissue) and completely infected inflorescence (diseased) were taken.

Altogether 13 amino acids were detected in all the three kinds of tissues studied, except the absence of γ-aminobutyric acid in the intermediate tissue. These amino acids were: leucine/isoleucine, β-phenylalanine, valine, proline, glycine, methionine, Dl-alanine, Dl-threonine, glutamine, homoserine, glutamic acid and aspartic acid. It was interesting to note that the amount of glycine, homoserine and glutamine was less while that of leucine/isoleucine, γ-aminobutyric acid and Dl-alanine was more in the healthy tissue when tested against the diseased ones. β-phenylalanine, proline, methionine, valine and glutamine showed a slight variation in their amount in healthy and diseased tissues. The amount of aspartic acid and Dl-threonine decreased gradually with the age of the plant, although it was higher than that in the diseased region.

Post-infectional variations in amino acid content are reported in a number of crop plants but the mechanism of amino acid metabolism is yet to be fully understood (6). Here we report post-infectional variations in amino acids in a crucifer, Raphanus sativus, caused by 'white rust' pathogen.

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A NOTE ON TRANSFER OF RESISTANCE TO WHITE RUST FROM ETHIOPIAN MUSTARD TO INDIAN MUSTARD

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In India, two species of genus Brassica namely B. Campestris (L) and B. juncea L. Czern & Coss are grown as oil crops. These species suffer from number of diseases viz. white rust, Alternaria, Downy mildew and Powdery mildew. During last two years due to white rust epidemic the losses in seed yield upto 30 per cent have been reported. Various genotypes of Ethiopian mustard (Brassica carinata Braun) possess resistance to different races of white rust, whereas resistance has not been observed in Indian mustard. The two species can be crossed successfully and transferring of desirable genes from B. carinata to B. juncea is a practical possibility. The alternative procedure, to synthesize B. juncea (2n = 36 AABB) from B. campestris L (2n = 20 AA) with B. nigra (2n = 16 BB), is recognized to be very difficult. If accomplished it would necessiate long term breeding approach to improve thus evolved unadapted synthetic. Therefore, the present procedure adopted was to transfer disease resistance from B. carinata to B. juncea. The interspecific hybrids had shown immunity for white rust.

The interspecific F_1's were advanced to F_2, F_3 and F_4, and plants intermating within the population continued to F_5 and F_6. The selection for increased fertility and disease resistant plants was done in the F_7. The progenies were grown for further selection. The number of progenies in the back ground of most cultivated species of B. juncea possessed resistance to white rust. The back crossing with recurrent parent and intermating in backcross plants is presently underway to develop genotypes of Indian mustard for white rust resistance. Interestingly, a few progenies from above cross material have also exhibited high degree of tolerance to Alternaria leaf spot disease.

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BREEDING FOR RESISTANCE TO BLACK ROT, DOWNY MILDEW AND CURD BLIGHT IN INDIAN CAULIFLOWER

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Epiphytotics of Black rot -BR (Xanthomonas campestris pv. campestris) -XCC, downy mildew - DM (Peronospora parasitica) - PP and Curd blight/ Curd drying (Alternaria brassicae / A. brassicicola) have become limiting factors both in vegetable and seed crops of cauliflower in the hills and north Indian plains (Rao and Srivastava, 1964; Sharma et al., 1975). A systematic breeding programme was, therefore, initiated at the Indian Agricultural Research Institute, New Delhi and its Regional Station, Katrain, Kulu Valley (H.P.).

For screening against BR, the XCC inoculum was prepared by harvesting 48 h bacterial growth from YG - CA medium in de-ionised sterile water. Large germplasm collections were initially screened by seed soaking in XCC suspension followed by incoulations at nursery and adult plant stages adopting the standard method for BR inoculation (Bain, 1955; Sharma et al., 1972; William et al., 1972).

A sporangial suspension ($5x10^2$/ ml) wqs prepared from infected leaf tissue of Improved Japanese, a susceptible variety in dewy conditions and spray inoculated in misty weather. The inoculation temperature ranged beween 5-8° C (min.) and 16-20° C (max.).

Alternaria spp. were multiplied on PDA at 25 ± 1° C and inoculum potential was adjusted to 10^4 conidia / ml of water by harvesting 10 days old growth from the culture medium and spray inoculated twice, first at 20° C and second at 25-27° C at pre-bolting and bolting stages respectively.

The disease reactions were scored in five categories for all the diseases i.e. 0 to 4, 0 and 1 being resistant, 2 tolerant, 3 susceptible and 4 highly susceptible.

On the basis of XCC reactions, MGS-2-3, Pua Kea and S.No. 445 showed that the resistance to BR was governed by dominant polygenes. However, curds of these lines were not acceptable as such, hence MGS-2-3 was crossed with a commercially accepted line 15-1-1. Selected F_3 plants were again crossed with a well established cv. D-96. One of the lines selected from this multiple cross was released as Pusa Shubhra after multilocation testing. This variety also possesses resistance to Alternaria species and riceyness. From the same cross two sister lines 1-6-1-2 and 1-6-1-4 were also found to be resistant to DM in addition to the BR and Alternaria blight. However, they could not be released because of their poor curd quality. They can be used as donor parents.

In a similar attempt to incorporate BR resistance in the Snowball group, E.C. 12012 was crossed with S. No. 445. Sel-12 from this cross was

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found to carry high degree of resistance to this disease. It has been recognised as resistant donor after testing its performance under All India Coordinated trials both in the field and under artificial inoculated conditions. Incidentally, a selection from E.C. 12012, later named as Pusa Snowball K-1 was also found to possess field resistance to this disease with excellent curd characters and has been released for commercial cultivation (Gill et al., 1983). This is the only commercial cultivar of Snowball group which carried field resistance to BR.

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RESISTANCE OF RADISH (RAPHANUS SATIVUS L.) TO DOWNY MILDEW, PERONOSPORA PARASITICA

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Downy mildew of cruciferous plants, Peronospora parasitica de By f. brassicae Thüm, causes serious damage in France to crops of radish subjected to excess moisture ; the optimal infection temperature is 20°C. The symptoms are, first, a yellowing of cotyledons, then yellow spots on the upper leaf surfaces with white powdery down on the lower surface. The fleshy roots can also be affected : they show discoloration of the cuticle, followed by brown and black spots. Infection results from germ tubes which directly emerge from sporangia.

Artificial inoculation of young seedlings

Artificial inoculation according to the KLUCZEWSKI method (1980) is being used to study resistance to downy mildew. This consists in spraying a sporangial suspension (10^5 sp./ml.) on 25 young seedlings at the spread cotyledons stage. Inoculum issued from leaves of unhealthy radishes collected in the Avignon district. Then seedlings are kept in a humid chamber at $\pm$ 20°C.

Reading of symptoms and sporulation is made after 10 days ; a note of 0 to 4 (0 = no symptom, 4 = very abundant sporulation) is assigned to each plant. Testing of 14 lines of the small European radish has made possible the definition of a range of susceptibility to the parasite ; we have kept the following references :

line RB 2.1.17 = very abundant sporulation, susceptible line.
line 5 = medium sporulation, intermediate resistance
line 7 ms = very low sporulation, good level of resistance

The "Flamboyant" variety is also relatively resistant.
SHIRAISHI (1974) recorded the resistance to downy mildew of the Japanese radish variety "Tokinashi". That variety, also named "All Season", as well as an another Japanese variety, "Okura", showed no symptoms after inoculation.

