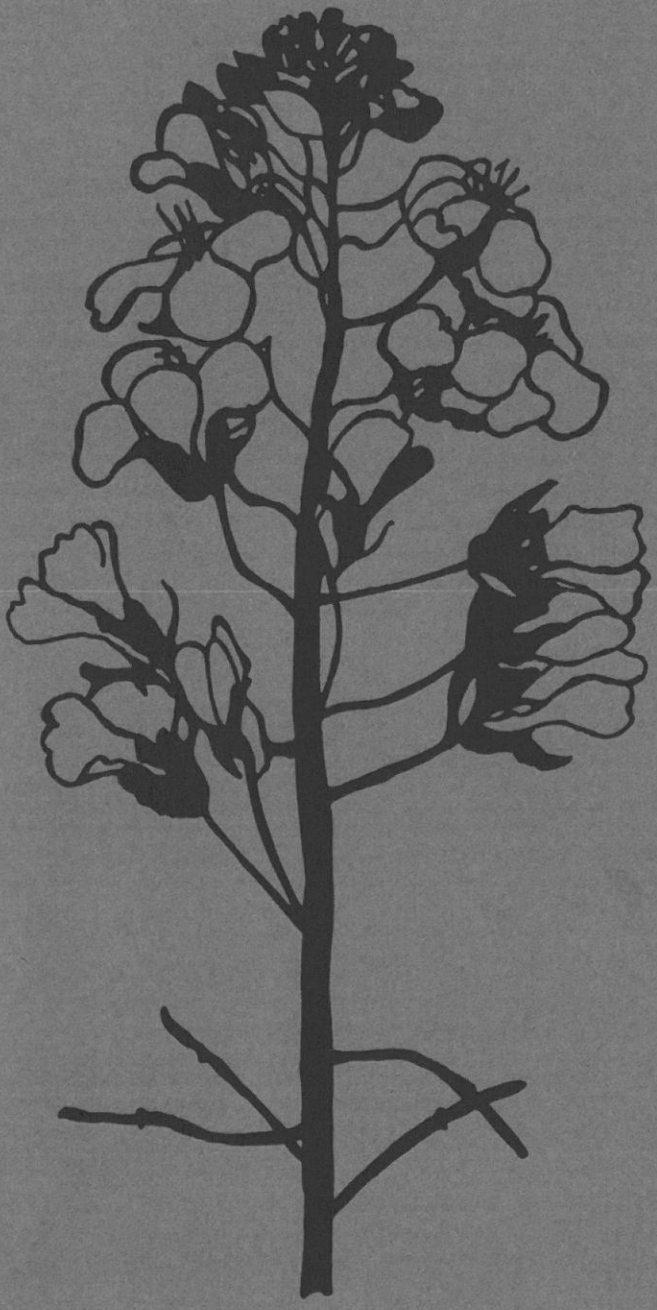
CRUCIFERAE NEWSLETTER



Nr. 16
May 1994

EUCARPIA

Ed. INRA Rennes

CRUCIFERAE NEWSLETTER

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EDITORIAL

Dear colleagues and members,

We are glad to announce you officially the re-birth of Cruciferae Newletter and to provide you an issue of Nr . 16 as the beginning of a new series ! We hope that the task we have proposed and been accepted for by Eucarpia will correspond to the request of most of you.

This publication is yours and I'd like to remind you some of the principles of its publication.

The publication is opened to everybody interested in Cruciferae (Brassicaceae). We have tried to organize Nr.16 in different topics but others may be opened (animal uses, agronomy...). The structure of Nr.16 covers only some of the Crucifer research areas..

The principle of 2 page-short notes will be maintained.
All papers are published on their authors' responsibility.

The publication committee will take care of the regular publication of the Cruciferae Newsletter but everybody must take seriously into account the deadlines of each Nr.

We have been mainly supported by our institute INRA for the re-start of the review.
Besides, we would like to maintain the idea of a free publication (no fee per issue). For that purpose, we need the financial help of all of you (private and public companies) if we want to maintain regular publication. You will find a financial support form for sending your free contribution or the one of your company. Thanks in advance for your help for Nr 17.

Please, don't forget that it is the only way to go on with our common interest!

The committee would like to change the illustration of the cover. If some of you have suggestions, we'd like to examine them for the preparation of Nr 17.

We hope everybody will keep interest and trust for Cruciferae Newsletter.

Grégoire THOMAS
Coordinator

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The Gatersleben *Brassica* collection -- an actualized survey

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The genus *Brassica* contains 41 species. 11 of them are related to the *Brassica oleracea* group. 6 species are of high economic value, 5 of which have a worldwide distribution. These are characterized by a complicated infraspecific structure, and they are containing an enormous variability. The Gatersleben genebank maintains an important collection, containing about 1,600 accessions from 25 species.

A key for the species and for all sufficiently known infraspecific taxa had been provided by GLADIS and HAMMER (1990, 1992). The *Brassica oleracea* group is treated by GLADIS (1989) so far. Taxonomy and infraspecific structure of this complex are extremely complicated and will be presented in a separate paper.

Genebank material plays an important role in enlarging the genetic background of highly domesticated crop plants HAMMER (1993). The *Brassicas* of the Gatersleben genebank may be an representative model for collecting, documentation, and description, for maintenance "ex situ" and storing, of large and very diverse living plant collections. It is, in connection with cultivation experience accumulated here, the basis for reconditioning and potential enrichment of the spectrum of our crop plants. For maintaining and population management of endangered wild relatives, primitive forms, introgressions etc. "in situ" or "on farm" respectively, there should be given more attention to the experience and methods developed by farmers as well as by genebanks.

Literature

GLADIS, TH. 1989: Die Gattung *Brassica* L. und die Reproduktion entomophiler Pflanzensippen in Genbanken. Diss. AdW, 123pp.

GLADIS, TH. and K. HAMMER 1990: Die Gaterslebener *Brassica*-Kollektion -- eine Einführung. Kulturpflanze **38**, 121-156.

GLADIS, TH. and K. HAMMER 1992: Die Gaterslebener *Brassica*-Kollektion -- *Brassica juncea, B. napus, B. nigra* und *B. rapa*. Feddes Repert. **103** (7-8), 469-507.

HAMMER, K. 1993: The 50th anniversary of the Gatersleben genebank. FAO/IBPGR Plant Gen. Res. Newsl. 91/92, 1-8.

ACTIVITIES IN THE USDA-ARS VEGETABLE CRUCIFER COLLECTION, GENEVA, NY

James R. McFerson, USDA-ARS Plant Genetic Resources Unit, Cornell Univ., Geneva, NY 14456-0462, USA

Service activities

Current holdings of vegetable crucifers are summarized in the table below. Accessions with G number are undergoing regeneration and characterization before Plant Introduction (PI) numbers are assigned and the accessions incorporated into the National Plant Germplasm System.

PI and G number accessions of vegetable crucifers held at the USDA-ARS Plant Genetic Resources Unit, Geneva, NY, March, 1994.

Prefix	Taxon	Total
PI	*B. juncea*	0
G	*B. juncea*	60
PI	*B. rapa*	130
G	*B. rapa*	144
PI	*B. oleracea*	1090
G	*B. oleracea*	417
PI	*R. sativus*	591
G	*R. sativus*	74
Total		2506

All regenerations are performed using controlled pollination and large population sizes (approximately 100 plants). Comprehensive facilities improvements now allow regeneration of around 150 accessions per year. In 1993 the entire collection was tested for germination, repackaged into triple-laminated seed storage envelopes, and preserved at 0°C and 25% RH. The local database has been completely overhauled. The rewrite of software used by the Germplasm Resources Information Network (GRIN) is proceeding on schedule and expected to come on line in June, 1994. A version of GRIN written for use on personal computers (PC-GRIN) is available for *Brassica* and *Raphanus*. Over the past five years, we have distributed an average of around 500 accessions per year.

Research activities

To manage our germplasm collections more effectively and efficiently, we require more genetic information on accessions. The primary goal of the research is to obtain this information through the integration of strategies and techniques from conventional and molecular approaches. Results can then be applied to establish and maintain more useful and representative crop collections based on the identification of DNA sequences, genes, and genotypes. The selection of molecular diagnostic strategies ("fingerprinting") is predicated on adaptability, cost-effectiveness, and desired level of resolution necessary for solving problems associated with either ex situ or in situ genetic resource conservation and use. This approach and its related methodologies may be used to resolve and establish: (1) genetic identity, redundancy, or property rights regarding plant genetic resources; (2) minimum genetic distance for patent protection; (3) identification and tracking of DNA sequences, genes, and genotypes; and (4) link between molecular markers, genetic diversity, and agricultural useful traits.

CATALOGUE OF OILSEED RAPE CULTIVARS

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Oilseed rape is a major crop grown throughout the temperate regions of the world. There is an extensive and ever growing array of oilseed rape cultivars and an up-to-date listing of these cultivars is handy to have for reference purposes. To help meet this requirement, the author began the compilation of a catalogue of oilseed rape (Brassica napus and Brassica rapa) cultivars in the early 1980s and has continued to update this catalogue annually.

The "Catalogue of Oilseed Rape Cultivars - 1994 Edition" (Sernyk 1994) is a worldwide listing of oilseed rape cultivars arranged by:

1. Growth habit (Spring vs Winter),
2. Species (Brassica napus or Brassica rapa) and
3. Country of registration.

For each cultivar entered in the catalogue, the following information is provided:

1. Cultivar name,
2. Year of registration,
3. Owner of the cultivar,
4. Erucic acid and glucosinolate contents and
5. Pedigree (if available) or other comments.

The catalogue contains a world map showing the countries represented. It also contains a list of owners of the cultivars in the catalogue with contact addresses, phone and FAX numbers, and email addresses. The owners can be contacted to find out additional information or obtain seed of their cultivars.

This catalogue is updated annually using information provided by breeders, researchers and regulators in the countries listed in the catalogue. This information is essential to keep the catalogue up-to-date and the author appreciates the many responses received from these sources.

Copies of the "Catalogue of Oilseed Rape Cultivars - 1994 Edition" can be obtained by writing or FAXing your request to the author.

REFERENCE

Sernyk, J.L. 1994. Catalogue of Oilseed Rape Cultivars - 1994 Edition.

GUIDE TO THE WILD GERMPLASM OF BRASSICA AND ALLIED CROPS

S.I. Warwick, Centre for Land and Biological Resources Research, Agriculture Canada, K.W. Neatby Bldg., C.E.F., Ottawa, ON, CANADA K1A OC6

This note announces the availability of a new guide to the wild germplasm of *Brassica* and allied crops (Tribe Brassiceae, Cruciferae). The information provided in this guide is intended to be useful in providing direction for future genebank needs for *Brassica* crops and for assisting biotechnologists and breeders wishing to utilize these genetic resources in their research programs.

The guide is divided into five PARTS (I to V) which are published as Agriculture Canada Research Branch Technical Bulletins:

I. WARWICK, S.I. 1993. Guide to the Wild Germplasm of *Brassica* and Allied Crops. Part I. Taxonomy and Genome Status in the Tribe Brassiceae (Cruciferae). Agriculture Canada Research Branch Technical Bulletin 1993-14E, 33 pp.

[Provides a complete list of genera and species in the tribe and their genomic status, containing cross references for commonly confused names.]

II. WARWICK, S.I. & J.K. ANDERSON. 1993. Guide to the Wild Germplasm of *Brassica* and Allied Crops. Part II. Chromosome Numbers in the Tribe Brassiceae (Cruciferae). Agriculture Canada Research Branch Technical Bulletin 1993-15E, 22 pp.

[Provides a complete list of all published chromosome counts for species in the tribe, including recent counts obtained in our laboratory.]

III. WARWICK, S.I. & L.D. BLACK. 1993. Guide to the Wild Germplasm of *Brassica* and Allied Crops. Part III. Interspecific and Intergeneric Hybridizations in the Tribe Brassiceae (Cruciferae). Agriculture Canada Research Branch Technical Bulletin 1993-16E, 31 pp.

[Summarizes literature on inter-cytodeme hybridizations between members of the tribe, including information on whether the hybrid was obtained sexually, or artificially. Its also provides a summary of the 45 diploid cytodemes or crossing groups and six amphidiploid taxa described for *Brassica* coenospecies.]

IV. WARWICK, S.I. 1993. Guide to the Wild Germplasm of *Brassica* and Allied Crops. Part IV. Wild Species in the Tribe Brassiceae (Cruciferae) as Sources of Agronomic Traits. Agriculture Canada Research Branch Technical Bulletin 1993-17E, 19 pp.

[Reviews the potential of wild members of the tribe as sources of agronomic traits. Examples are presented under the sections: North American germplasm, morphological characters, chemical traits, photosynthesis, cytoplasmic male sterility, soil adaptation, cold and drought tolerance; herbicide, disease, insect, and nematode resistance, and new crops.]

V. WARWICK, S.I. & A. FRANCIS. 1994. Guide to the Wild Germplasm of *Brassica* and Allied Crops. Part V. Life History and Geographical Data for Wild Species in the Tribe Brassiceae (Cruciferae). Agriculture Canada Research Branch Technical Bulletin 1994-2E, 61 pp.

[Summarizes information on the life cycle, growth form, ecology, geography and phytogeographical status of each of the species indicated in Part I of the guide.]

These publications are available free of charge upon request.

THE PETAL PIGMENTS OF MUSTARD CULTIVARS AND ERUCA SATIVA Linn.

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[2]Plant Biotech.Section,B.A.R.C.Bombay-85,India

Mustard(Brassica juncea(L.)Czern and Coss. cultivars with yellow flower colour are grown in India. There are reports indicating that mustard with white flower colour does not attract alate aphid pests,during their initial anchorage on the plants. The objective of this study was to examine the pigments in yellow (Rai-5,Varuna,TM-4,Y-2)and white flower (B-85) cultivars of Indian mustard. E.sativa producing yellowish-white flowers was also included in this study for comparison. Petals(300 mg) from freshly opened flowers were collected in the morning and crushed in a mortar with 6ml methanol. Supernatant (100µml) was used for thin layer chromatography (TLC) using 9:1 chloroform and ethyl acetate as solvent. The absorption maxima(nm) and the optical density (OD) of the different pigments available in the petals were studied using Aminco UV-Vis spectrometer (at wavelengths 400-800 nm).

The colour of petals can be due to the presence of anthocyanin,flavanoids, carotinoids etc. The chromatogram for pigments in the petals revealed four spots in all the yellow petal cultivars under visible light whereas in B-85 only two spots were visible (Fig.1).The absorbance and OD between the yellow flower cultures were not significant. However, the OD between the yellow and the white flower types were different(Fig.2). There were three peaks at 416,436 and 466 nm in all the samples. In the light yellow flowers of E. sativa, the OD at the three peaks were much lower and in the white flowers of B-85,the OD was at the lowest. From the peak heights of OD,it is clear that the three compounds in B-85 and E.sativa are much less in concentration compared to the yellow flower types. The white flower character in B-85 may be the result of both quantitative and qualitative differences in the pigments.

REFERENCE

Chatterjee. S.D. and K. sengupta 1987: Observation on reaction of mustard aphid to white petal and glossy plants of Indian mustard. Journ. Oilseeds Res. 4(1) : 125- 127,1987.

Fig.1 CHROMATOGRAM OF
PETAL PIGMENTS

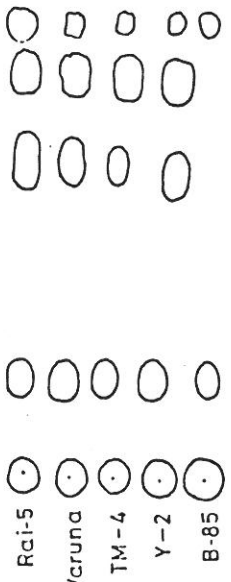
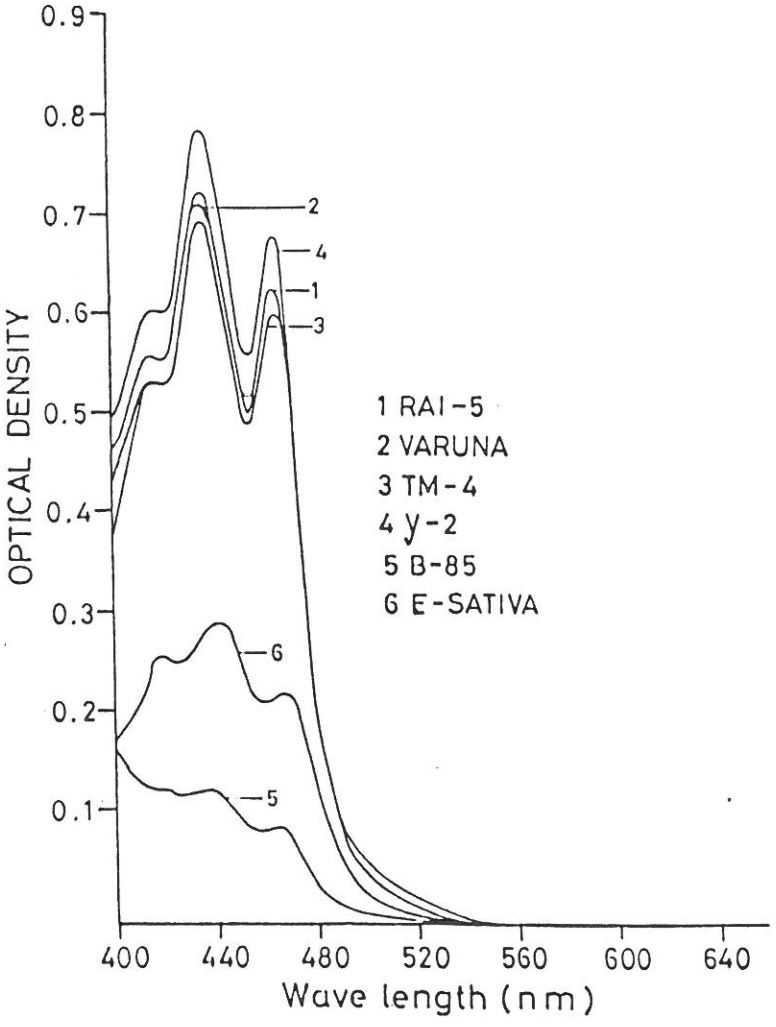


Fig.2 ABSORPTION SPECTRA OF
PETAL PIGMENTS



Effect of chemical mutagen (EMS) dose rate on seed germination of *Sinapis alba* and two *Brassica* species

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Rapeseed (primarily *Brassica napus* and *B. campestris*) is grown worldwide as a source of edible and industrial oil. The development of new oilseed crops adapted to more agricultural regions of the world would increase the yield potential and acreage of oilseed crops. *Sinapis alba* (commonly known as yellow mustard), is a close relative to the genus *Brassica*. This species has proven to be a potential rotation crop for the Pacific Northwest region of the USA with yields exceeding 3,600 kg ha^{-1} in dry land regions (Gareau *et al.*, 1990). In addition, the high levels of pest resistance in this species reduces the amount of insecticide required for successful crop production (Brown and McCaffrey, 1993). Yellow mustard has traditionally been grown as a condiment crop, although it has been suggested that this species could be grown as an oil crop. One limitation of *S. alba* is that available genotypes have oil content considerably lower (28%) than desired. In addition, available germplasm is too high in seed meal glucosinolate content to be considered useful as a livestock feed supplement.