Artificial inoculation of excised cotyledons

A test on excised cotyledons has been developed at the Plant Pathology Station of AVIGNON (INRA). The method consists in taking cotyledons 9 days after sowing and placing them face downwards on a damp filter-paper in a transparent plastic box. This is then put in a growth chamber at 20° day and 18° night, hygrometry 90 %, illumination 12 hours at 2 000 lux.
With a micropipette, 50 µl of a sporangial suspension (16.000 sp./ml.) are set down on the underside of cotyledons. After 5 days, the 2 cotyledons of each plant are rubbed with a brush in 2 milliliters of water in order to collect sporangia ; the concentration of the suspension is then measured using a Malassez hematocytometer.
Inoculations carried on 15-days-old plants have proved the excellent correlation between the number of sporangia obtained and the symptoms

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on leaves.

Cotyledon age has a very marked influence on the sporulation rating ; this last increases linearly from 6th to 9th day, then it decreases and becomes stable after 14 days.

The concentration of inoculum strongly affects the sporulation rating, thus permitting clear differenciation between the 3 reference lines ; the best discrimination is obtained with a concentration equal to 16.000 sp./ml..
The kinetics of sporulation also differs greatly between each of these 3 lines ; the 7 ms line has scant sporulation and produces a very low quantity of sporangia during the first 8 days ; in the most susceptible line, RB 2.1.17, sporulation begins as early as the 3th day and may reach 241.000 sp./cotyledon after 8 days.

Study of genetic determinism of the resistance

A study of genetic determinism of the resistance to downy mildew of the 2 Japanese radish varieties, "All Season" and "Okura", was made on excised cotyledons. These 2 varieties were crossed with the susceptible line RB 2.1.17 and F2 and BC1 progenies were produced. If all the plants having at last 1 sporangium per microliter are considered as susceptible, the following segregations are obtained :
parents "All Season" and "Okura" = all resistant
parent RB 2.1.17 = all susceptible
F1 = all resistant
F2 = 137 resistant out of 150, compatible with segregation 15-1 (X^2 = 1,495)
BC1 with resistant parent = all resistant
BC1 with susceptible parent = 40 resistant out of 60, compatible with segregation 3-1 (X^2 = 2,222).

The resistance of these 2 varieties thus appears to be controlled by 2 dominant and independent genes.
These 2 varieties are very different from the small European radish type. They form their tubers late, blossom only during long days and are very autoincompatible. After inoculation, their resistance is shown as small brown necrotic spots ; these may express a hypersensibility reaction already mentioned for the Brassicae by DIXON (1979).

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IN VITRO SELECTION OF FUSARIUM WILT RESISTANCE CABBAGE LINES

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Wilt of Brassica species caused by Fusarium oxysporum f.sp. conglutinans has already become the most important problem in Hungary during the past twenty years. Test method have been adapted according to Williams et al. (1968) using the most agressive isolate of race 1, originated from CMI (IMI 141 150). One of the Hungarian open pollinated cultivars named "Szentesi korai" proved to be fully sensitive, the remaining ten cabbage cultivars behaved heterogenious population having resistant level 5-85 %. Behaviour of sensitive and resistant cultivars were studied at callus level using the culture filtrate of the most aggressive isolates mentioned above and different concentrations of fusaric acid. The purpose was to develop a selection system obtaining resistant line from fully sensitive cultivars on the basis of somaclonal variation, which proved to be promising in numerous cases (Hartmann et al. 1984). Induction of callus from hipocotyl was carried out according to Murashige (Murashige, 1977) on a medium containing 5×10^{-6} M 2,4,5-T and 10^{-6} M BA. Culture filtrate of the patogen was prepared by the method of Hartmann et al. (1984) using the following concentrations 1,5,10,15,20,25, 30 and 50 %. In paralell experiments the fusaric acid concentrations were 25,30,40,50,60 and 75 mg/l. For the comparison of the fully sensitive "Szentesi korai", the partly resistant "Harmat", having a resistant level of 85 % and one of the resistant F_1 hybrides named "Drumhead" and for the selection of the resistant lines 50 % of survival rate was applied.
Results can be seen in Table 1.

Table 1.

The concentrations of culture filtrate of Fusarium oxysporum f.sp. race 1 and fusaric acid resulting 50 % survival rate of callus units.

Cultivars	Culture filtrate (%)	Fusaric acid (mg/l)
1. Szentesi korai	10	25
2. Harmat	20	50
3. Drumhead	30	60

Seed Production & Trading Co., H-6601 P.O. Box 41, Szentes, Hungary.

Plant regeneration experiments were carried out by Bajaj and Nietsch (1975), the micropropagation was slightly modified on the basis of Kartha (1974) results on medium contained $5\text{-}10^{-6}$M BA.

The results of tissue culture experiment showed that the cultures of resistant hybrid are able to tolerate the toxic filtrate and the fusaric acid at a much higher level than the cultures of the sensitive cultivar. These dates may suggest the importance of tolerance of plant cells against the fungal toxins in the host patogen interaction. The 30-40 % of the survival cultures resulted plants. These plants are being studied for resistance by the conventional test methods.

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THE STUDY OF RESISTANCE TO DIFFERENT VIRUS OF CHINESE CABBAGE(B.campestris ssp. pekinensis).

Yang Rui and Tao Guohua

There were always differences between seedling identifying in greenhouse and observation from the field on the disease resistance of chinese cabbage in previous studies. So three types of virus,TMV,TuMV and CMV were used to identify the resistance of eight chinese cabbage materials.

Materials and Methods

Eight materials were used: 5 self-incompatible lines,2079, Shuang Xiao,234,2157 & 2039-5;2 F1,Beijing 100 & Xiaoza 55 and Jiao Xian,a local variety of Shan Dong province. Generally 2079,Shuang Xiao,234 & Beijing 100 are virus resistant in the field,and 2157,2039-5,Xiaoza 55 & Jiao Xian are sensitive. Materials were inoculated in 13 days after sowing. The inoculating solution contains ¼ pestled diseased leaves of chinese cabbage in 0.05M,pH7.0 phosphate buffer. Each material was sowed 6 portions,and inoculated with TMV, TuMV,CMV,TMV+TuMV,TMV+CMV & TuMV+CMV. Plants were grown in greenhouse with temp. 25-28°C in days and 15-18°C in nights. The disease degrees were divided into 9 levels. The materials were divided into 5 types,high resistant indexes were 0-5.55;resistant ones' were 5.56-11.11;tolerant ones' were 11.12-33.33;sensitive ones' were 33.34-55.55 and high sensitive ones' were 55.56-100.

Results and Discussions

The results adapted in 17 days and 32 days after inoculation are shown in Table 1 and Table 2. It is seen in Table 1 that 2079,Shuang Xiao & Beijing 100 had stronger resistance than others,while each one has different level of resistance to different virus sources. The general tendency is the virus source TMV+TuMV has stronger inoculating ability. But from Table 2 we can see that strongest virus source is TuMV,it is also in other stronger mixing virus sources.

With these results it is clear that the differences between seedling identifying and observation from the field are probably due to the differences between virus sources. The material 234 has strong virus tolerance in the field normally. In this experiment it appeared serious symptom in 17 days after inoculation. But it's disease level had not any advance after that. It is shown in Table 2 that 234 had lowest rate of seedling death. As for the material Jiao Xian,it is a virus sensitive variety normally. But it was resistant to most

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virus sources as shown in Table 1. However its diseases had developed after that,and in 32 days after inoculation, its seedling death rate were much more than 234. So the better way to identify the resistance of materials is to combine the results from observations in the period that every materials appear most distinct symptom and advanced appearance.

Table 1. The result of resistance to different virus sources of chinese cabbage materials in 17 days after inoculation.