Mutagenesis has been used successfully by many plant breeders working on different crops to produce genetic variability in oil and meal characteristics (Auld *et al.*, 1992; Browse *et al.*, 1986). The chemical mutagen ethyl methanesulfonate (EMS) has been extensively used in several crops to produce genetic variation, since it produces primarily point mutations (Medrano *et al.*, 1986). Several mutant lines with low levels of polyunsaturated fatty acids were also isolated in *B. campestris* and *B. napus* using EMS (Auld *et al.*, 1992).

The aim of mutagenesis is to cause genetic change in specific genotypes using a set mutagen. The effect of these mutagens causes damage to a number of individuals. Too low a dose level will result in almost no damage, but will be associated with no genetic change. It is, therefore, necessary to identify the correct dose level which will maximize genetic change while minimizing damage which may cause adverse effects. These results have shown differences between the damage rate of *S. alba* (as examined by germination) compared to either *B. napus* or *B. juncea* lines. If mutagenesis is to be successfully applied in *S. alba* to create new variation then it will be necessary to identify the exact dose level which will maximize variation but minimize mutation damage.

The objective of this project was to determine the effect of increased doses of EMS on seed germination in *S. alba* compared to two *Brassica* species.

The three species examined were *S. alba* ('Tilney' and 'Gisilba'); *B. juncea* ('Lethbridge 22A' and 'Forge') and *B. napus* ('Westar' and 'Legend'). EMS concentrations of 0.0, 0.5, 1.0, 2.0, and 4.0% v/v were used. The buffer solution was prepared using dibasic potassium phosphate (K_2HPO_4) at the rate of three grams per liter to maintain the treatment solutions at neutral pH. Seed was imbibed for 16 hours in aerated water before exposure to various EMS doses for two hours (Auld *et al.*, 1992). Treated seed was then rinsed twice with distilled

water, and one hundred M_1 seeds per replication from each dose along with control were germinated on soaked filter paper in petri dishes, in a randomized complete block design with four replications. Percent germination was recorded after two weeks by counting the number of germinated seeds.

Increased EMS dose was linearly associated with reduced germination in all genotypes (Table 1). The major difference between genotypes was accounted for by the difference between the two *S. alba* lines and the *Brassica* species cultivars. The greatest differences were also noted at higher dose rates. It would therefore be suggested that lower EMS rate would be necessary for *S. alba* than have been used for *B. napus* or *B. campestris.*

Table 1. Mean germination of *Brassica* species as affected by different EMS doses (i.e., 0.0, 0.5, 1.0, 2.0, 4.0% v/v)

Genotype	EMS treatments					
	0.0%	0.5%	1.0%	2.0%	4.0%	Average
S. alba						
Tilney	96.0	90.6	77.2	33.9	12.0	61.9[b*]
Gisilba	96.0	89.2	88.2	32.2	2.9	61.7[b]
B. juncea						
Lethbridge	94.6	91.5	86.0	89.0	32.4	78.7[a]
Forge	96.4	94.1	79.2	84.6	62.4	83.3[a]
B. napus						
Westar	96.7	89.5	87.2	79.5	15.4	73.7[a]
Legend	98.9	96.6	94.9	85.1	28.0	80.7[a]
Average	96.4[a]	91.9[a]	85.1[b]	67.4[c]	48.6[d]	

* Letters indicate significance at 1% probability level using Duncans Multiple Range test

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Brown, J., and J.P. McCaffrey. 1993. Yield reduction in spring planted *Brassica* species as affected by cabbage seedpod weevil (*Ceutorhynchus assimilis* Paykull). Proceed. 74th annual meet. Pacific Div. AAAS. p. 36.

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Three pairs of satellited chromosomes in *Brassica nigra* (L.) Koch and *Sinapis arvensis* L.

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Introduction

Brassica nigra (genomes: BB, 2n=16) and *Sinapis arvensis* (SS, 2n=18) are diploid species. Previous mitotic analyses have revealed that two pairs of chromosomes carry satellites (Sikka, 1940; Lan et al., 1991) and are involved in the formation of nucleoli (Sikka, 1940) in *B. nigra* . To our knowledge, the number of satellited chromosomes has not been reported in *S. arvensis*. The somatic chromosomes of *Brassiceae* species are small and when contracted at metaphase, it could be difficult to identify all chromosomes with satellites if the quality of the preparations is not good enough. In the present paper, we report a new number of satellited chromosomes in *B. nigra* and *S. arvensis* observed after Giemsa staining, which was further corroborated by silver-staining.

Materials and methods

Seeds of *B. nigra* cultivar "Giebra" was provided by Svalöf Weibull AB, Svalöv, Sweden, while seeds of *S. arvensis* accession No7006 originated from a wild population collected from the northwestern part of China. These seeds were harvested from open-pollinated plants. The methods used for mitotic analyses, that air-dried chromosome preparations were stained with Giemsa or silver nitrate, are described in detail in Cheng et al. (in preparation).

Results and discussion

After Giemsa staining, we observed that at prometaphase three pairs of chromosomes, of submedian type, had secondary constrictions and satellites on their short arms in both *B. nigra* and *S. arvensis*. The results are inconsistent with the previous reports in *B. nigra* (Sikka, 1940; Lan et al., 1991). This discrepancy might be due to the differences in the mitotic stages analysed and/or the quality of the preparations. It is likely that secondary constrictions and satellites are not detectable in the maximally contracted metaphase chromosomes. Since silver-staining reveals the number of active ribosomal DNA (rDNA) loci (Jimenzen et al., 1988), we use this technique to test our observation on the number of

chromosomes with secondary constrictions and satellites. Indeed, silver-stained nucleolar organizing regions (NORs) were localized on three pairs of chromosomes in *S. arvensis* and *B. nigra*. However, the silver-stained bands varied in size and intensity among the chromosomes, thus indicating differences in nucleolar organizing activity. Therefore, we conclude that the three pairs of chromosomes with secondary constrictions and satellites in *B. nigra* and *S. arvensis* carry active rDNA loci. In *B. nigra*, only two pairs of chromosomes with rDNA loci have been identified by *in situ* hybridization (Maluszynska and Heslop-Harrison, 1993). Whether this discrepancy is due to the differences of techniques or the variations of the materials remains to be investigated.

It has been reported that diploid *Brassiceae* species are secondary polyploids which have evolved from a common ancestor of x=6 (Röbbelen, 1960). However, a lower basic number of x=3 was also suggested (Chen and Heneen, 1991). The presence of three pairs of chromosomes with secondary constrictions and satellites in *B. nigra* and *S. arvensis* supports the basic number of x=3 in the *Brassiceae* genome.

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ISOELECTRIC FOCUSING ANALYSIS OF RUBISCO IN THE GENUS *ERYSIMUM* L.

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The genus *Erysimum* is perhaps the most taxonomically difficult genus in the Brassicaceae comprising between 200 and 350-420 species, depending on estimations (AL-SHEHBAZ 1988). Several intrageneric subdivisions in sections or series have been suggested. These classifications however, are controversial and mostly fractional, as only a few species have been analyzed in the respective local analyses (AL-SHEHBAZ 1988, BALL 1990) and not all of the species studied could be merged in the intrageneric segregates (see BALL 1964 in Flora Europaea). The distribution of secondary compounds, i.e. glucosinolates, cardenolides, fatty acids, sterols (cited in AL-SHEHBAZ 1988), seems not to be of great significance for intrageneric grouping.

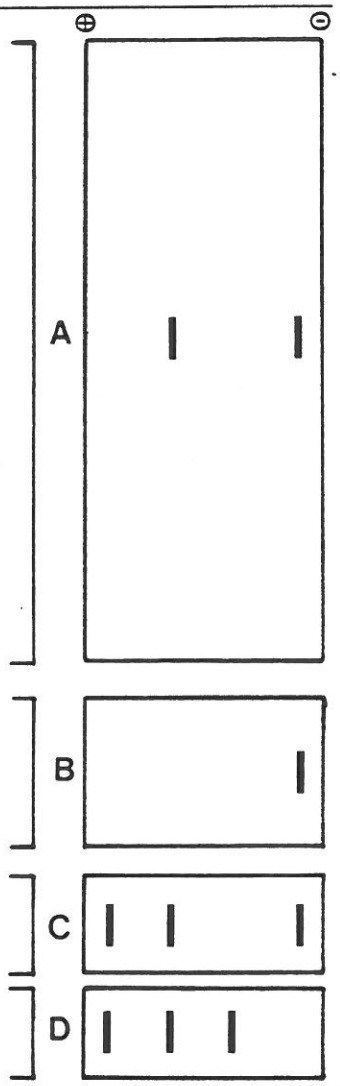
Recently we have provided evidence that differences in the polypeptide composition of Rubisco (ribulose-1,5-biphosphate carboxylase/oxygenase; EC 4.1.1.39) subunits is useful for intrageneric classification in the taxonomically difficult genera *Lepidium* and *Thlaspi* (MUMMENHOFF and HURKA 1991, MUMMENHOFF and ZUNK 1991). Rubisco is composed of eight large subunits (LSU) encoded by a single gene in the multicopy chloroplast genome, and eight small subunits (SSU) encoded by a small multigene family in the nuclear genome. Thus, isoelectric focusing (IEF) analysis of Rubisco subunits provides information on both the chloroplast and nuclear genome. In the present study we have analyzed the polypeptide composition of the LSU and SSU of 28 *Erysimum* species from N. America, Europe and the Macaronesian region (Canary Islands, Madeira, Cape Verde Islands). A detailed protocol of the techniques employed in this study is given in MUMMENHOFF and HURKA 1990).

All species analyzed in this report are characterized by identical IEF patterns of the LSU. On the basis of differences in the IEF pattern of the SSU *Erysimum* species studied fall into four groups. Species of each group are characterized by identical Rubisco SSU patterns. Two of these groups (A, B) do not coincide with previous systematic arrangements. Groups C (*E. bicolor*, *E. caboverdianum*) and D (*E. scoparium*) representing species from the Macaronesian region seem to be well differentiated from the other *Erysimum* species by morphological characters (woody subshrubs, purple petals) and unique Rubisco IEF patterns, thus supporting the separate status of taxa from the Macaronesian phytogeographical region.

Species delimitation in *Erysimum* is highly controversial. Numerous "species" have been described on the basis of minor variations in populations of previously recognized taxa (AL-SHEHBAZ 1988, BALL 1990). Identical IEF patterns found in so many *Erysimum* species would, therefore, support the view that the definition of species may be too narrow.

Table 1. Classification of IEF patterns of Rubisco small subunits (SSU) from *Erysimum* species under study. ⊖ = Cathode; ⊕ = Anode.

Taxon	Accession no.	Origin	IEF pattern of Rubisco SSU
E. capitatum Green	90-17-119-10	N. America	
E. concinnum Eastwood	90-17-118-10	N. America	
E. franciscanum Ros.	90-17-120-10	N. America	
E. insulare Green	90-18-012-10	N. America	
E. nivale Rybd.	90-10-076-50	N. America	
E. cheiranthoides L.	90-01-033-10	Eurasia	
E. decumbens Dennst.	90-18-011-10	Europe	
E. grandiflorum Desf.	90-18-041-10	Europe	
E. helveticum (Jacq.)DC.	88-13-053-10	Europe	
E. hieracifolium L.	90-17-101-10	Europe	A
E. hungaricum Zapal	90-18-075-10	Europe	
E. montesicola Jordan	90-18-048-10	Europe	
E. odoratum Ehrh.	86-34-007-10	Europe	
E. perofskianum Fi. & Mey.	88-35-034-10	Asia	
E. pulchellum (Willd.) Gay	88-11-103-70	Europe	
E. pumillum Gaudin	90-18-018-10	Europe	
E. sylvestre Scop.	88-05-102-70	Europe	
E. virgatum Roth.	90-18-046-10	Europe	
E. wahlenbergii Borbas	87-04-154-74	Europe	
E. witmannii Zawadzki	90-18-037-10	Europe	
E. cuspidatum DC.	86-12-086-10	Europe	
E. diffusum Ehrh.	90-18-019-10	Europe	
E. leptostylum DC.	90-01-010-70	Europe	B
E. microstylum Hauskn.	90-18-034-70	Europe	
E. senoneri Wettst.	90-18-019-10	Europe	
E. bicolor (Hornem.)DC.	90-18-030-10	Canary Islands	
E. carboverdianum Sund.	89-34-001-40	Cape Verde Islands	C
E. scoparium Brouss.	90-18-019-10	Canary Islands	D



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CROP BRASSICAS AND PHYTOREMEDIATION - A NOVEL ENVIRONMENTAL TECHNOLOGY

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We have coined the term "phytoremediation" to describe the use of plants to remediate soils and waters contaminated with inorganic and organic pollutants. Removal of toxic metals from soils is a particularly promising application of this novel technology (Raskin et al. 1994). Plants with an increased ability to concentrate metals in their shoots and roots have been collected from metalliferous soils throughout the world. Many of these metal-accumulating plants are members of the Brassicaceae family and are endemic to metalliferous soils (Baker & Brooks 1989). Some of the best metal accumulators belong to the genera *Thlaspi* and *Alyssum*. Unfortunately, the usefulness of these plants for metal remediation is limited by their low biomass, slow growth and lack of established agronomic practices (Baker et al. 1993). On the other hand, some of the crop species of the Brassicaceae are known for their high biomass and well established agronomic practices.

We have tested all the six crop species of the the genus *Brassica* for their ability to take up lead in a research green house under controlled environmental conditions. Ten day-old, uniform seedlings were transplanted into plastic pots containing a sand and perlite mixture (1:1 v/v). After seven days, plants were treated with 625 μg Pb/g DW substrate. Fourteen days after treatment plants were harvested, washed, oven dried and ashed at 500 ^{o}C. The ash was acid extracted and analyzed by direct current plasma spectrometer. The lead content of roots and shoots is presented in Table 1. Tested brassicas had almost similar amounts of lead in their roots, however, they varied greatly in their ability to transport the metal into the shoots. *B. juncea* had the highest and *B. oleracea* had the lowest capacity to concentrate metals in shoots. Currently, we are screening different cultivars of *B. juncea* for their ability to translocate lead into the shoots. We hypothesize that the AB genome is a better enhancer of the metal transport than the C genome alone or in combination with the A or B genome. In addition, we are trying to further improve the ability of *B. juncea* to grow on heavy metal containing soils through transfer of genes from other metallophytes belonging to the Brassicaceae. Conceivably, several sequential crops of laboratory-improved metal-accumulating plants could reduce soil concentrations of toxic metals to environmentally acceptable levels. *B. juncea* , a high biomass producing plant, is presently grown as an important oilseed crop in Asia and in Canada. As a

result of our work, *B. juncea* may also be used, some day, for soil remediation.

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Table 1. Lead uptake by different crop brassicas

Plant (Genome)	Tissue	ug Pb/g Dw tissue
Brassica juncea (AABB)	Shoot	18,812
	Root	91,666
B. carinata (BBCC)	Shoot	8,757
	Root	115,461
B. campestris (AA)	Shoot	6,941
	Root	100,354
B. napus (AACC)	Shoot	3,877
	Root	60,768
B. nigra (BB)	Shoot	2,489
	Root	110,011
B. oleracea (CC)	Shoot	1,416
	Root	51,399

Attempts of receiving yellow seeded Brassica napus recombinants as the result of interspecific crosses B.juncea (L.) Czern. et Coss.x B.carinata Braun

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Aiming at obtaining yellow seeded B.napus, 217 offsprings of the F_2 generation have been obtained as the result of crosses between two yellow seeded parental forms B.juncea (2n=36;AABB) x B.carinata (2n=34;BBCC). According to the expected segregation there should appear recombinants in following ratios: 4AABB(2n=36) : 4ABBB(2n=34) : 4AABC(2n=37) : 10ABBC(2n=35) : 4ABCC(2n=36) : 1AACC(2n=38) : 1BBBB(2n=32) : 4BBBC(2n=33) : 4BBCC(2n=34). Cytological investigations undertaken in F_3 generation, did not reveal, until now, any B.napus recombinant having 2n=38 and yellow seed coat. May be, that the idea of this crossing combination is based on wrong assumptions and therefore the expected result will never appear. It is possible, that the genomes A, B and C during their coexistence in alloploid species B.juncea and B.carinata have been so differentiated, that they are not able to create B.napus(2n=38;AACC), like the elementary species B.oleracea(2n=18;CC) and B.campestris(2n=20;AA) do. But we have still the hope - the somatic chromosome numbers ranging among 2n=32 to 2n=35 until now, did not switch off our expectations.

Backcross derivatives of *Brassica napus* x *Sinapis alba* hybrids

J. S. BIJRAL, T. R. SHARMA AND K. S. KANWAL

Sinapis alba L. (2n=2x=24) is reported to be highly resistant to nematodes (Grene *et al.*, 1988) and black spot. Besides being sufficiently rich in erucic acid which finds considerable technical use as a lubricant, plasticizer and anti-foam agent (Nieschlag & Wolff, 1971), this species is also visualized as a potential source of proteins in human diets (Kirk & Pylietis, 1976), and could be valuable for *Brassica napus* L. (2n=4x=38) cultivar improvement. While hybrids of *S. alba* with *B. napus* both through protoplast fusion and by sexual crossing via embryo rescue have been obtained (Primard *et al.*, 1988; Ripley and Arnison, 1990), production of sexual hybrids between the two species by conventional means is only a recent accomplishment (Bijral *et al.*, 1993). The *B. napus* x *S. alba* sexual hybrids were successfully crossed back to both parental species. The present communication, however, deals with the cytogenetics of first backcross derivatives of *B. napus* x *S. alba* interspecific hybrids to *B. napus*.