Materials	Virus Sources					
	CMV	TuMV	TMV	TMV+CMV	TuMV+CMV	TMV+TuMV
2079	R	R	T	R	R	R
2157	T	HS	HS	S	T	HS
Shuang Xiao	R	T	T	T	T	HS
2039-5	HS	HS	HS	HS	HS	HS
234	HS	T	HS	HS	HS	HS
Jiao Xian	T	R	S	T	T	HS
Xiaoza 55	T	T	S	HS	T	HS
Beijing 100	T	T	T	HS	T	HS

* R:resistant; T:tolerant; S:sensitive; HS:high sensitive

Table 2. The death rate of chinese cabbage materials in 32 days after inoculation(%).

Materials	Virus Sources					
	CMV	TuMV	TMV	TMV+CMV	TuMV+CMV	TMV+TuMV
2079	66.7	100	16.7	0	66.7	0
2157	100	100	100	100	100	100
Shuang Xiao	100	100	83.3	100	100	100
2039-5	0.17	100	33.3	0	100	100
234	33.3	16.7	0	0	0	0
Jiao Xian	100	100	83.3	100	83.3	100
Xiaoza 55	83.3	50.0	50.0	100	66.7	100
Beijing 100	16.7	16.7	0	0	33.3	0

GROWTH AND NUTRIENT UPTAKE OF EARLY CAULIFLOWER VARIETY "BRİO" (Brassica oleracea var.botrytis cv.BRİO)

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It is interesting to see the significant differences between the results of experiments made as to the amount of macronutrients removed from soil by cauliflower plants in both production systems, for market and seed production. Sevgican (1981) reported that cauliflower plants removed 200 kg/ha N, 75 kg/ha P_2O_5 and 250 kg/ha K_2O from soilwith the 50 tonnes/ha marketable curds. According to the Nieuwhof (1969), these amounts were 210 kg/ha N, 67 kg/ha P_2O_5 and 220 kg/ha K_2O. On the other hand, Raut and Kedar (1980) pointed out that to apply 50-100 kg/ha N, 25-50 kg/ha P_2O_5 and 25-50 kg/ha K_2O were increased the seed yield of cauliflower. In Great Britain, the applications of N, P_2O_5 and K_2O with the amounts of 125-250 kg/ha, 25-175 kg/ha and 60-300 kg/ha respectively were advised to the farmers (Anonymous 1983). As pointed out by Nieuwhof (1969), the biggest similarity of these results is that the amount of K_2O removed from soil by cauliflower plants was highest among the macronutrients and N and P_2O_5 follow it respectively.

With the aim of clarification to this subject, the results of the experiment realized with Brio, early cauliflower variety (Brassica oleracea var. botrytis cv.Brio), is seen on Table 1. On this table, it is seen that the growth rate of the young cauliflower plants is slow at the 35$^{th.}$ day after transplanting and total dry matter of the plant is 103,9 kg/ha at this period. Between the 35$^{th.}$ - 93$^{rd.}$ days after transplanting, the growth rate of plants increase so fast and total dry matter reaches to the 5042 kg/ha. In the third period (93$^{rd.}$ - 137$^{th.}$ days), the rate of dry matter accumulation of plants desreases and at the bolting stage reaches to 8339 kg/ha. %52.8 of this amount is in the generative organs.

The total amount of nitrogen removed from soil increases fastly up to the bolting stage and reaches 254,8 kg/ha and %49.1 of it accumulates in the generative organs. When the curd harvest begins, namely, at the curd maturation stage, it is seen that cauliflower plants remove 169,7 kg/ha N from soil with the 40 tonnes/ha marketable yield.

The total amount of P_2O_5 removed from soil is 83.0 kg/ha at the bolting stage, although it is 49,0kg/ha at the curd maturation stage. %58.5 of it accumulates in generative organs. Total K_2O received by the plants has similar trend with P_2O_5 along the growing period and at the 93$^{rd.}$ day reaches to 175.4 kg/ha value. At the bolting stage (137th day) this amount is 280.6 kg/ha which is the maximum P_2O_5 value received by plant.

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As a result, it is determined that cauliflower plants remove 169.7 kg/ha N, 49.0 kg/ha P_2O_5 and 175.4 kg/ha K_2O from soil with the 40 tonnes/ha marketable curds. In the cauliflower seed production , plants remove 254,8 kg/ha N, 83,3 kg/ha P_2O_5 and 280,6 kg/ha K_2O from soil.

TABLE 1. Dry matter accumulation and nutrient uptake of cauliflower plants at different growth stages

Part of Plants	Growth Stage (x) Seedling 35th. day	Curd Maturation 93rd. day	Bolting 187th. day	Seed Maturation 248th day	% (xx)
Dry matter (xxx) (kg/ha)					
Root	25.1	356.4	614.2	754.2	7.4
Stem	13.7	540.0	716.8	668.0	8.6
Leaf	145.1	2606.7	2606.7	2606.7	31.2
Curd	-	1537.3	4401.7	4591.2	52.8
Total	183.9	5042.1	8339.4	8620.1	100.0
N (kg/ha)					
Root	0.48	4.30	8.56	7.15	3.4
Stem	0.49	11.08	14.55	9.60	5.7
Leaf	7.31	106.60	106.60	106.60	41.8
Curd	-	47.78	125.13	87.97	49.1
Total	8.28	169.76	254.84	211.32	100.0
P_2O_5 (kg/ha)					
Root	0.14	1.39	2.83	1.74	2.4
Stem	1.13	4.32	5.51	4.05	6.6
Leaf	0.61	27.08	27.08	27.08	32.5
Curd	-	16.21	48.32	43.26	58.5
Total	1.18	49.00	83.34	76.83	100.0
K_2O (kg/ha)					
Root	0.93	11.00	17.57	17.51	6.3
Stem	6.24	29.97	35.38	33.46	12.6
Leaf	6.76	82.27	82.27	82.27	29.3
Curd	-	52.20	145.45	102.95	51.8
Total	13.53	175.44	280.67	236.19	100.0

(x) After transplanting. (xx) At the bolting stage (xxx) Air dry basis

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EFFECT OF PRESOAKING TREATMENT OF SEEDS BY INDOLE-3-ACETIC ACID ON GROWTH AND YIELD OF *BRASSICA JUNCEA* L. UNDER SALT STRESS

Rupa S Dhawan

A screening of Brassica varieties for salt tolerance has revealed that *B. juncea* (Linn.) Czern & Coss subsp. juncea (Linn) var. Prakash is sensitive to the presence of salts in the medium (Dhawan et al., 1987). Its yield is reduced to about 50% at an EC of 6 mmhos/cm and the plants are unable to grow and form pods at an EC of 10 mmhos/cm in the medium. A seed treatment with growth regulators like indole-3-acetic acid (IAA) and napthalene acetic acid is known to improve yield in some crop plants. The possibility that these regulators may enhance salt tolerance was investigated in order to alleviate the adverse effects of salinity in *Brassica*.

Seeds of *B. juncea*(L) var. Prakash were presoaked with 500 mg/l IAA for 4 hrs. Polythene lined earthen pots containing 5 kg sand (27% field capacity) were irrigated with Hoagland solution containing 0 and 60 meq/l NaCl. The electrical conductivity of the solutions was 2.4 and 6.0 mmhos/cm respectively. 5 seeds were sown in a pot. These were thinned after 10 days to retain one seedling in each pot. The pots were irrigated at weekly intervals. The plants were harvested at maturity and the observations were recorded.

Data presented in the Table 1 indicates that the presence of salts in the medium reduces seed yield. A presoaking seed treatment with 500 mg/l IAA promotes the growth and yield of plants. This enhancement is observed on all the parameters like plant height, number of branches, number of siliquae/plant as well as seed yield/plant under saline as well as nonsaline conditions. Other concentrations of IAA and also 500 and 1000 mg/l NAA showed no effect.