At metaphase I, the backcross-I derivatives showed a mean chromosom pairing relationship of 5.3 I, 12.8 II and 1.3 III per meiocyte. Higher associations (trivalents) were recorded in 90% of the cells analysed. Chromosome number of the BC_1 plants varied from 32 to 39 thereby suggestin that both hypo-and hyperploid female gametes made their contribution to zygote formation. A high frequency of bi- and trivalent associations reflects intergenomic affinity to a considerable extent. High seed fertility of BC_1 plants further suggests that useful genetic traits contai ed in *S. alba* can be stabilized in *B. napus* background by repeated select-ion and backcrossing.

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Brassica cosseneana x *Brassica carinata* hybrids

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Many wild cytodemes of the genus *Brassica*, including *Brassica cosseneana* Biess. & Reut. (2n=4x=32), are potential sources of genes conferring resistance to biotic and abiotic stresses. Whereas interspecific hybrids of *B. cosseneana* with *Brassica juncea* and *Brassica napus* have been realized and extensively studied (Harberd and McArthur, 1980), interspecific hybridization between *B. cosseneana* and *Brassica carinata* Braun. (2n=4x=34) has not been accomplished so far. The present communication reports the cytogenetics of the sexual hybrids between *B. cosseneana* and *B. carinata*.

At Metaphase I, the trigenomic amphihaploids showed a mean chromosome pairing relationship of 7.4 I +9.88 Ring II + 1.20 Rod II + 0.92 III + 0.12 IV per microsporocyte. Of the 26 cells analysed, higher associations (tri- and tetravalents) were recorded in 22 cells (84.6%). Some of the cells (19.2%) contained upto 2 trivalents. The presence of 11.08 (ring + rod) bivalents in conjunction with a high frequency of multivalent associations in 33-chromosome hybrids strongly suggests homoeologous pairing between the parental genomes, and consequently provides an effective way for successful transfer of economically important genes from *B. cosseneana* to *B. carinata* through meiotic recombination. Further, the development of *B. cosseneana* x *B. carinata* sexual hybrids also opens up the possibility of developing alloplasmic *B. carinata* with *B. cosseneana* cytoplasm.

Acknowledgement:

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GENETICS OF BASAL BRANCHING IN INTRA- AND INTER-SPECIFIC CROSSES OF *Brassica*

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Basal branching in mustard is a desirable trait to be bred for in Indian conditions. The presently grown varieties are tall with branches initiating upto 1 m above ground. Most of the branches are located in the top 30-40% of the plant height. Most of the seed yield is realised from siliquae in dense secondary and tertiary branches. The plants before maturity have a dense canopy with top heavy branches. Such branches of adjacent plants inter-twine due to the 30-45 cm between-row spacing adopted in India. Hailstorm/heavy winds usual during harvest (April-May) in most parts of North India cause heavy lodging of plants resulting in substantial yield losses.

It was found essential therefore to examine whether basal branching (defined as, at least one effective primary branch emanating upto a height of 30 cm from the base of the plant) can be associated with a compact plant frame with primary branches distributed uniformly through the height to result in a balanced plant canopy resisting lodging.

Three sets consisting of 6 crosses each were therefore made with parents whose branching status was earlier identified : [Branching : B - Basal; NB - Non-basal]

Female	B/NB	Male	B/NB
(*B. juncea*)		**Set 1** (*B. juncea*)	
Pusa Barani	B	NC 57347	B
		BDSM 7	B
YN 3	B		
DIRA 313	NB	**Set 2** (*B. napus*)	
		ISN 706	NB
		BO 54	B
		Set 3	
(*B. napus*)		(*B. campestris*)	
BEC 247	NB	NC 63647	B
BEC 266	NB	P Kalyani	B
BEC 273	NB		

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Partial sterility was observed in F_1. In Sets 1 and 2, the branching status of F_1 was the same as that of the maternal parent. In Set 3, four crosses followed this rule and were non-basal branching while two provided exceptions and were basal branching.

F_2 population sizes were variable depending upon the cross and the species to which the parents belonged (Table 1). As would be expected, the F_2 populations in intra-specific crosses were larger than in inter-specific crosses. The data indicated that basal branching was likely to be governed by two genes with epistasis in inter-specific crosses. Due to variable population sizes, distortion of segregation ratios from 9:7 to 10:6, from 13:3 to 12:4 and 14:2 and from 49:15 to 54:10 was observed. It was however possible to figure out the probable genetic constitution of the basal and non-basal parental genotypes based on the F_2 segregation data (Table 1). The results confirmed that basal branching is a quantitative trait and hence amenable for utilization in reconstructing desired plant ideotypes in Indian mustard.

Table 1 The genetics of basal branching in intra- and inter-specific crosses in *Brassica*

F_2 ratio fitted	No. of crosses	F_2 population size	Genotypes		Number of genes
			Basal	Nonbasal	
		B. juncea x *B. juncea*			
13 : 3	3	945	$B_1B_1B_2B_2$	$b_1b_1B_2B_2$	2
		2339	$B_1B_1b_2b_2$		
		1256	$b_1b_1b_2b_2$		
49 :15	3	1774	$B_1B_1B_2B_2B_3B_3$	$b_1b_1B_2B_2B_3B_3$	3
		1509	$B_1B_1b_2b_2b_3b_3$		
		1554	$b_1b_1b_2b_2b_3b_3$		
		B. juncea x *B. napus*			
13 : 3	3	132	$B_1B_1b_2b_2$	$B_1B_1B_2B_2$	2
		132		$b_1b_1B_2B_2$	
		84		$b_1b_1b_2b_2$	
9 : 7	3	114	$B_1B_1b_2b_2$	$B_1B_1B_2B_2$	2
		135	$b_1b_1B_2B_2$		
		135	$b_1b_1b_2b_2$		
		B. napus x *B. campestris*			
13 : 3	4	37	$B_1B_1B_2B_2$	$b_1b_1B_2B_2$	2
		110	$B_1B_1b_2b_2$		
		166	$b_1b_1b_2b_2$		
		204			
9 : 7	2	330	$B_1B_1B_2B_2$	$b_1b_1b_2b_2$	2
		760			

AN ASSAY OF BASAL BRANCHING F_2 SEGREGANTS FROM INTRA- AND INTER- SPECIFIC CROSSES IN *Brassica*

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Basal branching plant types with good productivity are desirable in *Brassica* under Indian conditions. Currently cultivated *B. juncea* varieties are tall with no branching even upto 1m above ground. The plants have weak stem supporting profuse siliquae-bearing branches at the top. The top heavy plants are prone to lodging during hailstorm usual before harvest in India resulting in heavy seed loss. It was therefore planned to assay the F_2 generation from intra- and inter-specific crosses for productive basal branching segregants.

The F_2 generation of three sets of crosses were studied during 1990-91. Six crosses made between 3 female parents of *B. juncea* and 2 male parents of *B. napus* constituted Set 1. Six crosses between 3 *B. napus* female and 2 *B. campestris* male parents provided Set 2. Six intra-specific crosses between 3 *B. juncea* female and 2 *B. juncea* male parents constituted Set 3.

Reasonably large F_2 populations of inter- and intra-specific crosses were evaluated for a number of yield components and seed yield. A multiple regression index using them was used to assign a selection score for each F_2 segregant. Only basal branching F_2 plants were used for this study. A basal branching plant was defined as one which had at least one effective primary branch upto a height of 30 cm from the ground level. Based on the selection scores, the plants were arranged in ascending order of magnitude giving a ranked F_2 distribution (RFD). The RFD was partitioned into four equal strata denoted by T1 to T4. The frequency of F_2 segregants and their average seed yield in various strata were used to analyse the potential of various crosses in producing basal branching but productive F_2 segregants.

The following significant results emanated from the analysis (Table 1).

1. The productivity of basal branching segregants from the inter-specific crosses, *B. juncea* x *B. napus* was as good as that from the intra-specific, *B. juncea* x *B. juncea* crosses. The study provided no reason why the alleged high sterility in F_1 should result in poor segregation in F_2.

2. Most of the productive segregants occurred in the top 25% - 50% of the ranked F_2 distribution. Taken along with comparable evidence from extensive studies on groundnut (Bandyopadhyay *et al.*, 1985), it would appear that selection in further generations need be confined to stratum T1 and when needed, T2.

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3. An analysis of variability for seed yield and related traits in the basal branching segregants provided substantial evidence that some of them were as productive as the best non-basal branching ones, removing the suspicion that basal branching would be associated with poor yield.

4. This study which examined both basal and non-basal branching genotypes in the species, *B. juncea*, *B. napus* and in inter- and intra-specific crosses using them provided sound evidence against the deficient inferences from previous studies (Bhargava and Tomar, 1982; Chauhan *et al.*, 1987) that basal branches in *B. juncea* were parasitic sinks and that plants with dormant basal nodes or uniculm plants would need to be developed.

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Table 1 Distribution of productive basal branching F_2 segregants in intra- and inter-specific crosses of *Brassica*

Set		Stratum	Mean values		
			PB	SY	HI
1	*B. juncea*	T1	6.7	46.2	28.4
	x	T2	9.8	28.4	16.0
	B. napus	T3	8.7	15.5	11.9
		T4	7.9	7.3	8.8
2	*B. napus*	T1	8.8	17.8	12.1
	x	T2	7.6	10.6	11.2
	B. campestris	T3	6.9	4.1	7.6
		T4	7.7	2.5	4.4
3	*B. juncea*	T1	7.3	49.7	28.9
	x	T2	6.5	33.7	27.2
	B. juncea	T3	6.3	25.3	24.9
		T4	5.9	17.9	21.5

PB : Primary branches; SY : Seed yield (g/plant);
HI : Harvest index (%)

INTER-GENERIC HYBRIDIZATION IN CRUCIFERAE

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Hybridization between **Brassica tournefortii** Gouan and **Raphanus caudatus** L. is now not a utopean phenomenon but a plausible possibility. Pollination and fertilization was carried out between these two genera at Agricultural Research Station, Sriganganagar in rabi, 1991-92. The experiment proved as surprising success but its reciprocal pollination did not prove as successful combination.

Seeds of F_1s scattered for growth in earthen pots which were kept in cage house. Plants of F_1s after growth attained the height of 92 cm. and had 10,760 flowering buds. Out of which some buds in their early stage fell down and rest 6000 flowering buds changed into full fledged flowers. Flowers of the F_1 s resembled with the flowers of **R. caudatus.** The plant was completely normal with male sterility and not at all deficient in chlorophyll. Each flower had four sepals, four petals, six sterile stamens and normal pistil which evidenced by normal fertilization with pollens of **B.tournefortii** and **B. napus.** Though, plant was male-sterile having some slender siliquae but without any seed. The length of siliquae was 3.7 cm.

Later, some seeds of F_1s were treated with colchicine (1%) and scattered for growth in a earthen pot in the month of february, 1993. The plant which grew, was of 55 cm. height. Twelve siliquae of 8 cm length were recorded and rest of the siliquae were smaller and seedless. Each siliquae had 5-6 seeds. Out of these seeds, only two seeds were found normal and rest were undeveloped. The resultant is expected to bring about a significant development which will surily and substantially improve the quality and raise productivity of oil seed corp.

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CREATION AND CHARACTERIZATION OF *Brassica napus- Diplotaxis erucoides* ADDITION LINES

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Alien addition lines provide informations on the genetic organization and relationships between genomes. In *Brassiceae* species, addition lines with *Brassica napus* (AACC, 2n=38) as genetic background and *B. nigra* (BB, 2n=16) chromosomes have been described (Jahier *et al*, 1989; Struss *et al*, 1991). The creation of *B. napus - Diplotaxis erucoides* (DeDe, 2n=14) addition lines is reported in the present paper. The comparison of the chromosomic map of *D. erucoides*, which is one of the species with the lowest chromosome number known in the tribe, n=7, to the one described for a species with n=8, *B. nigra* (This *et al*, 1990; Chèvre *et al*, 1991; Struss *et al*,1992) might provide informations on the genome evolution.

Manual crosses between *D.erucoides* (population collected in Spain) and a spring oilseed rape variety, RP1, followed by fertilized ovary culture allowed to produce F1 interspecific hybrids and their backcross progeny with *B. napus* (Delourme *et al*, 1989). One F1 hybrid (DeAC, 2n=26 = 12,87 I + 6.25 II + 0.12 III + 0.07 IV) and its two BC1 plants (AACCDe, 2n=45) were selected. The meiotic behavior of these two last plants is not close to the expected one because of the high frequency of cells with less than 7 univalents (32% of the cells) and with mutivalents (36% of the cells). After the third backcross with RP1, 8 plants with 19 bivalents and 1 or 2 univalents lines were obtained. From their selfing progeny, 15 monosomic (1 I + 19 II) and only one disomic (0.9 I + 19.22 II + 0.16 IV) addition lines were observed.

From the 9 isozyme systems studied, three genes (Pgm-1, Aco-1 and Idh-1) were localized on the same chromosome (fig. 1). For RAPD (random amplified polymorphic DNA) (Quiros *et al*, 1991) analyses, 29 primers, out of the 37 studied, revealed polymorphism. Nine of them allowed to characterize 17 loci distributed on 4 different chromosomes (fig. 1). On an other hand, the addition lines carrying the chromosome 1 were distinguishable by the light yellow color (l.ye.) of their flowers.

Figure 1 : Chromosomic map of *Diplotaxis erucoides*

Chromosome 1	Chromosome 2	Chromosome 3	Chromosome 4
Pgm-1			
Aco-1			
Idh-1			
l.ye.			
OPA01.1	OPA01.2	OPA05.1	
		OPA05.2	
	OPA08.1		OPA08.1
		OPA14.2	OPA14.1
OPB08.1	OPB13.1	OPB08.2	
		OPB18.1	
		OPB18.2	
OPC02.1	OPC02.1		
OPC02.2	OPC02.2		

By comparison of different addition lines from the same mother-plant, we sometimes observed that loci of the additionnal chromosome were missing. This result could be explained by deletions. On an other hand, the high frequency of multivalents during the different backcrosses could lead to recombinations between *D. erucoides* and *B. napus* genomes. For these reasons, it will be difficult to provide the complete set of addition lines. The possibility of allosyndesis between the genomes of the species used is in agreement with the hypothesis that *D. erucoides* might be the ancestor of the two progenitors of oilseed rape.

Few markers are common to the two chromosomic maps of *B.nigra* and *D. erucoides*. However one syntenic group was found with Pgm-1 and Aco-1 linked on *B. nigra* (22 cM, Chèvre com. pers.) and localized on the chromosome 3 of *B. nigra* (Chèvre *et al*, 1991) and on the chromosome 1 of *D. erucoides*. Comparisons with RAPD markers are in progress.

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Intergeneric hybridization between *Brassica juncea* and *Diplotaxis virgata*, and their cytology

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Wild relatives of the crop are often a valuable source of genes in crop improvement. It is thus essential to investigate the possibility of gene transfer from wild relatives of the crucifer to *Brassica* crops. Gene transfer from interspecific and intergeneric plants to *Brassica* crops were investigated in some hybrid progenies (Inomata, 1985a, 1991, 1992, 1993). For further enlargement of gene source of *Brassica* crops, intergeneric hybrids were produced between *Brassica juncea* and *Diplotaxis virgata* and the meiotic chromosome configurations were investigated.

The materials used in the experiment were *Brassica juncea* ($2n$=36) and *Diplotaxis virgata* ($2n$=18). Pollination was carried out by conventional method after emasculation of the flowers of *B. juncea*, and the ovary culture was made according to the previous papers (Inomata, 1985b, 1990). Chromosome configurations at the first meiotic division and the pollen fertility were examined by acetic carmine.

Ovary culture was carried out in 3 days after pollination in the cross of *B. juncea* × *D. virgata*. Three seeds were obtained in 73 ovaries cultured. All seeds germinated and grown up. Conventional pollination was made as a control of ovary culture and no seed was obtained. The chromosome numbers of all hybrids was 36 and the morphology of the leaf was similar to *B. juncea*.

Table 1 shows the results on the pollen fertility and chromosome configurations at the first meiotic division of the F_1 hybrids. Mean pollen fertility was 15.3%. Different types of division in microspore mother cell were observed in 1011 microspore mother cells and 84.1% of them was tetrad. Others were dyad, triad and pentad. The mode of chromosome pairing showed $11_{II}+14_{I}$ followed by $10_{II}+16_{I}$ and $12_{II}+12_{I}$, and reached 66.3%. The chromosome configurations for the PMCs were $(0-1)_{IV}+(0-2)_{III}+(7-12)_{II}+(12-20)_{I}$. The range of bivalent formation was from 8 to 12, with a mean of 10.3.

The hybrid with 36 chromosomes may consist of one genome of *B. juncea* and two genomes of *D. virgata*. The male gamete of *D. virgata* may diplodize soon before or after fertilization. If genome of *D. virgata* might be paired, maximum bivalent formation was 9_{II}. Number of maximum bivalents were 12 and it may be considered that there are some homology of the meiotic chromosomes between the genome of *B. juncea* and *D. virgata*. Number of bivalents in the F_1 hybrid of *D. virgata* × *B. juncea* with 27 chromosomes ranged from 1 to 8, with a mean of 5.0 (Harberd and McArthur, 1980). These F_1 hybrids may be useful for the breeding of *B. juncea*.

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Table 1. Pollen fertility and chromosome configuration at the first meiotic division of the F1 hybrids with 36 chromosomes in the cross of *Brassica juncea* × *Diplotaxis virgata*

Plant No.	Pollen fertility[1] (%)	First meiotic vision				
		No. of PMCs observed	$10_{II}+16_{I}$	$11_{II}+14_{I}$	$12_{II}+12_{I}$	Other types
1	19.7	36	7	11	6	12
2	14.8	30	12	4	3	11
3	11.4	32	2	11	9	10
Total or mean (%)	15.3	98	21 (21.4)	26 (26.5)	18 (18.4)	33 (33.7)

[1]: From 506 to 512 pollen grains were counted.

Hybridization of *Brassica tournefortii* and cultivated Brassicas

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Brassica tournefortii (2n=20; TT) is a wild species sometimes cultivated for oil. One of the cms systems in *B. juncea, B. napus* and *B. carinata* has the cytoplasm derived from *B. tournefortii* (1, 2). However lack of effective restorer lines prevents its exploitation (1). Hybrids between *B. tournefortii* and oilseed Brassicas are required for transfer of restorer genes and other characters (tolerance to aphids, leaf miner, *Alternaria* blight and salt stress) to oilseed Brassicas. The nature of incompatibility barriers in crosses of *B. tournefortii* with other *Brassica* species have not been studied before.

Our objective was to determine if the incompatibility barriers are pre-or post-fertilization and then obtain hybrids between *B. tournefortii* and four oilseed Brassicas (*B. juncea, B. napus, B. carinata* and *B. campestris* var. brown sarson) using appropriate methods. In all, 8 crosses were made, four using *B. tournefortii* as the female parent and four reciprocals. Pollen germination, pollen tube growth and its entry in ovules was studied. Thirty pistils were fixed 5 days after pollination in each cross and observed using Aniline Blue Fluorescence method.