An increase in the seed yield by presoaking IAA treatment is in confirmity with earlier results (Dua and Bhardwaj, 1979) where a foliar application with IAA is seen to increase grain yield. The stimulation in growth and yield by IAA under saline conditions may be by raising the endogenous auxin level which gets reduced by enhanced IAA oxidase activity under saline conditions(Naqvi and Ansari, 1974; Shukla and Baijal, 1977).

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Table 1: Effect of 4 hr presoaking treatment with IAA on plant height, number of branches, number of siliquae and seed yield/plant under non saline and saline conditions in B.juncea (L)prakash.

Growth regulator (mg/l)	Treatment	Plant height (cm)	Number of branches	Number of siliquae	seed yield/ plant
Control	NS	96.3	16	90	1.128±0.02
	S	95.0	15	85	0. 741±0.06
IAA	NS	110.0	29	140	2.396±0.06
	S	104.0	21	91	1.455±0.07

NS=Non Saline; S=Saline; ± = Standard error

FIRST REPORT OF TRIAZINE RESISTANT POPULATIONS OF RAPHANUS RAPHANISTRUM

G Baillargeon

Atrazine resistant populations of bird's rape (Brassica rapa = B. campestris) the wild and weedy biotype of the spring turnip rape cultivated on a large scale in western Canada, have been known to occur in the province of Quebec (eastern Canada) for more than ten years (Maltais & Bouchard 1978). Until recently (Bandeen & al. 1982), the infestation was thought to be restricted to a small 50-ha area near Bromptonville (45°28'N 71°57'W). It appears now that the resistant biotype is slowly but steadily extending its range in southern Quebec, populations being yet scattered over 900 km² (Maltais, pers. comm.).

The discovery of a triazine resistant weedy Brassica ten years ago quickly led to a very intensive research effort in order to breed triazine resistance into Brassica-crops for low cost chemical control of weeds (Souza Machado & al. 1978, Souza Machado 1982, Souza Machado & Bandeen 1982, Mapplebeck & al. 1982). So far, psbA, the chloroplast gene responsible for the resistance (Xiao & al. 1986, Reith & Straus 1987) has been transferred from wild bird's rape into cultivated spring turnip rape and Chinese cabbage (all B. rapa), as well as into spring rapeseed and rutabaga (B. napus), and Indian mustard (B. juncea) (Beversdorf & al. 1980, Souza Machado & al. 1983, Beversdorf & Hume 1984, Hobbs, 1986).

Unfortunately, the usefulness of these new cultivars may be relatively short-lived as other cruciferous weeds respond to herbicide selection resulting in a build up of triazine resistant biotypes. This has been reported as early as 1983 for Sinapis arvensis in Ontario (Ali & al. 1986) and would appear to be the case with Raphanus raphanistrum in Quebec. In many maize fields near Sorel (46°02'N 73°07'W), Richelieu Co., in southern Quebec, populations of this weed treated for years with atrazine are no longer killed by heavy applications of the herbicide. Laboratory studies are currently in progress documenting the resistance level of these populations. Until now, R. raphanistrum (wild radish) was considered easy to control, but with the establishment of this new biotype it may become a serious agricultural problem again, at least in maize.

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GROWTH STUDY OF WINTER CAULIFLOWER

V. RUFFIO-CHABLE , Y. HERVE

A growth study of 6 winter cauliflower genotypes was undertaken in North Finistère (West Brittany) under field conditions. Some conclusions on two hybrids representing two different heading dates are reported here.

This work is a preliminary to further studies of cauliflower physiology. Their interest is for both production (study of physiological disorders, optimisation of farming practices) and breeding (heterosis study, cultivation of plants for seed production).

The first results gave indications about growth patterns for various organs, in terms of heading date.

The two cauliflower hybrids studied were sown on the 8th of June and transplanted on the 3rd of August. Random samples of 8 plants were taken every two weeks before heading, then every week after appearance of the head. The fresh and dry weight of organs was measured.

Leaves and roots reach their maximum weight during the vegetative phase in autumn. Stems show a different growth pattern and reach their maximum weight at crop maturity (Fig 1 a and b).

For these winter genotypes, total dry weight of the leaves decreases during the head growth; this decrease is due to leaf senescence and has already been descrided by WURR et al (1981).

Fig 2 a and b distinguish several groups of leaves; however, there is a continuous distribution between groups :

- a first group whose growth is rapidly restricted
- a second group containing the largest leaves with the same maximum size.
- a last group whose growth follows that of the curd up, until around crop maturity (after which the curd loses compactnes and begins elongation).

Therefore, an allometric relationship between the increase in growth of the head and that of the leaves is not seen in late heading varieties as in early heading ones (SALTER, 1960 ; WIEBE , 1972), except for the 3rd group of leaves.

The growth of the first two groups of leaves and the growth of the roots seems independant of that of the head.

Its remains to examine more precisely how growth is modified as soon as curd initiation takes place.

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Fig. 1 : Growth curves of each organ, related to accumulated day - degrees above 0°C from sowing date, as the time scale .

L : leaves ; S : stem ; R : root ; C : curd; ↓ : time of curd maturity

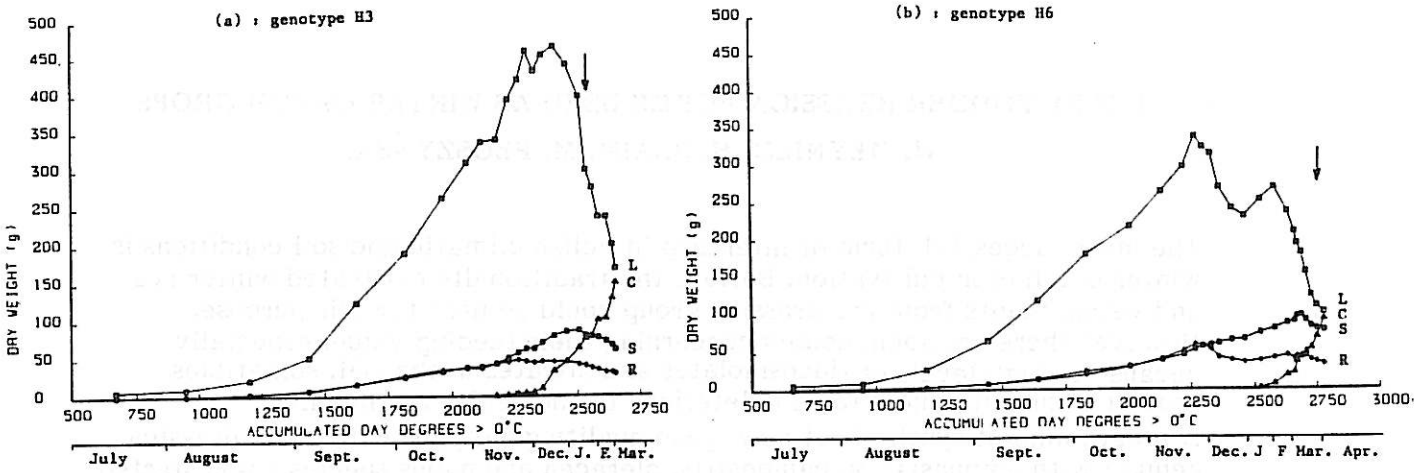
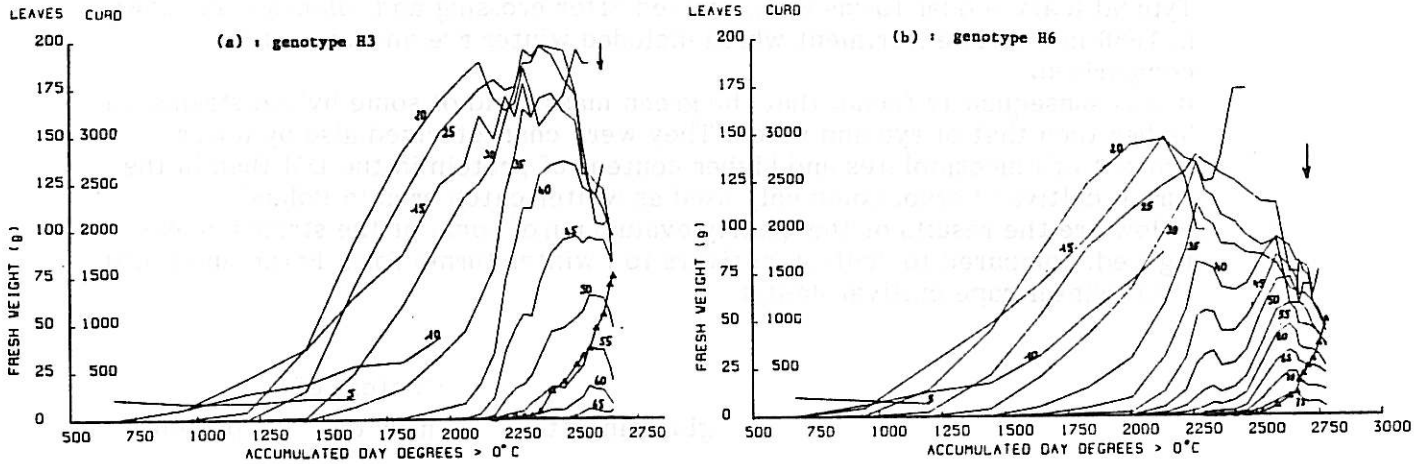


Fig 2 : Growth curves of each leaf (one out of five) and curd growth curve (⟷) ↓ time of curd maturity



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LEAFY FODDER BRASSICA FORMS USED AS WINTER CATCH CROPS

W. MŁYNIEC, H. BLAIM, M. PŁOSZYŃSKI

The most successful form of intercrop in Polish climatic and soil conditions is winter catch crop cultivation. Besides the traditionally cultivated winter rye and vetch, plants from the Brassica group could be used for this purpose. However there are some doubts concerning their feeding value, especially because of high levels of glucosinolates and nitrates in the DM, sometimes reaching amounts likely to be deleterious to the health of animals.

A programme of synthesis of new, good quality genotypes within the Brassica genus / with emphasis on campestris, oleracea and napus species / was started some years ago in the Plant Genetics Institute of Polish Academy of Sciences in Poznan / Cruciferae Newsletter No.6 : Balicka p.35, Barcikowska, Szyld p.43 ; Młyniec : p.38, Zwierzykowska : pp. 36-37/.

Typical leafy fodder forms were derived after crossing and selection and sown in 1986 in a field experiment which included winter rye and vetch for comparison.

It was subsequently found, that the green mass yield of some hybrid strains was higher then that of rye and vetch. They were characterized also by lower content of glucosinolates and higher content of protein in the DM than in the turnip cultivar Perko, commonly used as winter catch crop in Poland.

Below are the results of the quality evaluation of some of the strains investigated, compared to control cultivars to : winter turnip form Perko and Polish "OO" winter rape cultivar Jantar.

		Content of glucosinolates mm/g DM	nitrates % DM	protein % DM
B.n. cv Jantar		5.2	0.019	24.45
B.c. fo Perko		11.6	0.041	20.77
Synt.B.n.Fo.	1.1.2. Strain 12	6.3	0.032	24.86
	1.1.3. 11	9.3	0.017	24.70
	1.2.1. 22	5.9	0.017	23.47
	1.2.1. 85	5.9	0.030	25.27
B.napocamp.Fo.	2.1. 24	6.2	0.017	23.47
	2.2. 19	6.1	0.018	21.64
B.n./intersp./	3.2. 38	6.2	0.017	21.64

In the further selection carried out in the field and in pot experiments, in collaboration with the Institute of Plant Cultivation, Manuring and Soil Science in Pulawy, winterhardiness, high green matter yield of high DM content, low quantity of glucosinolates and nitrates and high content of protein are the main selection criteria.

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WILD CRUCIFER SPECIES AND 4-HYDROXYGLUCOBRASSICIN

J. P. Sang and P. A. Salisbury

With the glucosinolate content of rapeseed being reduced to levels below Canola specifications, the content of indole glucosinolates, particularly 4-hydroxyglucobrassicin, has become more important.

While much information on seed glucosinolate content of cultivated Brassica species is now available (Gland et al. 1981), few studies have been made on the distribution of glucosinolates in wild crucifer species. Recently, levels of alkenyl and sulphur-containing glucosinolates in the seed of some wild Brassica species were reported but no information on indole glucosinolates was given (Horn and Vaughan 1983).

In order to obtain data on the distribution and content of glucosinolates (particularly 4-hydroxyglucobrassicin) a survey of crucifer weed seeds in Australia was undertaken. The genera in the survey was Brassica, Camelina, Capsella, Carrichtera, Diplotaxis, Hirschfeldia, Myagrum, Raphanus, Rapistrum, Sinapis and Sisymbrium.

The profiles of glucosinolates in the seed meals were obtained using HPLC (Sang and Truscott 1984) and examination of them showed marked differences between the species. Preliminary results indicate that 4-hydroxyglucobrassicin is found in differing concentrations in the Brassica, Hirschfeldia, Raphanus and Rapistrum seed meals but was not detected in the seed meals of other species.

The variation in concentration of 4-hydroxyglucobrassicin in the seed of these wild species may be utilised by plant breeders to lower the contents of this glucosinolate in Brassica oilseed crops.

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COMPARISON OF NEAR-INFRARED REFLECTANCE ANALYSIS WITH MICROKJELDAHL AND KJEL FOSS FOR ANALYSIS OF PROTEIN IN RAPESEED

M. R. O'Grady, D. I. McGregor and R. K. Downey

The relatively high oil content of rapeseed can lead to variable result in Kjeldahl determinations of protein when analyses are carried out on whole seed. Even when the oil is removed and oil-extracted meal digested, variability can be high compared to cereals and other grains (Starr and Smith 1978). Recently Bengtsson (1985) noted that near-infrared reflectance (NIR) spectroscopy of whole seed gave more reliable results for protein content than either Biuret or Kjeldahl. The suitability of NIR for protein analysis of whole rapeseed has been substantiated in a comparison of NIR with Kjel Foss, and a modified Kjeldahl procedure in which the nitrogen released by digestion was measured by automated colourimetric analysis.

Six Brassica napus L. strains and cultivars from six locations of the 1986 Canadian Co-Operative Trials, S83-748, S83-4520, SVO-2272, SVO-2403, Regent and Westar, were chosen on the basis of preliminary analysis to represent the available range in protein content.

Kjel Foss analysis was performed in duplicate on oil-extracted air-dried meals prepared by the Swedish tube method (Tröeng 1955). MicroKjeldahl analysis was performed in duplicate by colourimetry on a Technicon AutoAnalyzer (Gehrke et al. 1968) of oil-extracted air-dried meals prepared by the Swedish tube method. Both the Kjel Foss and microKjeldahl analyses were repeated in duplicate to yield a second set of results. NIR analysis was performed on a Technicon InfraAlyzer model 500 using a 3 wavelength calibration previously developed for the 1985 growing season. Duplicate samples of whole seed were loaded into open cups. Each sample was measured once at the three discrete wavelengths and protein predicted.

Table 1.