In three crosses, *B. napus* x *B. tournefortii, B. carinata* x *B. tournefortii* and *B. campestris* x *B. tournefortii* there was either no pollen germination or the few pollen that germinated did not show pollen tube growth in the stigma or style. This indicates that here the incompatibility is pre-fertilization. Stump pollination (stigma excised) did not help pollen tube growth in these 3 crosses and the pistils degenerated 5-10 days after pollination. Next, irradiated mentor pollen technique (1000 Gy γ-rays) was used (3). It helped embryos develop normally in all the 3 crosses compared to the incompatible crosses where pistils abscised. However, embryo culture was necessary to obtain hybrids.

In the other five crosses pollen tubes entered ovules. This indicates that here the barriers are post-fertilization and ovary/ ovule culture would help in embryo rescue.

In all the 8 crosses (5 where fertilization was normally seen and 3 where irradiated mentor pollen helped fertilization) the stage of embryo growth was studied 5, 15 and 30 days after pollination. Pollinated pistils were cultured 6-10 days after pollination. The following hybrid plants were obtained after ovary/ovule culture in MS + BAP (0.5 mg/l) medium:

B. tournefortii x *B. campestris*

B. tournefortii x *B. juncea*

B. juncea x *B. tournefortii*

The leaf morphology of the hybrids was intermediate between the two parents. These plantlets have been multiplied using shoot meristem/axillary bud culture in MS + BAP (0.5 mg/l) medium. The true hybrid nature was also established by esterase and peroxidase analysis of leaf samples of parents and hybrids. The hybrid plants are also being screened for fertility restoration and tolerance to *Alternaria* blight, white rust, black leg and water and salt stress.

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Hybridization of *Diplotaxis* and *Erucastrum* with crop Brassicas

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Intergeneric hybridization has been used in crop breeding for gene transfer, to investigate species relationships and to provide new species and alien cytoplasm lines. There is a need to develop diverse cms lines in *Brassica*. *Diplotaxis* and *Erucastrum* are genera closely related to *Brassica*. Yet, only a few hybrids have been reported between these two genera and *Brassica*[1,2,3] and of these only *D. muralis* has provided a cms system[4].

We studied the crossability relationship of three wild species *D. siettiana* (2n=16), *D. tenuisiliqua* (2n=18) and *E. abyssinicum* (2n=32) when used as female parent in crosses with six cultivated species of *Brassica, B. campestris, B. nigra, B. oleracea, B. juncea, B. napus* and *B. carinata*. We report here our studies on pollen germination, pollen tube growth and its entry into ovules and growth of embryo in these 18 crosses to decided whether the barriers are pre-fertilization or post-fertilization. The following three hybrids designated EJ, FO and DC respectively are reported for the first time:

E. abyssunicum x *B. juncea*

E. abyssinicum x *B. oleracea* var. alboglabra

D. tenuisiliqua x *B. campestris*

Forty pistils from each cross were studied using the Aniline Blue Fluorescence method. Pollen germination, pollen tube growth and percent ovule fertilization was observed 5 days after pollination (dap).

Strong pre-fertilization barriers were found when *D. siettiana* was used as the female parent in crosses with *B. campestris, B. nigra, B. oleracea, B. juncea* and *B. carinata* and in the cross *D. tenuisiliqua* x *B. carinata*. In these 6 crosses pollen tubes did not grow beyond the stigma. In other 12 cross combinations barriers were presumably post-fertilization i.e. pollen tubes were seen entering micropyles. The per cent ovule fertilization ranged from 0.02% in *D. siettiana* x *B. napus* to 24.8% in *E. abyssinicum* x *B. oleracea*. For the cross showing pre fertilization barriers, techniques such as bud pollination, stump pollination, mentor pollen are being used to overcome such barriers On the other hand for the crosses showing post fertilization barriers, techniques such as ovary/ovule culture are being used to rescue embryos.

Irradiated mentor pollen technique was used in the cross *D. siettiana* x *B. juncea*, var. Pusa Bold to overcome per-fertilization barriers. Four sets of pistils were pollinated (50 in each sets). Three controls (C_1, C_2, C_3) and one experimental (E). C_1: pistils pollinated with normal *D. siettiana* pollen, C_2: pistils pollinated with irradiated *D. siettiana* pollen (mentor pollen), C_3: pistils pollinated with normal *B. juncea* pollen, E: pistils pollinated with irradiated *D. siettiana* pollen followed by normal *B. juncea* pollen after 30 min.

D. siettiana pollen was used as mentor pollen (irradiated with 1000 Gy dose of γ rays from a Co 60 source). Twenty five pistils in each set were fixed at 1 dap and 5 dap, hydrolyzed and observed with Aniline Blue fluorescence technique. In addition 25 pistils in each set were fixed at 10 dap and squashed in 2% acetocarmine to observe the presence of embryos. At 5 dap, 17% ovules showed entry of pollen tubes in E. However no pollen tubes entered the ovules in C_3. No embryos were observed in control (C_2 & C_3) pollinations but in E at 10 dap 27% ovules showed small globular embryos which grew no further. This suggests that irradiated mentor pollen brings about fertilization in this cross and hybrids can be obtained if embryos are rescued.

Of the 12 crosses showing pollen tube entry in ovules, 3 crosses were selected for ovary/ovule culture to overcome post fertilization barriers. Ovary culture followed by ovule culture was used. The crosses were *E. abyssinicum* x *B. juncea* showing 16.57% ovules fertilized, *E. abyssinicum* x *B. oleracea* showing 24.80% ovules fertilized and *D. tenuisiliqua* x *B. campestris* showing 0.06% ovules fertilized. Ovaries at 10 dap were cultured in MS medium + casein hydrolysate (400 mg/l). After 15-20 days of culture ovules were dissected out and cultured on MS medium + sucrose (5%), Kinetin (1 mg/l) and CH (400 mg/l). In the cross *E. abyssinicum* x *B. juncea*, 121 ovaries were cultured and 4.1% of cultured ovules germinated and 2 hybrids were raised up to maturity. In the cross *E. abyssinicum* x *B. oleracea*, 96 ovaries were cultured, and 86.4% of cultured ovules germinated and 3 hybrids were raised up to maturity. Seedling obtained in both crosses were multiplied by culturing shoot tips and nodal segments on MS medium + BAP (0.5 mg/l). Rooting was induced on MS medium + NAA (0.1 mg/l). Mature hybrids flowered inside the culture tubes and pods were formed under continuous light. EJ hybrids had yellow flowers, and EO hybrids had white flowers. There was no seed set in either cross. The hybrid nature was confirmed morphologically, biochemically and cytologically.

Esterase and peroxidase isozymes were studied in the hybrids and parents using starch gel electrophoresis. Bands unique to both parents were observed in the hybrids thus confirming their true hybrid nature.

For cytological studies flower buds were fixed in Carnoy's solution (1 part acetic acid : 3 part Chloroform : 6 part ethanol) and anthers were squashed in 2% acetocarmine. Pollen fertility was studied using acetocarmine staining. EJ hybrid had 34 chromosomes, the commonest association of chromosomes being 1 IV + 12 II + 6 I. Pollen fertility was 10.94%. EO hybrids, on the otherhand had 25 chromosomes, the commonest association of chromosomes being 1 IV + 7 II + 7 I. Pollen fertility was 6.5%. Presence of 12 II and 7 II in most of cells in the EJ and EO hybrids respectively indicates the existence of homology between these two genera. We are attempting back crosses with *B. juncea* to obtain seed set in EJ.

In the third cross *D. tenuisiliqua* x *B. campestris* 2 hybrids were obtained from 40 ovaries cultured. The leaves of these hybrids were intermediate in morphology compared to both the parents. However they did not flower. Esterase isozyme bands confirmed that they were hybrids.

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LIGHT SEEDED FORM OF SYNTHETIC *BRASSICA NAPUS* L.

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In the course of the crossing programme of elementary *Brassica* species to obtain synthetic *B.napus* forms with the *B.oleracea* cytoplasm, a hybrid from the combination *B.oleracea* var. *sabauda* × *B.campestris* ssp. *oleifera* was obtained. In F_2 generation individuals with light-coloured testa were found.

Material and methods

Savoy cabbage - *B.oleifera* var. *sabauda* cv."Predzvest" (2n=18) was used as a maternal form, whereas a paternal component was the winter form of turnip rape - *B.campestris* ssp. *oleifera* cv."Tobin" (2n=20). Crossing was performed under greenhouse conditions by pollinating emasculated flower buds and isolating them with aluminium foil tubes. Plants of F_1 and F_2 generations were analysed for the somatic chromosome number. Root tip meristems were fixed in AA fixative after previous chromosome shortening in icy-water. Squashes were prepared by orcein method. Pollen grains viability (male fertility) was determined by staining them in a mixture of acetocarmine and glycerol. Seed colour was estimated on attaining full maturity. With reference to the testa colour, three groups of seeds were distinguished: 1) light, including yellow seeds, yellow with a darker hilum and brown seeds, 2) mixed-brown, containing besides typically brown seeds also those with a spotted testa having lighter-stained sectors, 3) black.

Results

650 ovaries were pollinated and only 252 of them (38.8%) set siliques. Out of the total number of pollinated ovaries 127 were used for *in vitro* culture. 125 siliques remained on the mother plants were found to have five brown-coloured seeds, which gave rise to five plants of F_1 generation (Table 1). One of these plants was a completely sterile amphihaploid (2n=19). Of the remaining four plants, three were spontaneous amphidiploids (2n=38) with pollen grains viability from 39% to 63%, and one plant had the chromosome number 2n=28. Results of observations on the testa colour segregation are presented in Table 2. Among 31 seeds obtained none had a black testa.

In F_2 generation, containing 17 individuals, spontaneous amphidiploids (2n=38) were dominant. Three plants had 2n=28 chromosomes, one was an amphihaploid (2n=19). There were two plants, in which we failed to determine the chromosome number. Pollen grains viability in F_2 generation ranged from 0 to 93% (mean 55.7%). Seed setting was poor, which was caused by anomalies in the structure of both male and female generative organs. Among 17 plants analysed only one set seeds with a black testa.

In F_3 generation only male fertility was studied. It ranged from 15% to 83.3% (mean 54.2%). Two out of 35 plants analysed were completely sterile. 23 plants set seeds. Like in F_2 generation, numerous anomalies in the structure of generative organs were observed, which made fertilization difficult or even impossible despite high pollen fertility. The number of individuals with light and black testa increased. The number of plants with mixed-coloured and brown seeds remained on the unvariably high level.

At the present time, further selection is being conducted in the direction of stabilization of light-seeded forms with the *B.oleracea* cytoplasm, which represent a supplement to the light-seeded forms of synthetic *B.napus* with the *B.campestris* cytoplasm in our possession.

Table 1. Somatic chromosome number and pollen grains viability in F_1, F_2 and F_3 generations of *B. napus* synthetic form

Generation	No. of plants obtained	Somatic chromosome number (2n)			Mean pollen grains viability (%)
		19	28	38	
F_1	5	1	1	3	37.4
F_2	17	1	3	11	55.7
F_3	35	-	-	-	54.2

Table 2. Segregation of testa colour in F_1, F_2 and F_3 generations of *B. napus* synthetic form

Generation	No. of plants analysed	No. of infertile plants	No. of plants with seed colour		
			light	mixed-brown	black
F_1	5	1	2	2	-
F_2	17	1	3	12	1
F_3	35	12	8	13	2

Mycogen Plant Sciences Brassica RFLP Linkage Maps

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Genetic linkage maps have been constructed for the three Brassica species *B. napus*, *B. rapa*, and *B. oleracea* based on RFLP markers detected by the same set of Brassica DNA clones. This set consists of 380 nonrepetitive clones from a PstI genomic library of the commercial *B. napus* cultivar 'Westar'. Using the methylation sensitive restriction enzyme PstI for cloning excluded most of the repetitive sequences in the Brassica genome. Further screening was done by colony hybridization with labeled total genomic DNA to eliminate highly repetitive and organellar sequences, as well as by hybridization to DNA samples of each species for clear and simple banding patterns.

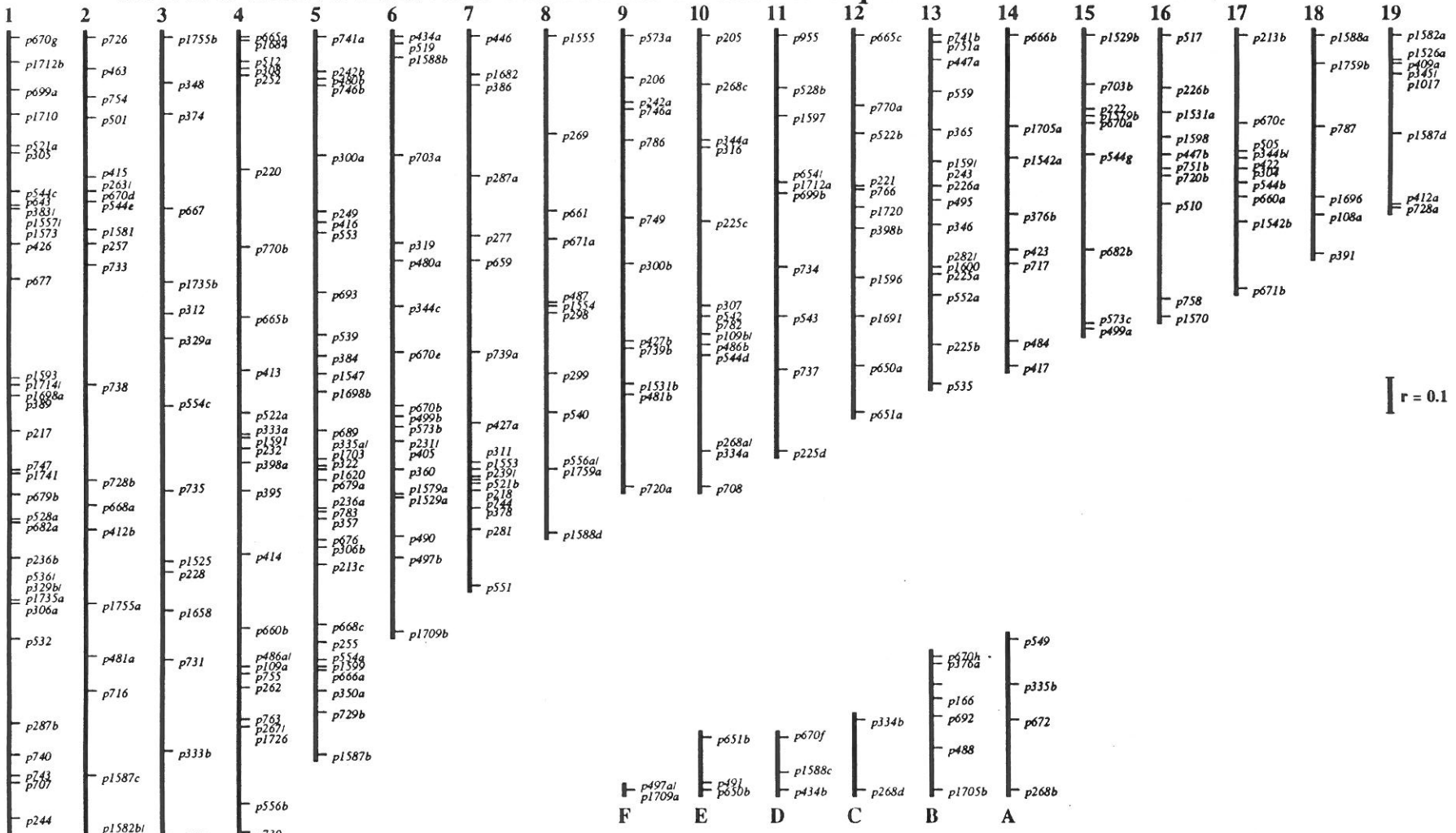
The following table summarizes our results on the three aforementioned Brassica species using this probe set. Due to our internal emphasis on oilseed crops, the cole crops were included at a later stage and hence the lower number of mapped markers. About 70% of the probes tested have been polymorphic for *B. oleracea*, and we expect a similar number of informative clones to be identified for *B. oleracea* as for *B. rapa*. The linkage maps for *B. napus* and *B. oleracea* are shown in the following pages, and the *B. rapa* linkage map can be found in Genome (35:746-757, 1992).