Method	Mean	Coefficient of variability
NIR	42.6	2.42
MicroKjeldahl 1	44.9	7.02
MicroKjeldahl 2	44.0	3.89
Kjel Foss 1	44.6	3.65
Kjel Foss 2	44.6	3.61

Statistical analyses showed NIR to have a coefficient of variability of 2.42, the lowest of the three methods (Table 1). The coefficient of variability of Kjel Foss method was somewhat higher at 3.6 and comparable to the second of the two sets microKjeldahl analyses. Analysis of variance indicated that genetic, environment and method of analysis effects all influenced the value for protein content (Table 2). However, the cultivar by method interaction was not significant indicating that the method did not influence the ranking of cultivars. Since NIR was the most precise and did not show bias towards cultivars it appeared to be the well suited for protein analysis.

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Table 2.

Effects	Degrees of freedom	Mean square	Significance
Location	4	452.0	**
Replicate (Location)	5	7.1	
Cultivar	5	47.5	**
Method	3	132.0	**
Method x Location	12	29.0	**
Cultivar x Location	20	18.0	**
Cultivar x Method	15	2.53	
Location x Cultivar x Method	60	3.73	
Duplicates (in sample)	120	5.48	**
Error	235	3.61	

NIR is a simple and fast technique enhanced by the fact that several constituents may be analyzed simultaneously. This has made NIR attractive for both plant breeding of agricultural crops and quality control analysis of agricultural products. An additional attribute is the non-destructive potential of the technique which permits reanalysis or analysis by another method or, in the case of plant breeding, planting and growing of selections to produce subsequent generations.

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THYMOL METHOD FOR GLUCOSINOLATE ESTIMATION

R.J.W. Truscott and S. Shen.

A method for the determination of total glucosinolate content in rapeseed meal using thymol reagent was first reported by Brzezinski and Mendelewski (1984). It has the advantages of being technically straightforward and inexpensive when compared with the HPLC method or GC/trimethylsilyl procedure.

A number of laboratories however, have reported difficulties in establishing the thymol method on a routine basis.

Amongst the problems encountered were unacceptable degrees of variation in replicate analyses of samples and technical difficulties with regard to the use of concentrated sulfuric acid. As part of a program to develop a new reflectance method for the measurement of total glucosinolate content in rapeseed we needed a reference procedure with which to compare our reflectance results. This paper outlines some of the results obtained in this study and the procedure which we have adopted for routine analysis of rapeseed using the thymol method. This procedure is based on that of Brzezinski and Mendelewski (1984),

Materials and Methods

(a) Analytical balance
(b) Glass test tubes, 20 x 150mm
(c) Spectrophotometer (505 nm)
(d) DEAE Sephadex A-25 columns (8 x 10 mm) in Bio Rad polypropylene Econo-Columns)
(e) Boiling water bath
(f) Plastic disposable cuvettes (e.g. Mallinckrodt)
(g) Ultraturrax.

Reagents

(1) Thymol, 6% (w/v) in Ethanol. Weigh 6g thymol (A.R.) in a 100 mL volumetric flask. Make to volume with absolute ethanol.
(2) Sulfuric acid, 78%. Add 800 mL of the conc. sulfuric acid to 200 mL of H_2O.
(3) Glucose solution, 10 mM. Weigh 180.16 mg into a 100 mL vlumetric flask. Make to volume with distilled water.
(4) Sinigrin solution, 10 mM Weigh 19.87 mg Sinigrin monohydrate into 5 mL volumetric flask. Make to volume with distilled water.
(5) K_2SO_4 solution, 0.3 M. Weigh 26.14 g K_2SO_4 into a 500 mL volumetric flask. Make to volume with water.

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Standard Curve

Into chromic acid-washed test tubes add 0.5 mL of glucose or sinigrin solution. (0.02-0.25 µmol). Add 0.1 mL 6% thymol and 2.0 mL 78% H_2SO_4 to each tube. Cover tubes with aluminium foil. Mix using vortex mixer. Heat in boiling water bath for 45 mins. Allow to cool to room temperature (or under running water). Read absorbance at 505 nm using plastic disposable cuvettes.

Preparation of Seeds.

1. 400 mg of seeds are weighed into a 10 mL graduted centrifuge tube.
2. Immerse in a boiling water bath. Add 2 mL of hot distilled water and leave for l0 mins.
3. Remove tubes containing boiled seed and homogenise while hot. Rinse shaft with hot water, into the centrifuge tube.
4. Leave a further 5 mins in heated water bath.
5. Cool tube and adjust total volume to 6.0 mL.
6. Remove 1.0 mL and load onto a DEAE-A25 Sephadex column and wash with 400 µL of 30% formic acid followed by two 400 µL aliquots of water or until no more colour eluted.
7. Elute sample with three 500 µL aliquots of 0.3M K_2SO_4 into a 5.0 mL volumetric flask. Make up to volume with H_2O.
8. For the thymol reaction, remove 0.5 mL of the column eluate and add 0.1 mL 6% Thymol and 2.0 mL of 78% sulphuric acid.
9. Process samples as described under Standard Curve.

Results

The colour development is complete by 35 mins incubation at 100°C but we routinely use 45 mins since the colour does not change to any significant extent up to 60 mins.

Figure 1 shows the influence of thymol concentration on colour development at two glucose levels; 0.25 µmol (top curve) and 0.0625 µmol.

The highest glucose level is equivalent to 2.5 µmol glucosinolate on column or 75 µmol glucosinolate/ g meal. We routinely use 6% thymol giving a final 0.23% thymol in the incubation tube. Other procedures [1,2] result in a final 0.11% thymol which may lead to an underestimate of glucosinolate content in rapeseed containing high levels of glucosinolates.

Using this procedure reproducible standard curves can be obtained (Fig. 2). In the range 0.2-2.5 µmol sinigrin added to the DEAE column, recovery was 97 ± 1%.

As the method is very sensitive, it is important to exclude dust, lint etc. For this reason tubes are first acid washed and also capped during incubation. All aqueous solutions are filtered through 0.45 μ filters prior to use. Vortex mixing of tubes after incubation at 100 °C reduces degassing, and schlieren effects. The use of plastic disposable cuvettes eliminates the need to rinse cuvettes between readings. The cuvettes can be rinsed with distilled water and reused.

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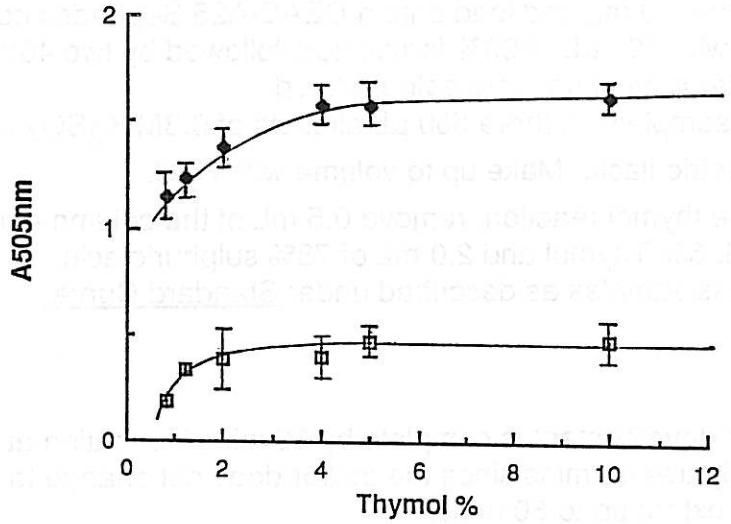

FIG 1

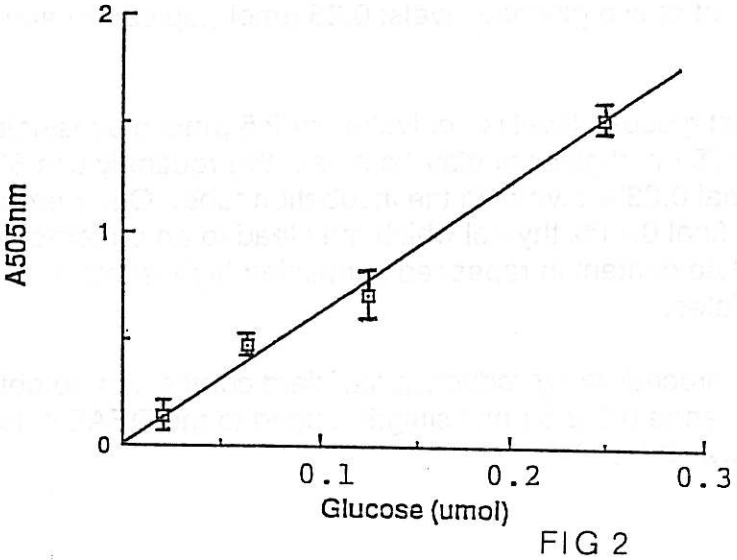

FIG 2

QUANTITATIVE LEVELS OF GLUCOSINOLATES IN RAPESEED POLLEN

S.G. DUNGEY

The recent finding of glucosinolates in rapeseed and Indian mustard pollen (1) suggested that these compounds may be accessible to pollen selection (2). To establish a quantitative basis for pollen selection it was necessary to determine whether the relative amounts of glucosinolates in pollen could be correlated with the relative levels of glucosinolates in seed. This report presents some preliminary quantitative data on glucosinolates in pollen and seed of two rapeseed cultivars Midas and Tatyoon.

Pollen and seed from phytotron-grown plants were extracted and analysed for glucosinolates according to Dungey et al. (1). Pollen samples were further concentrated by freeze drying to attain adequate sensitivity for HPLC detection.

The results (Table 1) indicated that the glucosinolate content of pollen generally reflected that of seed with respect to the Canola glucosinolates. The Indole glucosinolates were either absent from pollen or present only in trace amounts. The results also indicated that quantitative differences observable at the seed level were also evident at the pollen level. These very promising results, if extended to other cultivars, would provide the quantitative basis for the pollen selection of low glucosinolate cultivars.

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AN EFFECT OF OIL-SEED RAPE ON SEED PRODUCTION IN CAULIFLOWERS

Peter Crisp, Susan Angell and George Hargreaves

Summary

A high proportion of 'off-types' produced by a seeding crop of cauliflowers was probably due to an adjacent field of oil-seed rape attracting pollen-bearing insects from a long distance, rather than cross-pollination by the rape.

Introduction

The area of winter oil-seed rape in Britain expanded from 25,000 ha in 1974 to over ¼m ha in 1986 - five times the area of horticultural brassicas. This may have caused increases in diseases and pests on horticultural brassicas (eg. Alternaria - Humpherson-Jones, 1984; cabbage stem weevil - Wheatley and Finch, 1984).

Concern has also been expressed about cross-pollination by rape of swede (both B. napus) and turnips (B. campestris) (I.H. Munro, personal communication). Cross-pollination of B. oleracea by rape has not been considered a problem because of the difficulty in making this cross artifically (eg. Yamagishi and Takayanagi, 1982).

In spring 1985, anomalous plants appeared in biennial, spring-maturing cauliflowers grown from seed produced outdoors in Yorkshire, UK. In one plot of ca. 10,000 plants about 90% produced small branched inflorescences resembling sprouting broccoli. Their vegetative leaves were more rounded than typical cauliflower. The seed had come from 20 plants growing a few metres from a rape crop (cv. Jet Neuf) covering several ha. Both had flowered together in spring 1983. A seed crop produced from the same stock in the same year, but ca. 500 m from the rape, gave 100% cauliflower phenotypes in a plot adjacent to that containing the anomalous plants. Both seed crops had been at least 1 km from any other flowering B. oleracea (in a neighbouring village). That is, the only identifiable difference between the parents was their proximity to rape at the time of flowering. How did these anomalous plants arise, and how could they be prevented from occuring in future?

Observations and experiments

1. The anomalous plants smelt differently from cauliflowers when bruised, but comparisons of glucosinolate contents, using GLC, showed no consistent differences between these and other B. oleracea plants, and no consistent similarity to rape. (We are indebted to Dr Rosemary Cole for this investigation).
2. Six randomly selected, anomalous plants had high proportion of pollen stained by lacto-phenol cotton blue (the lowest count was 74%), and the grains were regular. All six set seed freely when selfed. There was, therefore, no evidence of infertility. Chromosome counts from root tips showed the normal 2n = 18 of B. oleracea.
3. A small experiment using rape cv. Jet Neuf to repeatedly pollinate spring cauliflower gave no evidence that this cross could give rise to seed.
4. Progeny from the selfed anomalous plants (in 2., above) showed apparent segregation for cauliflower/cabbage leaf characters, and no indication of non-oleracea characters; 18 of the 20 plants formed loose cabbage-like heads.

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Discussion

Were it not for the proximity of rape to the flowering cauliflowers, and the distance from other B. oleracea, our observations would indicate that the cauliflowers had simply been contaminated by pollen from other flowering coles - probably cabbages. The most convenient, and most probable, explanation is that the large area of flowering rape was highly attractive to pollinating insects, and these travelled long distances to reach it, carrying cabbage pollen with them, and fortuitously the first brassicas they alighted on were the nearby cauliflowers.

However, other explanations cannot be eliminated. The anomalous cauliflower plants appeared to possess other oleracea genes; but is it conceivable that these genes came by some mechanism from rape rather than other coles? Although under most experimental conditions this cross could be so improbable as to go undetected, the quantity of pollen in a rape crop must be huge, which may render unlikely events common, and may change normal biological relationships. To quote Stettler and Ager (1985): 'mentor effects in mixtures of pollen are merely an arbitrary zone in the continuum of pollen interactions', and these may range from the rare incorporation in ovules of a few paternal characters from damaged pollen (Pandey, 1978), to the effects of extracts from B. napus pollen on the incompatibility of B. oleracea (Roggen, 1975). Indeed, Chiang et al (1981) reported that ca. 1% of emasculated B. oleracea flowers pollinated by B. napus gave rise to seeds, none of which were identifiably hybrid.

Whatever the explanation, it must be in the interest of seed producers to locate B. oleracea seed crops well away from agricultural crops of rape.

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A recent survey of sib content in F_1 hybrid Brussels sprout varieties

E. Harvey & B.M. Smith

Seed of fourteen F_1 hybrid varieties of Brussels sprouts were tested for sib content to see whether or not sib percentages had improved compared to previously published results, and whether they fell below the desired level of 5% as indicated by seed companies and not above the maximum acceptable of 10%. A further objective was to see whether more recent F_1 hybrid Brussels sprout varieties are amenable to testing by this method of polyacrylamide gel electrophoresis (PAGE). Seed or cotyledon extracts were analysed for the isoenzymes of acid phosphatase by PAGE. Commercial seed was purchased in 1986 from seed merchants other than the breeder; the hybrids were Titurel, Rampart, Merlon, Pearl, Achilles, Sheriff, Aries, Cavalier, Roger, Acropolis, Porter, Rasmunda, Lunet and Cor.

Out of the fourteen F_1 hybrids tested, four, Merlon, Pearl, Acropolis and Rasmunda were found to be homozygous for the same acid phosphatase isoenzyme alleles and could not therefore be tested for sib content by this method. This proportion is similar to that found by Wills, Fyfe and Wiseman (1979) where out of nine F_1 hybrid Brussels sprout varieties tested, three were found to be homozygous for the same acid phosphatase isoenzyme allele.