Linkage Map Statistics for *B. rapa* (BR), *B. napus* (BN), and *B. oleracea* (BO)

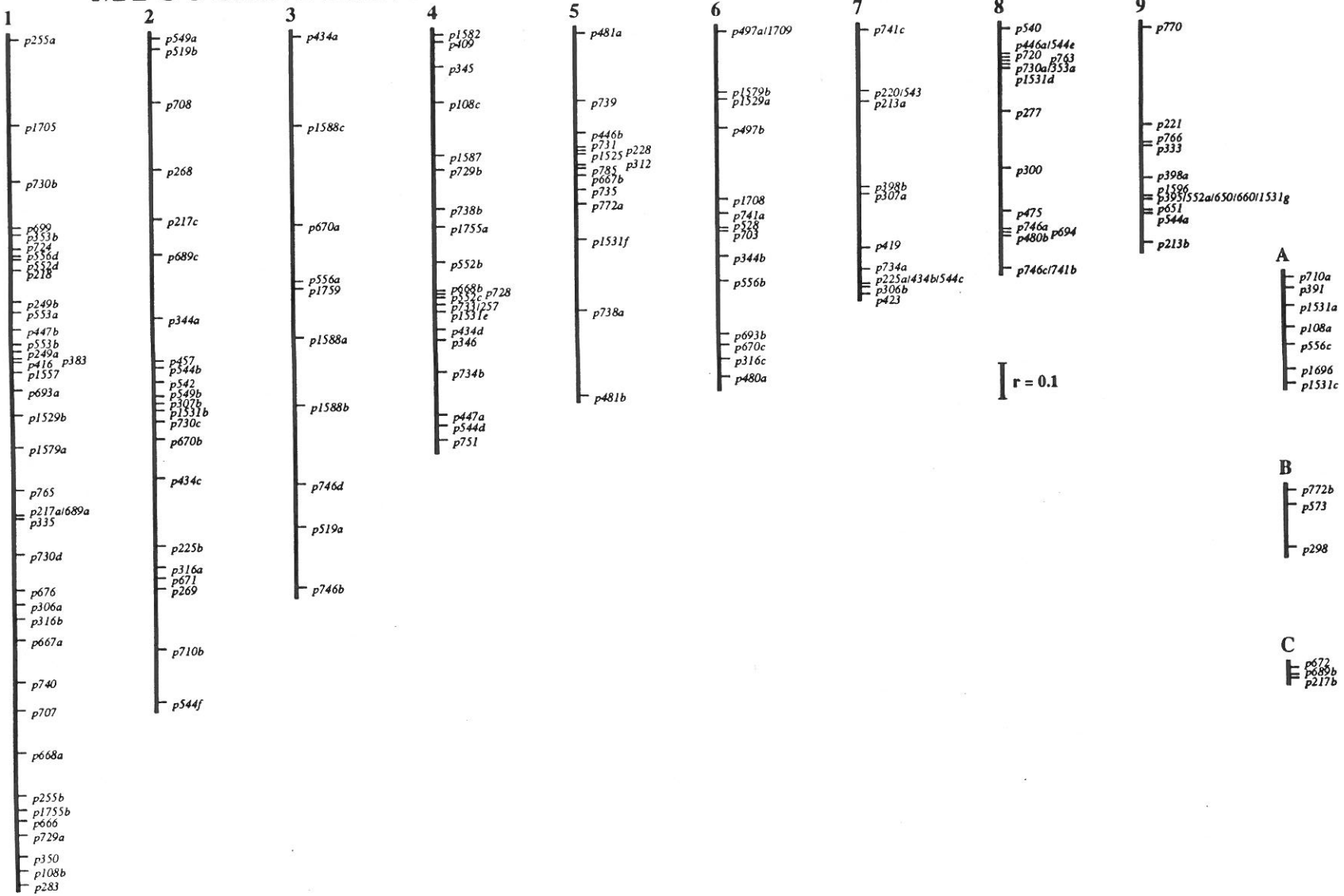
Genome	BR	BN	BO
Mapping cross	'Horizon' x 'R500'	'Regent' x 'SI271'	'Prime Crop' x 'Snowball'
Mapping generation	F_2	BC_1	F_2
Population size (# of plants)	104	105	105
Map size	1876 mu	2602 mu	1023 mu
Linkage groups	10	19+5*	9+3*
Number of clones tested	380	380	136
Number (%) of polymorphic clones	269 (70.8%)	225 (59.2%)	96 (70.6%)
Markers mapped	360	309	142
Markers unmapped	0	14	8

*unassigned linkage segments.

MYCOGEN PLANT SCIENCES *Brassica napus* RFLP LINKAGE MAP



MYCOGEN PLANT SCIENCES *Brassica oleracea* RFLP LINKAGE MAP



Chromosome Locations of the *SI* Gene in Three Brassica Species

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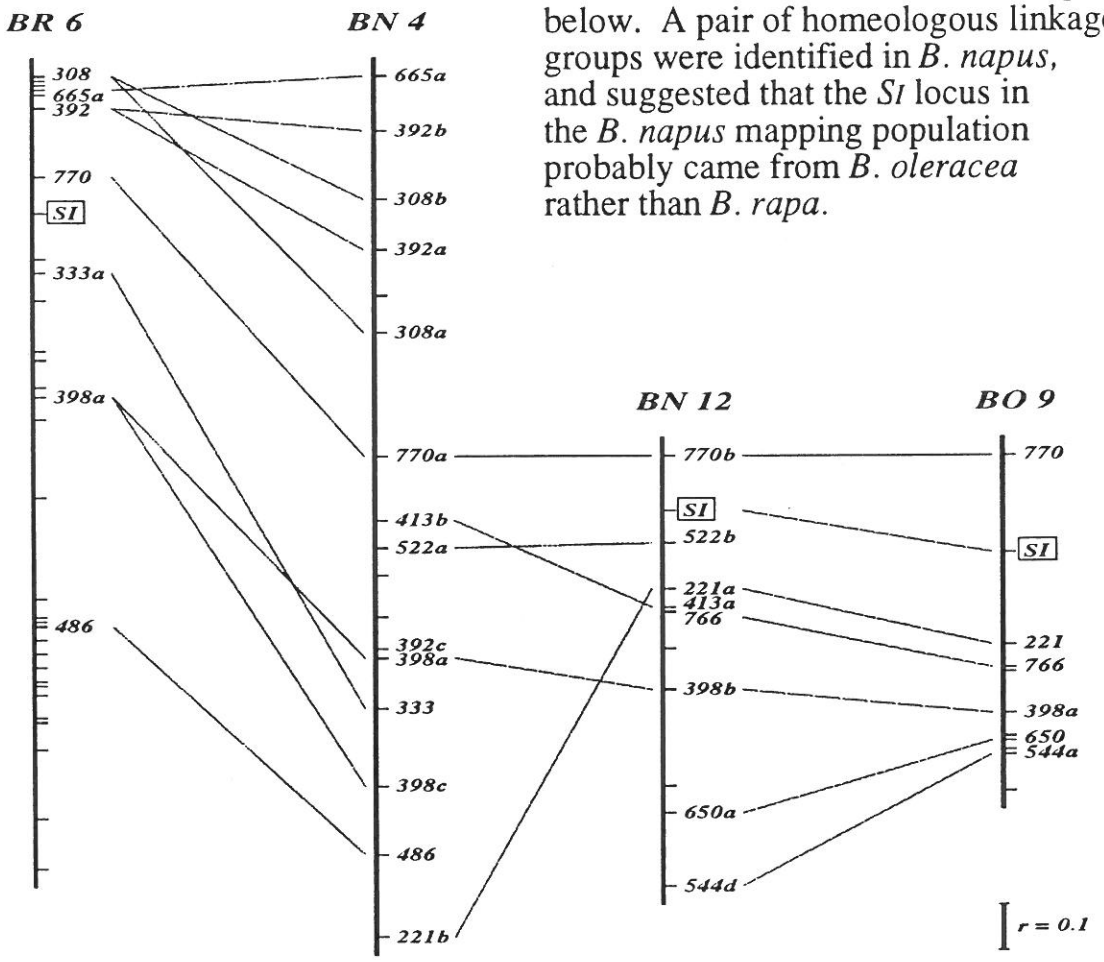
Locations of the self-incompability loci have been determined through linkage relationship with RFLP markers in *Brassica rapa* ('Horizon' x 'R500'), *B. napus* ('Regent' x SI271), and *B. oleracea* ('Prime Crop' x 'Snowball'), using segregating populations numbered over 100 in each species. The self-incompatible response was scored by both microscopic observation of pollen tube growth (Kho & Baer, 1968, Euphytica 17:298-302) and final seed set. The scores were then confirmed with PCR amplification of the *SI* sequence (Guilluy et al., 1991, Theor Appl Genet 82:466-472), followed by restriction digestion to reveal polymorphism. As expected, the linkage groups containing the *SI* locus showed a great deal of homoeology among the three species, as seen in the highly conserved linkage arrangement of common RFLP markers in the figure below. A pair of homeologous linkage groups were identified in *B. napus*, and suggested that the *SI* locus in the *B. napus* mapping population probably came from *B. oleracea* rather than *B. rapa*.



Location of *SI* locus in *B. rapa (BR)*, *B. napus (BN)*, and *B. oleracea (BO)*

TAGGING OF A GENE DETERMINING LINOLENIC ACID CONCENTRATION IN RAPESEED WITH DNA-BASED MARKERS

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High linolenic acid (LA) concentration is an undesirable trait affecting the flavor and quality of rapeseed oil (Galliard 1980). Therefore, lowering the LA concentration is an important objective for rapeseed quality improvement. Our goal is to tag the genes responsible for low LA concentration in *B. napus* with DNA-based markers. These markers will be used in rapeseed improvement to increase selection efficiency and in plant molecular biology to investigate the organization and regulation of these genes. We report here the tagging of a gene governing LA concentration in a *B. napus* .

MATERIALS AND METHODS: 132 F_3 families derived from the cross between a German rapeseed cultivar, 'Duplo' (10.4% LA), and a low LA line, 3637-1, were used in this project. LA concentration was determined on 132 plants from the F_2 populations grown in Germany, following the method of Thies (1971). Seeds from each individual F_2 plant of these populations were grown at Davis, California, for the gene mapping experiments. DNA extraction, RAPD and RFLP were carried out following the published protocols (Kianian and Quiros 1992 and Hu and Quiros 1991)

RESULTS AND DISCUSSION

SEGREGATION OF LA CONCENTRATION IN DUPLO X 3637-1 F_2 POPULATIONS The LA concentration for 'Duplo', 3637-1 and the F_1 hybrid were 10.4%, 2.2% and 4.7%, respectively. In the F_2 LA concentration exhibited a slightly skewed continuous distribution, thus making impossible discrete classification of the individuals. Maternal effect, partial dominant action of the genes involved, and environmental influence may be responsible for the deviation between F2 and the mid-parent value (6.3%) and the F_2 skewness. We concluded that LA concentration was mainly controlled by nuclear genes since the F_2 mean (6.2%) was practically identical to the mid-parent value (6.3%). This is in agreement with previous reports (Brunklaus-Jung and Robbelen 1987; Diepenbrock and Wilson 1987; Pleines and Friedt 1989; Chen and Bevesdorf 1990).

IDENTIFICATION OF MARKERS ASSOCIATED WITH LA CONCENTRATION Preliminary screening with RAPD primers and RFLP probes revealed very low level of polymorphism between the parents. Among 38 DNA probes tested, only 4 detected polymorphic bands. Forty RAPD primers produced 19 informative markers. Upon this observation, we adapted the trait-based approach (Lebowitz et al 1987) to tag the genes determining LA concentration. Twelve F_3 families at each end of the distribution (12 lowest, 2.1-4.4%, and 12 highest, 8.1-10.3%, LA concentration), two parental lines and the F_1 were used in the screening with the RAPD primers previously tested as polymorphic between the parents. Eighty-one out of the 82 markers amplified with 71 primers did not show difference in frequency between high and low LA groups. One markers, K01-1100, was present in 11 of the 12 high LA families and only in 5 of the 12 low LA families, indicating that it could be associated to LA concentration.

SEGREGATION OF THE RAPD MARKERS IN THE POPULATION AND THE ASSOCIATION BETWEEN K01-1100 AND LA CONCENTRATION A total of 19 RAPD primers disclosing 30 markers were tested for segregation in the 132 families representing the F_2 population. Twenty-four markers fit the expected 3 : 1 ratio and 6 deviated from expected Mendelian segregation. A significant regression (Haley and Knott,

1992)was found between the presence of the K01-1100 and high LA concentration (r=0.357***). The values of the regression coefficient between LA concentration and other markers ranged from -0.144 to 0.136, all below the significant level. Based on the presence or absence of K01-1100 the population could be divided into two groups. The individuals of homozygote recessive genotype (lacking K01-1100 band) occupied the tail of low LA concentration of the distribution. Comparison of the LA means of the two groups (101 plants with K01-1100, 6.5%; and 31 plants without K01-1100, 5.2%) revealed a statistically significant difference (t=4.06, $p < 0.001$). The variance in LA concentration within this genotype suggests the involvement of other genes and environmental effects on this trait. However, the association of K01-1100 to LA concentration is clear, indicating that one of the genes governing LA is tagged by this marker. We estimated that this marker accounts for about 12.8% of the genetic variation of LA concentration in this population.

RFLP ANALYSIS OF K01-1100 When K01-1100 was used as a probe, it detected two bands (6.5 and 7.5 kb) segregating in 1: 2:1 ratio (22:57:18, $x2$= 3.3, and $p > 0.1$). Thus 3 genotypes were recognized. The LA concentration means for each genotype were 4.8% (low LA parent, homozygote for 6.5 kb band), 6.4% (heterozygous), and 7.5% (high LA parent, homozygote for 7.5 kb band), respectively. However, there was enough variation in each of the three genotypes for LA concentration to result in the slight overlapping of the two homozygous genotypes and the wide range of the heterozygotes. Using the regression model proposed by Haley and Knott (1992) we estimated that this marker accounts for about 26.5% of the genetic variation of LA concentration in this population. The rest of the variation could be attributed to environmental effects, the interaction between the genotypes of the embryo and the maternal plant, and possible other genes governing LA concentration. In any case, the fact that the two tails of the F2 distribution are occupied by the homozygous individuals of the two parental genetypes confirms that K01-1100 is cosegregating with a major gene determining LA concentration. Since no linkage was detected between K01-1100 and the 80 mapped markers, we are adding markers to that segregating population. The K01-1100 fragment has been cloned into pCR II vector.

CONCLUSION

Our results clearly demonstrate that combining trait-based approach and RAPD technique can be used for tagging genes of agronomic importance. LA synthesis involves sequential enzymatic desaturation of oleic acid. Therefore, the genetic determination of LA concentration in *B. napus* is expected to be complex, especially because of its allotetraploid nature. Since 26.5% of the total variation for LA concentration in this population could be attributed to RFLP marker K01-1100, we believe that marker K01-1100 is associated with a major gene determining this trait. K01-1100 could be useful in marker-aided selection for identification of low LA genotypes among the populations derived from mutant 3637-1 in rapeseed breeding programs. It also could be used as a start point to isolate the genes responsible for low LA concentration and genetically manipulating this trait.

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RELATIONSHIPS AMONG CHINESE VEGETABLE BRASSICAS USING RAPD MARKERS

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Brassica rapa ssp. *pekinensis* (Lour.) Olsson (Chinese cabbage) is the most important vegetable crop in China. The leaves of the plants are usually eaten fresh, although they are occasionally preserved by fermentation. Another member of the genus, *Brassica juncea* (L.) Czern. (mustard), is also a popular vegetable in China. Stems, roots, and leaves of its various cultivars are eaten pickled, and in contrast to Chinese cabbage, are rarely consumed fresh. Because of the great importance, wide-ranging utility, and highly diverse morphologies of *B. rapa* and *B. juncea*, conservation of their germplasm is critical. To effectively and efficiently acquire and maintain material of these two species, genetic markers are needed that not only distinguish individuals and accessions, but also reflect the diversity and relationships among collection holdings (Kresovich and McFerson, 1992). The purpose of this study was to use random amplified polymorphic DNA (RAPD) markers to determine identity, variation, and relationships between accessions of these important species.

Fifty-two germplasm accessions of Chinese vegetable brassicas were analyzed for 112 random amplified polymorphic DNA (RAPD) markers generated by 5 primers (Table 1). The array of material examined spanned a range of morphological, geographic, and genetic diversity, and included 30 accessions of *Brassica rapa* L. (Chinese cabbage, pakchoi, turnip, broccoletto), 18 accessions of *B. juncea* (L.) Czern. (leaf, stem, and root mustards), and 4 accessions of *B. oleracea* ssp. *alboglabra* L.H. Bailey (Chinese kale).

All 52 accessions could be unambiguously identified using the RAPD markers that were found, since each accession could be distinguished from every other based on the presence/absence of a distinctive set of bands. In addition, using the presence/absence data, Nei and Li (1979) genetic similarities were computed and UPGMA cluster analyses were performed. In the analysis of accessions (Figure 1) both accessions and subspecies clustered into groups corresponding to species, but some accessions of some subspecies clustered most closely with accessions belonging to other subspecies. This was not unexpected in an outcrossing group of organisms where, historically, intentional and inadvertent crossing between subspecies has occurred. Such intraspecific hybridization also helps to account for the observed lack of correlation between geographic origin, genetic similarities and morphology that was found among these plants. Similar results were obtained from an isozyme diversity study of *B. oleracea* L. (Lamboy et al., in press).

Although no set of RAPD markers was discovered that distinguished between subspecies within species, fourteen RAPD markers were useful in unambiguously distinguishing between the species themselves (Table 2). Although RAPD markers are not usually used for identifications at the species level, in particularly difficult genera, such as *Brassica*, or in other groups, such as woody plants where characters needed for correct identification depend upon mature fruiting or flowering specimens, use of RAPD markers for identification can both save time and increase reliability. We have found that RAPD markers provide a fast, efficient method for identification at one or more taxonomic ranks, for assessing between and within accession diversity, and for determining genetic relationships in a genetic resources collection.

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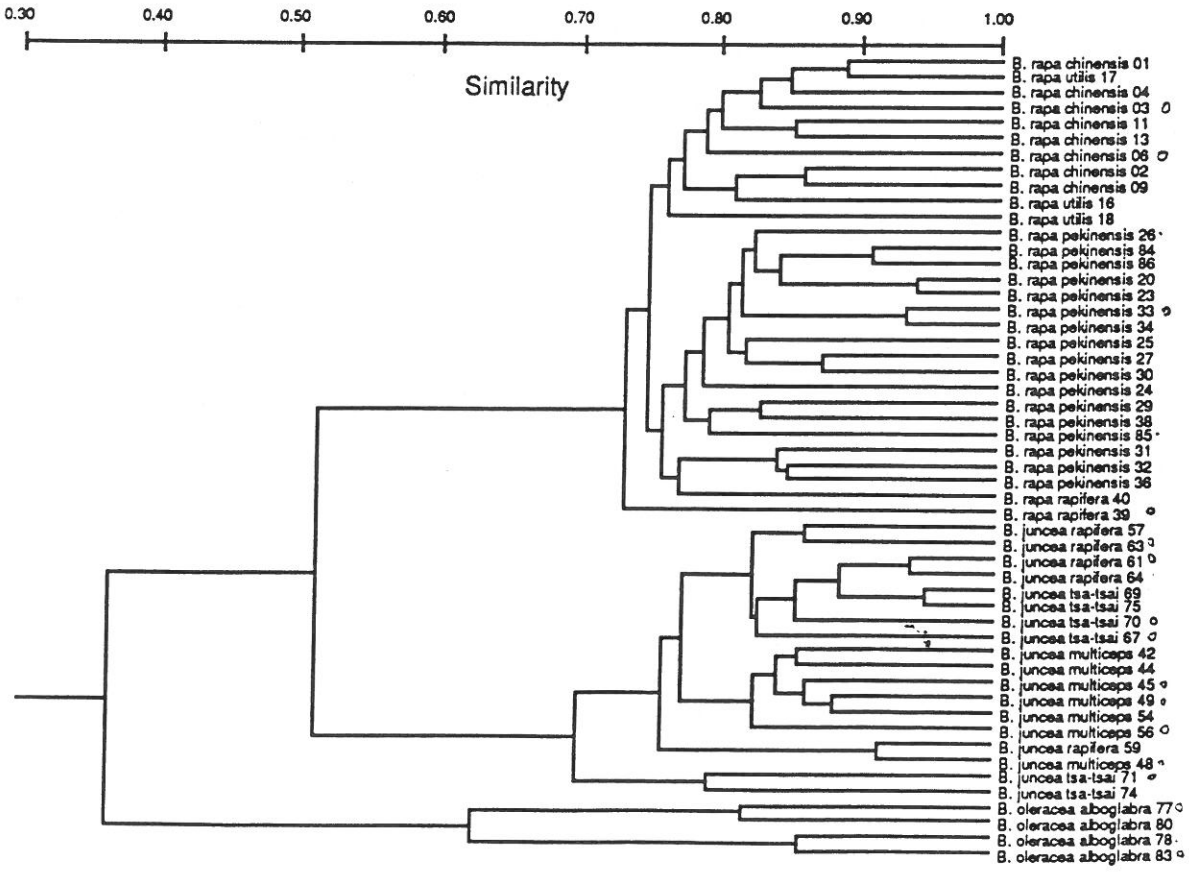
Table 1. Decamer oligonucleotides utilized as random primers.