Samples of 104 seeds or cotyledon extracts were tested for each of the remaining heterozygous hybrids. Seven hybrids were tested using seed extracts and three hybrids were tested using cotyledon extracts. Calculated sib percentages are set out in Table 1. They were calculated on the assumption that the parents of the F_1's in the table, were homozygous for different acid phosphatase isoenzyme alleles. Failure of this assumption would result in erroneous estimates of sib frequencies.

The estimates for percentage sibs were below 10% for all the F_1 hybrids and below the desired 5% level for four of them. However, these results were obtained from samples of only 104 individuals. The statistical test for indicating the level of sibbing in the bulk seed from a known sample size has been reviewed by Freeman and Johnson (1976) and Arus *et al* (1982), but we think it useful to repeat here. If N seeds are tested and the probability of a single seed being a sib is p then the probability of getting r sibs in a test is

$$\binom{N}{r} p^r (1-p)^{N-r}.$$

[If N is large the distribution of r is approximately normal with mean pN and variance Np(1-p). The distribution of $\frac{r}{N}$ is normal with a mean of p and variance $p\frac{(1-p)}{N}$.]

The results in Table 1 suggest that for some hybrids the true sib content of their bulk seed lots could be above 10%. For the hybrids

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Aries and Achilles the results suggest that the true sib percentage was no greater than 13% in the bulk seed lots (p = 0.95). Also for the hybrids Lunet and Cor the probabilities suggest that the true sib percentage was no greater than 11.8% in the bulk seed lots. Thus for four hybrids the sib content could be over 10% although it is likely that the seed companies would have tested a much larger sample before releasing seed for general sale. The six remaining hybrids all had sib levels below 10% but could be above the desired 5%.

The results indicate that sib content in F_1 hybrid Brussels sprouts is still a considerable problem and that the proportion of hybrids that can be tested by this method remains approximately the same.

Table 1: The percentage of sibs found in each hybrid and estimates of probable maximum percentage of sibs

Hybrid	Percentage of sibs found	Estimates of the likely maximum percentage of sibs in the bulk seed lot (p=0.95)
Titurel	4.8	8.1
Rampart	4.8	8.1
Achilles	8.7	13.0
Sheriff	5.8	9.3
Aries	8.7	13.0
Cavalier	2.9	5.4
Roger	2.9	5.4
Porter	5.8	9.3
Lunet	7.7	11.8
Cor	7.7	11.8

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MEASUREMENT OF YIELD PENALTY FOR TRIAZINE TOLERANCE IN OILSEED RAPE, BRASSICA NAPUS L.

G. Röbbelen

Triazine tolerance of oilseed rape has been developed by Beversdorf et al. (1980) using repeated backcrossing of a spontaneous accession of triazine tolerant bird's rape (2n = 20) into B.napus cv. 'Tower' (2n = 38). Already the first result of this breeding, the variety 'Triton', generated a lot of interest in certain regions of Canada, where otherwise hard-to-control weeds would have made rapeseed cultivation impossible (Downey 1984). However, even after further improvement triazine tolerant lines still proved to be inferior to their sensitive sister lines, i.e. they exhibited slower seed germination, lower seedling emergence and growth, and consequently less biomass production and seed yield (Mapplebeck et al. 1982).

Triazine tolerance has been shown to be maternally inherited (Souza Machado 1978). It is caused by a modification in the Photosystem II complex of the chloroplast thylakoid membranes in such a way that the triazine-binding protein loses its affinity for triazine. Evidently such change may dramatically affect the rate of electron transfer in photosynthesis (Arntzen et al. 1982). But unequivocal determinations of the resulting reductions can not be derived from mere comparisons of tolerant and sensitive lines; these differences may be characteristic of the given genotypes rather than the triazine tolerance trait. Therefore, lines with near-isogenic nuclear background were developed by repeated backcrossing and used to determine between them anatomical and physiological as well as fitness and yield variations (Vaughn 1986, Gressel and Ben-Sinai 1985, Forcella 1987).

Superior to such near-isoline approach is a comparative test with reciprocal hybrids which are truely isogenic in all nuclear genes. Moreover, they are easy to develop and much quicker to obtain. Therefore, in 1986 at flowering time material of a triazine tolerant winter oilseed rape BC_3 line, developed from the initial accession of Beversdorf et al. (1980), was reciprocally crossed with the German 00-cultivar 'Lirabon' in Göttingen. Flower buds were opened by hand when the anthers were still green and pollinated without emasculation in large numbers, so that sufficient seed was obtained for a usual performance test. The parents and hybrids were sown at mid of August in the same year at three locations (150 and 400 km apart, respectively) in 10 m² drilled plots each with 3 replications. Differences in development and plant height of the reciprocal combinations were evident throughout the season, the atrazine cytoplasm always marked by considerable reductions. Seed yields were determined after combine harvest and data were subjected to statistical analysis of variance. Effects of locations were not significant, but interactions between locations and candidates were significant at P = 0.05. Differences between the reciprocals were significant at P = 0.10 for the mean of the three locations, but they were evident in each case to the benefit of the triazine sensitive cytoplasm (Table 1).

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Table 1: Seed yield (mean of 3 replications, in dt/ha) of reciprocal hybrids of winter oilseed rape with and without triazine tolerant (ATR) cytoplasm harvested 1987 at three locations in the Fed.Rep. of Germany.

Genotype	Location			$\bar{x}$
	Göttingen	Thüle	Hohenlieth	
Lirabon x ATR-BC_3 triazine-sensitive	47.8	41.3	41.7	43.6
ATR-BC_3 x Lirabon triazine-tolerant	43.8	33.8	36.5	38.0
LSD 10%	5,4	12.6	5.8	4.9

Grant and Beversdorf (1985) have recently confirmed the inferior performance of triazine tolerant materials also for 8 different hybrid varieties as compared to their highest yielding sensitive parent. But in some combinations hybrids did reach higher yields than the parent lines of others of the 8 combinations tested. This indicates, that an improvement in performance of triazine tolerant varieties is possible through conventional breeding.

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STUDIES ON DETERIORATIVE CHANGES IN CAULIFLOWER SEED DURING AGEING

S.D. Doijode

Seed viability is largely affected by high temperature and humidity. The reduction in germination seems to be major criterion for the physiological manifestation of seed deterioration(Abdul-Baki and Anderson, 1972). However, it also involves certain changes in cell structure which enhance leaching of metabolites (Doijode, 1985). The present experiment was conducted to study certain biochemical changes associates with ageing of cauliflower seeds.

Materials and Methods

Cauliflower cv Pusa Deepali seeds were subjected to accelerated ageing at 40 C and 90% RH for six days. Seed viability and vigour were recorded as per the standard ISTA procedure. Seeds were surface sterilized with 0.1 per cent mercuric chloride, washed with sterile water, dried and soaked in 20 ml sterile water for 18 hours at 25 C. Electrical conductivity, soluble sugars and free amino acids were estimated in leachates and Dehydrogenase activity was measured in seeds.

Results and Discussion

There was drastic reduction of seed viability and vigour in aged seeds (Fig.1). The electrical conductivity of seed leachates was rapidly increased with decrease in seed viability. The leaching of soluble sugars and free amino acids were increased with the increase in ageing period. Dehydrogenase activity was low in aged seeds. The results suggest that loss of viability was associated with the leaching of metabolites from seeds which can be used for quick predicting of seed viability. The rate of leakage of total electrolytes increased with increase in ageing time. The quantum of metabolites in leachates was more from the less vigourous and non-germinable seeds. This increase in leakage was attributed to deterioration in cell membrane (Powell and Matthews, 1977). Thus it is advisable to keep the seeds at low temperature and low moisture or relative humidity in order to protect cell membrane and reduce physiological deterioration of seeds so that seeds remain viable for longer period.

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CONTENTS (Contd) Page

CONTENTS (Contd)

CONTENTS (Contd) Page

CONTENTS (Contd)