Primer ID	Sequence (5' to 3')	Number of Polymorphic Fragments	Band Numbers	Approximate size range
OPX-04	CCGCTACCGA	24	1-24	250-1650
OPX-06	ACGCCAGAGG	22	25-46	250-3200
OPX-07	GAGCGAGGCT	21	47-67	200-1550
OPX-16	CTCTGTTCGG	22	68-89	350-1650
OPX-17	GACACGGACC	23	90-112	300-3150

Table 2. RAPD markers that are diagnostic for the three species combination distinguish each species from the other two. 0 = band is never present in the species, 1 = band is always present in the species, * = band is sometimes present and sometimes absent in the species.

Species \ Band #	11	12	18	36	50	56	65	69	91	92	101	104	108	109
B. rapa	*	0	1	1	*	*	1	1	*	*	1	*	0	0
B. juncea	1	0	*	1	1	1	1	0	0	0	1	0	1	*
B. oleracea	0	1	0	0	0	0	0	0	1	1	0	1	0	1

Figure 1. UPGMA cluster analysis using Nei and Li similarity coefficients. Accession id numbers follow the subspecies names.



ASSESSING GENETIC IDENTITY AND RELATEDNESS IN CABBAGE WITH RAPDs

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Genebank curators need better information on genetic identity, relatedness, and structure among accessions in their collections. Molecular genetic markers offers a powerful tool to provide this information and thereby improve collection organization and management. We examined the applicability of the RAPD assay for quick, cost-effective, and reliable use in addressing the needs of genebank curators and potential users of these valuable resources. To establish measures of genetic identity and relatedness, fourteen closely related *Brassica oleracea* var. *capitata* L. 'Golden Acre' (cabbage) accessions were screened with nine decamer oligonucleotide primers. We generated 110 reproducible fragments. Eighty were polymorphic, ranging in size from 370-1720 bp and were sufficient to uniquely distinguish all fourteen accessions. A cluster analysis of genetic similarities reflected pedigree relationships, with one exception. In complement, we investigated the impact of various sampling strategies to reduce assay costs without sacrificing the quality or quantity of the information obtained.

Our results suggested the RAPD technique is a reliable, cost effective, powerful tool applicable to plant genetic resource conservation. The RAPD technique was able to assess genetic identity, relationships, and structure among very closely related cabbage genotypes. While our interest focuses on management of ex situ germplasm collections, a similar approach could be used to assess to natural populations (Chalmers et al. 1992). The results of sampling strategy experiments suggested a four-plant bulked tissue sample was sufficient to give an accurate DNA representation of individual cabbage accessions. This efficiency allows the large scale screening necessary to identify and deal with identical or redundant accessions. Accessions with high levels of similarity could be bulked or eliminated from the collection. This could allow curators to focus on the collection of new and valuable resources or lesser known species (Strauss et al. 1988).

Given limited resources, curators must consider reducing the overall size of plant genetic resource collections. Bulking genetically similar accessions and eliminating duplicated accessions allows this. Properly executed, selective bulking of genetically similar accessions retains the major amount of observed variation, while maintaining the greatest differences among the populations. Rumbaugh et al. (1988) characterized and evaluated 146 newly acquired alfalfa accessions. By utilizing discriminant statistical analysis of taxonomic and agronomic data the authors were able to pool the 146 accessions into just five populations. Based on our RAPD data and bulking samples with genetic identities <0.950, the fourteen 'Golden Acre' accessions in our study would be reduced to three bulked accessions. Though these accessions were selected for a particular trait or phenotype, it should be possible to represent those specific alleles in the bulked sample. When deciding to bulk accessions, a curator should use as many types of information as possible. In addition to molecular data, other sources of information should be employed, including: pedigree, agronomic/horticultural performance, pest resistance, etc.

Large scale assessment of genetic diversity, whether in natural populations in situ or collections maintained ex situ, would be greatly enhanced with the application of quick and easy molecular techniques such as RAPD. The RAPD technique can be utilized to select priority areas for conservation, to design and monitor management strategies in a genebank, and to identify duplicated or redundant accessions in the collection. Through the integration of molecular techniques with classical curatorial activities, curators will enhance their ability to increase the value of plant genetic resources for conservation and utilization.

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GENETIC AND PHYSICAL MAPPING OF AN *ARABIDOPSIS* GENE COMPLEX IN *BRASSICA* GENOMES.

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INTRODUCTION

Systematically, both *Arabidopsis* and *Brassica* species belong to the family *Cruciferae* , albeit to different tribes. Their relatedness has been confirmed by recent investigations at DNA level which revealed that *Brassica* species and *A. thaliana* display 80-90% homology in their coding sequences. However, the genome size of these species are very different, 145 Mbp for *A. thaliana* vs 468-662 for *Brassica* diploid species (Arumuganathan and Earle, 1991). DNA-based marker mapping demonstrated that the *Arabidopsis* genome is simple, while the *Brassica* genomes are highly duplicated in nature, up to 50% loci duplication among the diploid species (Quiros et al., 1992). A comparative studies of the *Arabidopsis* and *Brassica* genomes will help to understand how the duplication took place during *Brassica* genome evolution. The present studies concern the mapping of an *A. thaliana* gene complex in two diploid *Brassica* species, *B. nigra* and *B. oleracea*. The gene complex isolated from *A. thaliana* and subcloned in its components, consists of an *Em* gene (Gaubier et al., 1993) and three other genes (*At1*, *At3*, and *At4*) linked in a 10 Kbp sequence. This complex seems to be present in a single copy in *A. thaliana*. We report here the distribution of this *A. thaliana* gene complex in two diploid *Brassica* species, *B. nigra* and *B. oleracea* as determined by RFLP analysis and pulsed field gel electrophoresis (PFGE).

MATERIALS AND METHODS

GENETIC MAPPING **Plant Material** *B. nigra* F2 segregating population (83 individuals) and *B. oleracea* F2 population (60 individuals) were used for the construction of two *Brassica* maps.

RFLP DNA purification and RFLP were carried out as described earlier (Kianian and Quiros, 1992). The F2 linkage maps were constructed using computer program Joinmap (Stam, 1993).

PHYSICAL MAPPING Physical mapping by PFGE requires the following steps: 1. isolation of high quality protoplasts for preparing of mega-size DNA, 2. DNA digestion with methylation sensitive rare-cutting restriction endonucleases, 3. separation of mega-size DNA restriction fragments by PFGE and transfer to a membranes, 4. hybridization with probes specific to sequences closely linked on the chromosome.

Isolation of protoplasts Protoplasts were isolated from leaves of *B. oleracea* using the modified protocol of Crucefix et al.(1987). After purification, protoplast suspension was mixed with 1% LMP agarose solution and aliquoted to form agarose plugs.

DNA restriction Protoplasts embedded in plugs were treated with proteinase K (0.2 mg/ml), washed, and purified chromosomal DNA was digested with *BssH*II, *Mlu*I, *Not*I, and *Sfi*I restriction endonucleases following the manufacturer `s protocol (BioLabs).

PFGE Contour-clamped homogeneous electric field (CHEF) gels (Chu et al., 1986) were run at 6V/cm for 24 h with a switch time at a ramp from 50 sec to 90 sec at 14 C (for best resolution of 50-1000 Kbp restriction fragments). Alkali transfer to Zeta-Probe GT nylon membranes (Bio-Rad) and Southern hybridization were carried out as indicated by the manufacturer.

RESULTS AND DISCUSSION

GENETIC MAPPING

RFLP. Subcloned *At1*, *At2(Em)* and *At3* genes used as 32P-labeled probes in Southern hybridization showed several RFLPs both for *B. nigra* and *B. oleracea* F2 populations.

***Brassica nigra* mapping**. The *At* sequences were found at three linkage groups in four different locations. Similarly to *A. thaliana*, a conserved linkage of three *At* sequences was found in *B. nigra* B5 linkage group. Other *At* homologous sequences were found at the same linkage group (*At1* and *At2* at 0.0 cM)), at the B1 (*At1* and *At3* at 0.0 cM) and at B3 linkage groups (*At1*). These data confirm frequent duplication events in B. *nigra* as reported earlier (Truco and Quiros, 1993).

***Brassica oleracea* mapping**. Similarly to the *B. nigra* map, four different *At*-specific locations on three *B. oleracea* linkage groups were found. Tightly linked the *At1*, *At2*, and *At3* sequences (at 0.0 cM) were identified on the C1 linkage group. On the same linkage group at the distance of 1.1 cM from the triplet two more *At* loci: *At1* and *At3* were located. These five *At* loci are strongly linked (at 2 cM) with a gene family coding for storage proteins, napins and *Lea76*, coding for the late embryogenesis abundant protein. Interestingly, *Lea76* and *Em* (*At2*) belong to genes expressed during late embryogenesis and seed desiccation (Gaubier et al. 1993).

PHYSICAL MAPPING

In order to confirm *At* loci distribution and estimate physical distance between them on the *B. oleracea* C1 linkage group, we performed physical mapping by PFGE. The largest fragments up to 1500 Kbp were produced by *Not* I. The majority of fragments generated by *BssH*II and *Sfi*I were in the 100-800 Kbp size range, whereas *Mlu*I generated most restriction fragments in the range of 50-500 Kbp. Hybridization of *B. oleracea* digests revealed the presence of *BssH*II 230 Kbp and *Mlu*I 170 Kbp restriction fragments which hybridized with all three probes. In each digest there was only one restriction fragment hybridizing with *At1*, *At2* and *At3* probes. This is in agreement with results of RFLP analysis which showed the presence of only one *Arabidopsis*-like gene complex in *B. oleracea*. The maximal distance separating three *At* loci is 170 Kbp. Another hybridizing fragment of 40 Kbp were disclosed by *At*1 and *At*3 for both *BssH*II and *Mlu*I digests. This finding confirms the existence of tight physical linkage of *At1* and *At3* and lack of *At2* in this region of C1 linkage group. The physical mapping data strongly support the F2 linkage data. Further studies will aim at cloning long DNA restriction fragments carrying *At* gene complexes, identifying more genes within cloned DNA fragments, and estimation the intergenic distances among related genes in duplicated *Brassica* chromosomal regions. This will help us to understand relation between genetic (cM) and physical distances (Kbp , Mbp) in some evolutionary conserved regions in the *Brassica* genomes.

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MICROSATELLITES IN *BRASSICA NAPUS*

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Genome analysis, with the help of useful molecular biology techniques, can provide valuable information about an organism. We are currently utilizing the fundamental information and technology for conducting advanced genome analysis for the application of more effective conservation of plant genetic resources. One application of molecular genome analysis is toward increasing access to useful information and genetic markers to directly benefit crop improvement programs. Through the integration of molecular level diagnostics with classical curatorial, we can become more effective in preserving the crops, and enhance the value of the resources we desire to conserve and use. The purpose of this study was to find out how molecular diagnostic techniques may be utilized and modified to improve plant gene bank operations, specifically regarding the potential of molecular diagnostics based on the application of polymerase chain reaction-based DNA amplification, utilization of repetitive sequence DNA as a molecular marker, and fluorescence-based automated DNA detection. In addition, emphasis is placed on decreasing unit costs of assays through simplified protocolas and increased levels of automation.

Materials and Methods

Genomic DNA was isolated from leaf tissue of *Brassica napus* L. (canola) 'Jet Neuf' and a size-fractionated (100-500 bp) library was constructed. Two dimeric repeat- $(GA)_{10}$ and $(CA)_{10}$ and a single tetrameric repeat- oligonucleotides $(GATA)_6$ were used as probes to screen over 15,000 clones. Positive clones were characterized via automated DNA sequencing. Determination of the extent of polymorphism associated with specific loci was being performed using a modified procedure of 'Touchdown' PCR. The PCR products were electrophoresed on FMC NuSieve 3:1 agarose and visualized with ethidium bromide.

Results and Discussion

- Microsatellite loci with perfect GA- and CA-core repeat sequences may be abundant in canola, with GA-, CA-, and GATA-repeat loci approximately every 25-175 kb with possibly 5,000-30,000 per genome. These data are similar to an earlier report of Langercrantz et al., who estimated GA-core repeat loci to be found approximately every 125 kb and CA-core repeat loci to be found approximately every 350 kb in canola.

- Dinucleotide repeats appear to be more abundant than tetranucleotide repeats in canola. The dinucleotide frequency average is 1.5×10^4 per genome compared to average tetranucleotide frequency of 7×10^3 per genome.

- The GA-core repeat may be found at a similar frequency in both canola and humans. Although, the CA-core repeat sequences in canola may be less abundant than in humans. This corresponds with data reported by Morgante and Olivieri, which stated CA microsatellites are less frequent than expected in plants when compared to human CA microsatellite frequency.

- Perfect GA-core repeat are more frequent than imperfect or compound GA-core repeats. Imperfect GATA-core repeats appear more frequently than perfect GATA-core repeats. Further, the chance of identifying positive clones with perfect/compound core repeats and appropriate flanking sequences for primer design is relatively high (cumulatively 50-60%).

- Preliminary assays for primer verification and loci's informativeness suggest allelic differences at the species and subspecies level.

- In addition to a more extensive characterization of GA-, CA-, and GATÁ-repeat loci, modifications are also underway to simplify protocols, increase the level of automation, and decrease the unit cost of each assay.

- Assays for both di- and tetranucleotide-repeat loci informativeness will be performed via automated fluorescence-based detection with polyacrylamide gels (Genescan, Applied Biosystems, Inc.) and via manual fluorescence-based detection of tetranucleotide-repeat loci with agarose.

- Microsatellites are ideal markers for plant genetic linkage and physical mapping, population studies and varietal identification because of the amount of information they provide and their abundance. They have been shown by others to be highly polymorphic, somatically stable, and inherited in a co-dominant Mendelian manner. Microsatellite loci screenings may prove useful in plant gene banks for routinely establishing genetic identity, relatedness, and structure.

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CHROMOSOME DOUBLING OF MICROSPORE-DERIVED CANOLA USING TRIFLURALIN. Eric Eikenberry, Mycogen Plant Sciences, Madison, Wisconsin 53716 USA.

The antimicrotubule herbicide trifluralin was tested for it's ability to induce chromosome doubling in microspore cultures of canola (Brassica napus L.). Trifluralin in acetone solvent was added to the cultures immediately after isolation at levels of 0, 1, 5 and 10 µM. After 1 day the cultures were rinsed and subsequently regenerated into plants. All three of the trifluralin treatments resulted in at least 78% doubling as compared to 4.5 to 22.7% doubling in the controls. The frequency of polyploids rose with increased levels of trifluralin. We are now using 0.5 to 1 µM trifluralin routinely in our microspore regeneration system.

Figure 1 shows that trifluralin plus acetone does not cause a decrease in embryogenesis when compared to acetone alone. The solvent itself seems to account for a decrease in embryogenesis as acetone increases from zero to 100 µL acetone. Embryo germination can be used as a measure of embryo quality. Figure 2 shows that quality is not damaged when trifluralin is introduced into the culture medium as embryos germinate well regardless of the treatment used. Finally, Figure 3 shows excellent chromosome doubling when trifluralin is used, although polyploidy increases with higher levels of trifluralin.

Treatment Legend

Control = No treatment
10A = 10 µl acetone/plate control
10A+1T = 10 µl of a 1mM trifluralin stock (in acetone) to make 1 µM trifluralin
50A = 50 µl acetone/plate control
50A+5T = 50 µl of a 1mM trifluralin stock (in acetone) to make 5 µM trifluralin
100A = 100 µl acetone/plate control
100A+10T= 100 µl of a 1mM trifluralin stock (in acetone) to make 10 µM trifluralin

SUMMARY

Problems with conventional doubling techniques:

- ⊗ Low frequencies of spontaneous diploids
- ⊗ In vitro colchicine doubling frequencies inconsistant
- ⊗ In vivo colchicine (root or shoot soaking) results in:
  - stunting and delay of flowering
  - ploidy chimeras and often poor seed set
  - additional transplanting labor
  - use of large amounts of colchicine

Advantages with in vitro trifluralin treatments:

- ⊕ High doubling frequency
- ⊕ High quality diploids, as good as spontaneous diploids
- ⊕ Flower as fast as spontaneous diploids
- ⊕ No additional transplanting labor
- ⊕ Trifluralin used in micromolar quantities
- ⊕ Less toxic to humans than colchicine
- ⊕ Cheaper than colchicine

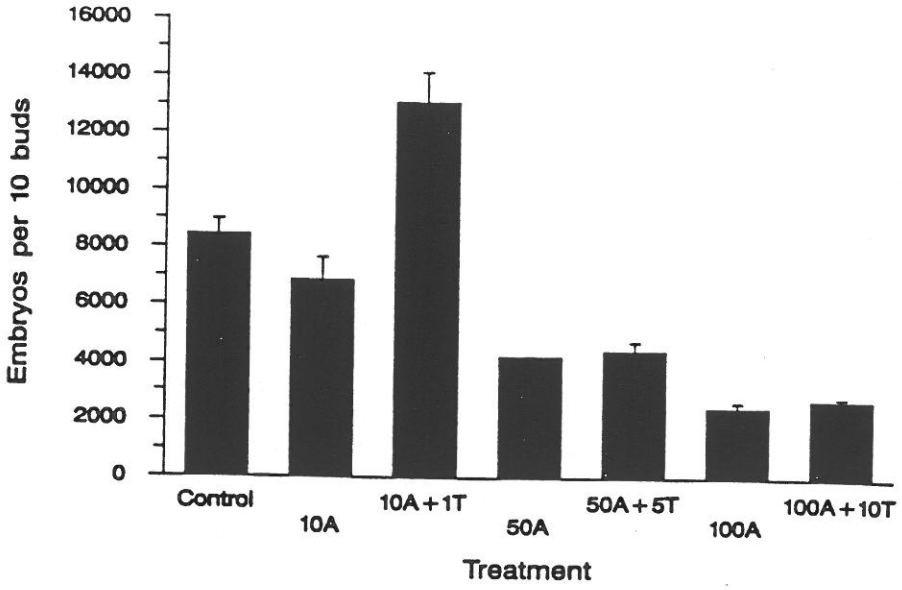

Figure 1. Embryogenesis from trifluralin-treated microspores.

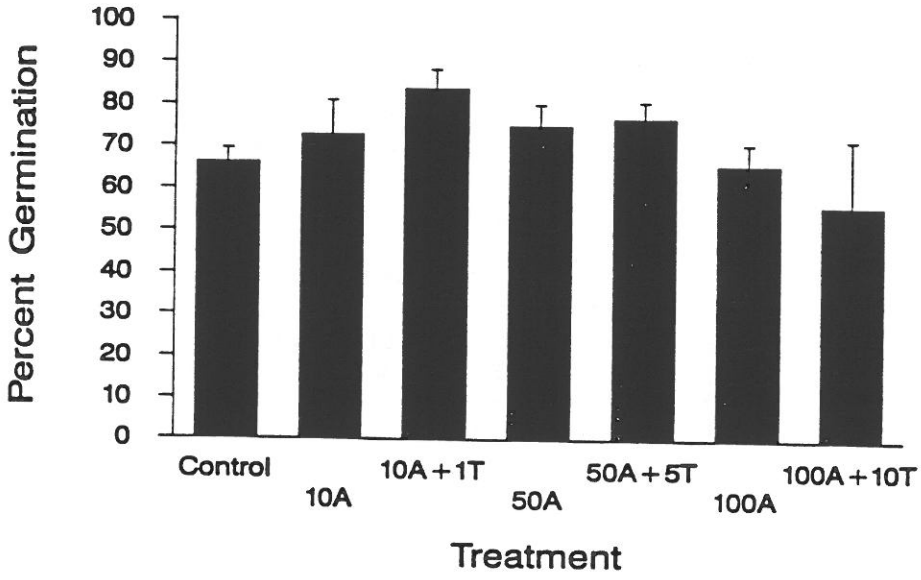

Figure 2. Embryo germination.

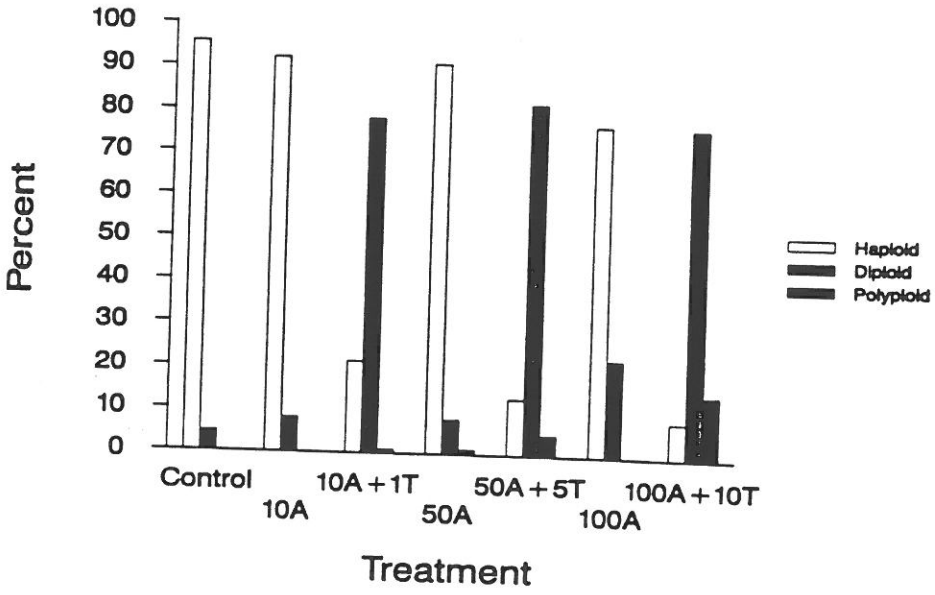

Figure 3. Chromosome doubling using trifluralin.

THE REGENERATION OF SHOOTS FROM SEEDLINGS EXPLANTS OF CAULIFLOWER

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Introduction

It would appear that cauliflower is very responsive to regeneration in tissue culture with regeneration from various explants of cauliflower being reported e.g. curd (Grout 1988), peduncle (Zarske 1988, Christey and Earle 1988), petals (Turton and Fuller 1989), internodes (Singh 1988) and cotyledons (Narashimulu and Chopra 1988). The use of regeneration from curd is now routinely used to micropropagate clones of cauliflower in breeding and seed production. Several laboratories are now interested in the transformation of cauliflower using Agrobacterium and microprojectile techniques but these techniques rely on an efficent regeneration system. We describe here the efficency of seedling explants of cauliflower in regenerating shoots in tissue culture.

Materials and Methods

Seed germination. Seed was surface sterilised by a 30 sec soak in 70% alcohol followed by 15 mins in a 10% bleach solution (0.6% sodium hypochlorite) and rinsed threee times with sterile distilled water. Seeds were then placed onto a germination medium (8g/l agar, M&S salts) in small 125 ml pots 10 seeds per pot in a growth room at 20 ^{o}C.

Explant preparation. Seedlings were cut up into the following explants; cotyledons, upper hypocotyl, lower hypocotyl, root/shoot joint, upper root, lower root. Explants were placed on regeneration medium coded S23 (8 g/l agar, M&S salts, 30 g/l sucrose, 170 g/l sodium dihydrogen orthophosphate monohydrate,0.4 g/l adenine sulphate, 30g/l thiamine,2.0 mg/l kinetin,1.0 mg/l IBA) in petri dishes and sealed with Parafilm. Petri dishes were incubated at 20 ^{o}C 16 h photoperiod for 7 weeks and scored for number of explants with shoots and average number of shoots per responding explant. The origin of the shoots was also recorded (proximal, distal end).

Experiment 1. Seeds of the variety Fanch germinated for 5,7,9 and 11 days grown under three conditions dark (D), 16h photoperiod (L) and 3 days dark followed by 16h photoperiod (DL).

Experiment 2. Seeds were germinated for 7 days with 16 h photoperiod. Varieties tested were:

Plana	F1 hybrid summer heading
Nautilus	F1 hybrid summer heading
Karfiol	Open pollinated summer heading
Dova	F1 hybrid autumn heading
Arbon	F1 hybrid autumn heading
Dagan	F1 hybrid autumn heading
Snowball	Open pollinated autumn heading
Alverda	Open pollinated, green curded, autumn heading
Fanch	F1 hybrid winter heading
Jakez	F1 hybrid winter heading
Parent 1	Inbred line
Parent 2	Inbred line
F1 (P1 x P2)	Experimental F1 hybrid winter heading
Arcade	F1 hybrid spring heading
Abina	Open pollinated spring heading
Marchpast	Open pollinated spring heading

Results

Experiment 1. Germination time influenced explant responsiveness with an optimum at 7 days germination but did not influence the number of shoots produced per responding explant (3 shoots). Light conditions during germination influenced explant responsiveness with germination in the dark significantly reducing responsiveness. Explant source influenced responsiveness. The most responsive explants were the hypocotyls where 70% of explants produced shoots. Roots were poorly responsive with shoots being produced only from sections closest to the shoot

and only 45% of these sections were responsive. Cotyledons were also poorly responsive with only 10% yielding shoots. Where explants did respond they generally gave about 3 shoots per responding explant although the upper hypocotyl gave 4 and the lower root only 1. Sites of shoot regeneration on responding explants were similar from explant to explant. For hypocotyls the majority of shoots originated from the cut ends of the hypocotyl particularly the end most proximal to the root/shoot joint. For roots shoot development was preceded by localised green swellings either at the cut ends or in the middle of the explant. For cotyledons, shoots appeared from callus which formed at the cut surfaces.

Experiment 2. All varieties tested responded to the protocol. With all varieties the most responsive seedling explants were the hypocotyls. There was some variability in responsiveness between varieties but all varieties gave over 60% of hypocotyls responding. The number of shoots per responding explant also varied between varieties with highly responsive varieties giving as many as 10 to 15 shoots per responding hypocotyl.

Discussion. When using seedling plants as sources of explants for transformation attention should be paid to the germination conditions. Explant responsiveness is enhanced if seeds are germinated under a 16 h photoperiod regime for 7 days with extended germination times depressing responsiveness. Our experiments clearly indicate that for cauliflower the most responsive explant from seedling plants is the hypocotyl and over 60% of explants can be expected to produce shoots using our regeneration medium. Furthermore, the regeneration from the hypocotyls is mostly from the cut ends which is important when considering the potential for transformation by Agrobacterium which needs a wound to infect. These factors indicate that hypocotyl explants are suitable for Agrobacterium transformation experiments.

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IN VITRO PLANT REGENERATION IN RADISH *RAPHANUS SATIVUS L.*

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Introduction

Radish is known to be very difficult object for in vitro plant regeneration. The main aim of the present work was to reveal optimal conditions for plant regeneration in various radish genotypes. A possibility of plant regeneration from primary explants and tissue cultures of radish *Raphanus sativus L.* was studied using a genetic collection of highly inbred lines (I_{30}-I_{35}). The lines marked by distinct genetic *in vivo* and *in vitro* traits (Narbut et al. 1985, Lutova et al. 1988) were divided into tumorous and non-tumorous ones on the basis of their ability to form spontaneous genetic tumors in the storage roots. Plant regeneration was shown to be genotype-dependent.

Methods

Radish *Raphanus sativus L.* inbred lines and their F_1 and F_2 hybrids and parental varieties 'Saxa' and 'Virovsky bely' were used. Cotyledons of the 10 days old axenic seedlings, were explanted onto Murashige-Skoog (MS, Murashige and Skoog 1962) or White cultural media without phytohormones or supplied with various concentrations of auxin naphthaleneacetic acid (NAA) or cytokinins 6- benzylaminopurine (BAP) or kinetin. Apical buds of the 10 days old seedlings and immature embryos were placed onto MS medium supplied with 2 mg/l BAP.

Results

Every of all 14 lines tested were able to form long term tissue cultures on MS medium supplied with 0,5 - 1 mg/l 2,4-D. Being replaced on phytohormone-free medium, such long term tissue cultures developed green morphogenic culture only in case of **LV-359 non-tumorous inbred line** which is likely to be ethylene-overproducing according to it in vivo phenotype characters. Excised cotyledons of this line did not possess high morphogenic response on phytohormone-free medium, but produced single buds on MS medium supplied with 0.5 mg/l NAA and 1 mg/l zeatin.

Excised cotyledons of the another **tumorous line, LS-337/76,** occasionally produced on phytohormone-free medium not buds but teratoma-like morphogenic tissues forming single buds. These tissues retains their morphogenic capacity after several years of cultivation on the MS medium without phytohormones or supplied with 0,1 mg/l kinetin. Similar teratoma-like tissues were obtained in cotyledons of several inbred lines and parental varieties when seeds were germinated on medium containing 2,4-D.

Being cultivated on White medium without phytohormones or supplied with 0.1 mg/l kinetin, excised cotyledons of all seven lines tested was shown to form rare single buds with frequency of bud formation less than 3%. In F_1 hybrids between the inbred lines ability to produce single buds on excised cotyledons was also low. Nevertheless, significantly higher frequency of bud formation on cotyledons was demonstrated for F_2 progenies derived from some of the 20 crosses where at least one of the parental lines was tumorous. In six such crosses analyzed when the tumorous parental line was represented by **LV-269 line**, the frequency of bud formation varied from 8 to 42%. The engaging of another **tumorous line LS-337/24** in analogous cross resulted in bud formation with frequency about 6%.

Embryogenic calli were also obtained by use of another type of explant than cotyledons. Namely, some inbred lines were shown to form calli in the bottom of excised seedling's apical bud on medium supplied with 2 mg/l BAP (Buzovkina et al., 1993). These auxin independent cytokinin-dependent calli could be embryogenic in case of the **LV-269 tumorous inbred line**. The calli formed on the same medium by F_2 progeny of LV-269 were also embryogenic.

In addition, efficient plant regeneration was obtained in case of embryogenic calli derived from immature radish embryos cultivated on MS medium supplied with 2 mg/l BAP. Such calli retained their morphogenic capacities after 6 month cultivation on the same medium. Cultivation of the mature embryos in the same conditions did not result in regeneration of the calli produced.

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DIALLEL ANALYSIS OF SHOOT REGENERATION IN *BRASSICA NAPUS*

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A shoot regeneration system using cotyledonary explants has been developed in *B. napus*, due to the high morphogenic ability (Moloney et al. 1989). However, we recently reported that the shoot regeneration responses were strongly influenced by genotype with wide range of variation (Ono et al. 1993, 1994). In this study, we attempted to obtain genetic information about the shoot regeneration from cotyledonary explants by means of diallel analysis.

Five cultivars of *B. napus*, 'Arabella', 'Doral', 'Bridger', 'Cascade' and 'Norin 30' were used. A full set of diallel crosses were artificially performed among the five cultivars. Four-day old cotyledonary explants were cultured on the regeneration medium which composed of MS medium supplemented with 4.0mg/l BA. After 3 weeks of culture, regeneration frequency (number of explants with shoots / total number of explants) was investigated. Calculation in the diallel analysis which is based on the method of Hayman (1954 a, b) and Griffing (1956) was carried out using a microcomputer program 'DIALL' developed by Ukai (1989).

The frequencies of shoot regeneration from cotyledonary explants of 5 parents and their F_1 hybrids are shown in Table 1. 'Arabella' and 'Doral' showed high frequency of regeneration (96.7% and 93.3%, respectively), while 'Bridger', 'Cascade' and 'Norin 30' had low or zero frequency (6.7%, 6.7% and 0.0%, respectively). The regeneration frequencies of their F_1 hybrids were the similar to those of the high responsive parent, and showed no difference between reciprocal hybrids. The variance (Vr) and covariance (Wr) graph of the shoot regeneration which is shown in Fig. 1 provides information about the genetic relationships among the parents. Coefficient of the regression of Wr on Vr was 1.029, suggesting the absence of epistasis. 'Arabella' and 'Doral' locating near the origin carry exclusively dominant genes for shoot regeneration. On the other hand, 'Bridger', 'Cascade' and 'Norin 30' located away from origin posses an excess of recessive genes. High shoot regeneration ability was dominant to low ability.

From analysis of variance and estimation of genetic variance components, the following results were obtained: (1) Additive and dominance effects were significant in shoot regeneration, with the additive effect being more important than the dominance effect, (2) The heritability for shoot regeneration ability was high (broad and narrow heritability was 0.973 and 0.819, respectively).

Our results suggest that shoot regeneration ability, which is controlled by major gene(s), can be transferred from high responsive genotype to low or unresponsive one by sexual crossing.

Acknowledgment

We thank Prof. Y. Ukai, the University of Tokyo, for providing the 'DIALL' program.

Table. 1. Shoots regeneration frequency (%) from cotyledonary explants of a set of 5×5 diallel crosses of *B. napus* cultivars.

No. Cultivar	♂	1	2	3	4	5
1 Arabella		96.7	90.0	90.0	83.3	93.3
2 Doral		96.7	93.3	78.5	86.7	80.0
♀ 3 Bridger		83.0	86.7	6.7	3.3	16.7
4 Cascade		83.3	78.5	6.7	6.7	6.7
5 Norin 30		93.3	73.3	33.3	0.0	0.0

The underlined figures represent parental values.

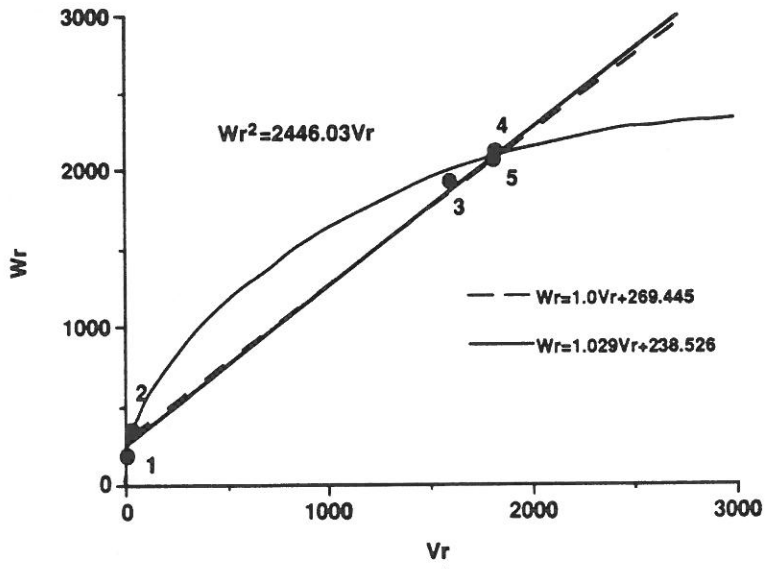

Fig. 1. Vr, Wr graph of the five parent diallel analysis for shoot regeneration. No. of cultivars refer to Table 1.

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TRANSGENE MOVEMENT BY POLLEN

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There is now international agreement on the need for a risk assessment before transgenic plants are evaluated outside a contained environment. This is not because transgenic plants are innately hazardous, but because transformation makes it possible to incorporate into plants, genes from unrelated plant species, microorganisms and animals (including humans). Various kinds of information are required before transgenic plants can be grown outside containment conditions; and includes a description of (a) the recipient crop plant (b) the transgene and how it modifies plant phenotype (c) the release environment and (d) how the field trial is to be carried out and the site monitored. This information is then used in an assessment of risks associated with the release. An evaluation of the proposed trial is made by the national regulatory authorities and if appropriate, given approval.

There have now been several hundreds of field release experiments with transgenic plants and there is considerable experience with the release of certain crops and transgenes (Dale, et al. 1993). The challenge for the regulatory authorities, is to devise efficient procedures for identifying those transgenic plants that may pose a particular risk to human health and/or the environment, and to provide a fast-track through the regulatory procedure for plants that present no greater risk than their non-transgenic counterparts. Simplified regulatory procedures are already being developed (eg. in the EU and USA) and it is likely that this will be extended to a wider range of crop species and transgenes with further experience.

Data on the likelihood of movement of transgenes by pollen is required as part of a risk assessment. This includes an evaluation of the possibility of the transgenes moving to adjacent crops of the same species or to sexually compatible wild species (including weeds). The provision of data on transgene movement by pollen makes it possible to estimate the optimum or minimum isolation distances, or to define other procedures (eg. non-transgenic buffer crops, asynchronous flowering, male sterile lines) for the experimental field evaluation of novel transgenic lines. The use of isolation procedures will mostly be impractical when a transgenic variety is in commercial production, so it is anticipated that the risk assessment leading to commercialisation will take this into account.

In a 1 hectare experiment in Norwich, UK, we studied the extent of pollen movement from a central plot of transgenic spring oilseed rape (variety westar) to surrounding non-transgenic plants of the same variety. The design is similar to that used by the group of M Renard at INRA, and the Norwich study was part of a collaborative project with INRA in Rennes and Plant Genetic systems in Gent. A 9 m diameter central plot contained transgenic plants carrying the Basta herbicide tolerance gene, surrounded by a 105 m square of non-transgenic plants. In the centre of the 9 m diameter transgenic plot was a 1 m diameter plot containing non-transgenic plants. Six honey-bee hives were introduced to increase the opportunity for pollen movement. A sample of bees from each hive was marked with a coloured spot on their thorax, to facilitate bee tracking. Data were collected on the flowering characteristics and bee movement throughout the flowering period. Samples of seeds were collected from different positions in the crop at harvest. Initially some seeds were germinated in a glasshouse; and later a larger sample of seeds was drilled into a 2 hectare field plot. Plants containing the transgene were identified by spraying with Basta. Samples of tolerant plants were progeny tested and analyzed by molecular methods to confirm they carried the marker transgene.

Figure 1. The proportion of seeds carrying the transgene, at varying distances from the central transgenic plot.

Distance from the central plot of transgenic plants (m)	Estimated number of plants tested	Estimated number of transgene containing seeds per million seeds(%)
Centre	7000[a]	47907 (4.8%)
1	12650	15940 (1.6%)
6	29206	1164 (0.12%)
12	110169	162 (0.016%)
24	117240	41 (0.0041%)
47	295176	3 (0.00034%)

[a] The sample harvested from the central 1 m diameter plot. In this case screening was in a glasshouse and the number corresponds to the number of seeds sown for herbicide spraying. Data shown for the five distances (1-47 m) were obtained from field screening.

As will be seed from Table 1, cross pollination with pollen from the transgenic marker plot declined sharply to 12 m and was 0.00034% at 47 m Samples were also taken from the corners of the hectare plot, and none contained the marker gene. The sample size from these locations was relatively small (70 m distance, 12884 plants screened) and the number of plants containing the transgene may have been below the limits of detection (Scheffler et al. 1993; Dale et al. 1993)

In conclusion, gene transfer was found to be limited at c50 m. The frequencies of transgene movement in pollen will depend, to some extent, on the design of the field plot, and other designs are being evaluated. These data are being used by regulatory authorities to assist with the risk assessment process.

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FIELD TRIAL OF TRANSGENIC CANOLA CONTAINING PHASEOLIN GENE

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The meal from rapeseed (Brassica napus) remaining after oil extraction has the potential to be an ideal feed for poultry, swine, and cattle. Factors currently limiting the suitability of rapeseed meal for this purpose include the lower protein content and amino acid balance in seed protein, higher fiber content and the presence of antinutritional components. These factors currently limit the proportion of rapeseed meal that can be used in feeds for non-ruminant animals (poultry and swine) and also result in a lower value for rapeseed meal as compared to soybean meal. Although considerable effort has been invested in the reduction of the antinutritional components, in particular the reduction of glucosinolates to a minimal level, less effort and success has been made in the improvement of the quantity and quality of seed storage proteins.

At Mycogen Plant Sciences, we have produced transgenic rapeseed cultivars Profit and Westar which contain either the native gene for phaseolin, a Phaseolus vulgaris seed storage protein, or a modified phaseolin gene with a methionine codon-rich synthetic oligonucleotide inserted into the coding region of the gene, under the control of the native phaseolin promoter. In mature seed of the transgenic plants grown in the greenhouse, native phaseolin accumulates to levels of up to 2% of total seed protein, while the high methionine-containing modified phaseolin accumulates to levels 10 fold less (Table 1). The reason for the differences in accumulation of the two forms of the phaseolin protein is still uncertain; however an obvious possibility is that the insertion of extra amino acids in the native protein has adversely affected proper folding and storage of the protein in seed storage bodies.

Field trials of transgenic Profit plants have been conducted in Saskatchevan in collaboration with the Plant Biotechnology Institute (National Research Council) and Mycogen Canada, to determine the level of expression of these gene constructs under field conditions. In particular, the levels of total, rapeseed and phaseolin seed proteins and the amino acid composition of seed storage proteins in each of the transformants is being compared to those observed in the Profit controls. Any other effects of the transgenes on agronomic performance, yield and other seed quality traits are also being analysed.

TABLE 1:

PHASEOLIN TRANSGENIC RAPESEED PLANTS

Plant #	*Variety*	*Construct*	*Phaseolin Protein (%)*	*Segregation Analysis*
H68-1a	Profit	Pneo	>1.0	15:1
H68-2a	Profit	Pneo	0.5	3:1
H77-1a	Profit	Pneo	0.5	3:1
H73-1a	Profit	HiPneo	0.1	3:1
H74-1a	Profit	HiPneo	0.1	3:1
H74-5a	Profit	HiPneo	0.2	15:1
H74-8a	Profit	HiPneo	0.1	3:1
H66-1a	Westar	Pneo	0.5	3:1
H65-1b	Westar	HiPneo	0.25	15:1
H65-3a	Westar	HiPneo	<0.1	3:1
H71-2a	Westar	HiPneo	<0.1	3:1
H72-1a	Westar	HiPneo	<0.1	3:1

Production of Transgenic *Brassica oleracea* Expressing *Bacillus thuringiensis* Insecticidal Crystal Protein Genes

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Introduction

Bacillus thuringiensis insecticidal crystal protein (*Bt* ICP) genes are being incorporated into many crop plants in order to improve insect control. Unfortunately, the benefits of these genetically-transformed plants may not be realized due to the development of insecticide resistance. The diamondback moth, an important pest of crucifers worldwide, has already developed resistance to *Bt* sprays in the field[1], and there is concern that other insects may also develop resistance to *Bt* ICPs in transgenic plants. Principles of managing resistance to plant-incorporated insecticides must be developed if they are to have a commercially viable future. We are using the only case of field-evolved *Bt* resistance as a model system to study different options for resistance management. We report the production of transgenic broccoli, cabbage and rapid cycling *B. oleracea* expressing a *Bt* ICP, under both constitutive and light-inducible control.

Materials and Methods

Both flowering stalk explants and *in vitro* grown seedling hypocotyl and petiole explants of broccoli, cabbage and rapid cycling *B. oleracea* lines were inoculated with an *Agrobacterium tumefaciens* strain containing the *nptII* gene and a *Bt cryIA(c)* gene optimized for plant expression. Culturing the explants on shoot induction medium containing 2 mg/L benzyladenine and 25 mg/L kanamycin allowed selective regeneration and growth of transformed shoots. Regenerated shoots were rooted on hormone-free medium containing 25 mg/L kanamycin. Leaf pieces from putative transformants were cultured on medium containing 50 mg/L kanamycin to confirm their transgenic nature[2]. Initial screening of transformants for *Bt* expression was conducted either on intact plants or on detached leaves using 5-10 1st instar larvae of a *Bt*-susceptible diamondback moth strain. Insect mortality was scored after 3, 4 and 5 days. Selected plants that gave 100% mortality of susceptible larvae were also assayed with 1st instar larvae from a strain of diamondback moth that had developed resistance to *Bt* in the field and with F_1 hybrids from a cross between the resistant and susceptible strains. Transformants derived from a cytoplasmic male sterile broccoli line were crossed to a non-transformed broccoli line. Progeny plants were analyzed for segregation of kanamycin resistance by spraying the plants with a 250mg/L kanamycin solution and of insect resistance, with 2-3 3rd instar *Bt*-susceptible diamondback moth larvae. Southern analysis was conducted to confirm T-DNA integration and to determine the number of transgenes present in each plant line.

Results

Both sources of plant material for inoculation produced good transformation results. Initial identification of transformants was determined by the ability of regenerating shoots to root on kanamycin containing medium. Placing leaf pieces of regenerated shoots on medium with kanamycin was used to identify occasional non-transformed shoots that regenerated roots. Leaf pieces from transformants remained green, producing callus and roots while controls and escapes bleached within a week. Transformation of flowering stalk explants produced 181 kanamycin resistant plants for an overall transformation frequency of 6.5%. Insect bioassays were performed on 164 of these plants; 112 (69%) were scored insect resistant (100% insect mortality). The seedling explant transformation yielded 102 kanamycin plants with a transformation frequency of 8.6%. 81 of 85 kanamycin resistant seedling explant transformants were scored as insect resistant.

32 insect resistant transformants were screened with a *Bt* resistant diamondback moth strain and the F_1 strain. *Bt*-resistant larvae showed >90% survival on 30 of 32 plants tested, whereas all F_1 larvae died. Analysis of progeny was conducted on 26

transgenic plant lines. Sixteen plant lines gave ratios of 1:1 for both kanamycin resistance and insect resistance, indicative of a single T-DNA integration event. Southern analysis showed 4 of the 16 lines with 1:1 progeny segregation contained a single copy of the transgene.

Discussion

A total of 283 transgenic plants were recovered in this study. Five broccoli lines, one cabbage and one rapid cycling *B. oleracea* line were successfully transformed by one or both of the transformation methods. Both methods of transformation proved to be reliable and efficient, and rooted transformed plants could be generated routinely in 3 months. The flowering stalk transformation method[3] is advantageous when plant material or seed supply is limited. Large numbers of explants can be derived from a single plant. The *in vitro* seedling explant method overcomes the problem of bacterial or fungal contamination often associated with flowering stalks derived from greenhouse or field plants, and is more convenient.

A high percentage of kanamycin resistant plants were also insect resistant. It was demonstrated that while these plants are able to kill susceptible diamondback moth larvae, *Bt*-resistant diamondback moth larvae are able to survive the level of *Bt* protein expressed in these plants. The ability of these transgenic plants to kill susceptible larvae while serving as a suitable host for resistant larvae makes them an excellent model for testing the various *Bt* resistance management strategies.

Insect resistant progeny plants were generated from male sterile transformants containing a *Bt* gene under constitutive control. Several lines containing a single copy of the transgene were identified by Southern analysis and segregation of progeny. These lines were judged to be the most appropriate for further studies, as multiple copies of the transgene may result in variable levels of gene expression[4]. Progeny plants from suitable transformants are currently being used in greenhouse resistance management experiments.

Broccoli and cabbage expressing the same *Bt* gene under light-inducible control were also produced. These transformants are currently being analyzed and may allow further tests of resistance management strategies involving control of gene expression.

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AN EFFICIENT TRANSFORMATION SYSTEM FOR RAPID-CYCLING *BRASSICA OLERACEA*

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We have developed a rapid and reliable transformation method for rapid-cycling *Brassica oleracea* based on an efficient regeneration system and analysis of the presence and integrity of the introduced DNA by PCR techniques. The key features of this protocol are that delaying the application of selection pressure results in increased numbers of both regenerants and positive transformants, and that selection using hygromycin rather than the more extensively used kanamycin is more efficient.

The plant material used was *B. oleracea* (accession 85 x 019) originally derived from the Crucifer Genetics Cooperative Type 3. The regeneration system employed was the stem internode method of Millam *et al.* (1991) using PS1 medium comprising Murashige and Skoog (M&S) medium supplemented with 50 mM sucrose, 17.74 μM BAP and 2.69 μM NAA, solidified with 8.0 g/l Difco Bitek agar. Cultures were maintained under conditions of 22°C in a 16 hr light, 8 hr dark regime, with a radiant flux density of 70 μe m^2s^{-1}.

The *Agrobacterium tumefaciens* construct C58 *rpoB rif* carrying the non-oncogenic Ti plasmid pGV3850 carrying two antibiotic-based selectable markers was used for these studies. Overnight cultures were established in TH medium, comprising 5 g/l tryptone, 1 g/l yeast extract and 6 g/l sucrose. Twenty 5 mm internodal explants of tissue-culture-derived *B. oleracea* were placed in 10 ml of liquid basal M&S and 1.0 ml of an overnight bacterial suspension added. After 45 minutes the explants were removed, blotted dry using sterile filter paper and plated on PS1 medium for 48 hours prior to subculture onto PS1 supplemented with 500 mg/l cefotaxime with no selection agents (control), 30 mg/l kanamycin or 15 mg/l hygromycin. The mean regeneration frequency per explant was assessed after 28 days culture.

Table 1. Mean regeneration frequency with standard error (20 plates per treatment, 10 explants per plate)

	No selection	Kanamycin	Hygromycin
control	12.6 + 2.3	0.0	0.0
treated	15.8 + 2.9	1.2 + 1.0	0.0

Mean regeneration frequency was significantly inhibited if explants were subjected to selection at levels as low as 30 mg/l kanamycin, and no regeneration at all was observed when 15 mg/l hygromycin was used in the PS1 regeneration medium. Consequently, selection was delayed until the shoots had developed after 14-21 days culture. At this stage shoots were excised and placed on the appropriate selection medium. The selection pressure was increased at each of three subculturing stages up to 50 mg/l kanamycin or 30 mg/l hygromycin. Following this selection (see Table 2) the surviving plants were maintained on

basal M&S medium supplemented with 25 mM sucrose, the appropriate selection agent, 500 mg/l cefotaxime and 8.0 g/l agar.

The rapid DNA isolation method of Edwards, Johnstone & Thompson (1991) was used to provide samples for PCR analysis. PCR reactions were performed using a programme of: 30 cycles of sample denaturation at 96°C for 90 s, primer hybridization at 68°C for 90 s and primer extension at 72°C for 90 s. From 10 to 30 ng of sample DNA was incubated in a 100 µl reaction volume and primers for NPTII (Beck *et al.*, 1982) were used at a final concentration of 1 µg/ml. The PCR products were separated by electrophoresis on an agarose gel and the bands visualised using ethidium bromide. Southern Blot confirmatory molecular analysis was performed by isolating DNA using the methods of Dellaporta, Wood & Hicks (1983), digesting the samples with EcoR1 and HindIII, and probing them using appropriate hybridizing fragments. The overall transformation efficiency following molecular analysis is shown in Table 2.

Table 2. Shoot survival on kanamycin (kan) and hygromycin (hyg) after three subcultures (200 shoots per treatment) and overall transformation efficiency as measured by the expression of antibiotic resistance genes following PCR and Southern Blot analysis

	Shoot survival		Positive selected shoots	
	kan	hyg	kan	hyg
control	0 (0%)	0 (0%)	0 (0%)	0 (0%)
treated	40 (20.0%)	82 (41.0%)	31 (77.5%)	57 (69.5%)

Since shoots formed after 14 days culture, the high efficiency and rapidity of regeneration using this system provides substantial numbers of conserved material for further analysis. It would appear that the use of hygromycin is a more efficient selectable marker in terms of putative transformants produced, but the number of false positives derived from this treatment was found to be slightly higher. Characterisation using PCR offers a number of advantages, notably the speed of obtaining results, the requirement for only small amounts of material, and also avoids the use of radioactive substances. The regeneration and initial characterisation techniques described have also been applied to a wider range of *Brassica* germplasm and *Agrobacterium* constructs and preliminary results suggest a broad range of applicability. The rapidity with which transgenic plants can be produced and analysed makes this rapid-cycling *Brassica* system of particular interest in plant transformation studies. When poorly characterised genes from exotic sources need to be introgressed into *Brassica* germplasm, the success of their integration can be easily followed using well-defined primers of the vector alone. The secondary analysis following selection of positive shoots can then be initiated with comparative ease because of the high number of shoots produced.

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In vitro transformation of radish by Agrobacterium rhizogenes

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Introduction

Agrobacterium rhizogenes induces the differentiation of genetically transformed roots on many of dicotyledonous plants. Root initiation and the in vitro growth characteristics of transformed roots are due to transfer of Ri plasmid genes to plant nuclei and their expression in plant cells (see rev. Birot et al., 1987). Therefore, Ri-T-DNA would be a convenient vector to introduce foreign genes into plants. Axenic hairy root cultures are also potentially valuable for the study of biotrophic fungi or for the production of secondary metabolites, especially alkaloids.

The interaction of *A. rhizogenes* wild type strain A4 with radish has been reported previously as a storage root infection (Tanaka et al. 1985). Here we presented our data on the transformation of radish inbred lines and varieties by *A. rhizogenes*.

Materials and Methods.

A. rhizogenes wild type strain 8196 (mannopine type) were used (Chilton et al. 1982). Axenic plants were inoculated by the overnight cultures grown in the liquid LB media.

The radish *Raphanus sativus L.* varieties Virovsky bely and Ledyanaya sosul'ka and highly inbred lines LV-265, LV-269, LV-342, LS-337/24 from genetic collection of St.Petersburg State University were used. Axenic plants were multiplied in vitro via lateral buds of the plant fragments on phytohormone-free MS medium. Three - six months old plants grown in vitro, apical or basal parts and internode fragments from the plants were inoculated. Whole plants were wounded in the stem; plants fragments, cotyledons and leaf disks were cutted by a sterile blade. The inoculum was smeared in the wound or cutting. After 6 days plants were transformed on MS medium without phytohormones supplemented with 500 mg/l claforan or carbenicillin. Mannopine synthesized in hairy roots were analyzed by paper electrophoresis (Petit et al. 1983).

Results

When *A. rhizogenes* 8196 was applied to the wounded stem of axenic radish plants, adventitious roots at the site of infection were visible in a month after infection. The intensity of the hairy root formation was similar for the various genotypes when the lower part of the whole plant or plant fragment were inoculated. In this case first hairy roots developed in 15 - 20 days, and an abundant root formation was observed in 30 - 40 days after inoculation.

When upper cuts of the plant fragments were inoculated, the

results differed for various genotypes. In two weeks after inoculation adventitious roots in cuts were visible in the plant fragments of the LV-269 and LV-342 inbred lines and varieties, and no roots were formed in cuttings of the LS-337/24 line. In this line first hairy roots became visible only in four - five weeks, and the intensity of the root formation was much lower than in another genotypes. As it has been shown earlier, LS-337/24 line does not form crown galls after infection of the intact plants growing in the field by *A. tumefaciens* strains A277 and C58 (Narbut et al. 1989).

Whole plants or plant fragment were used because of their higher viability. The inoculated cotyledons and hypocotyls of axenic seedlings had lower vital capacity and often necrotized in 30-50 days. Control uninoculated leaf disks themselves were characterized by vigorous root formation that makes difficult an observation of the transformed roots.

The percentage of inoculated plants forming mannopine positive hairy roots depends from the physiological status of the axenic plants and cultivation conditions. But, roughly, about 50% - 75% of in vitro inoculated plants of all genotypes tested formed hairy roots. Although the frequency of transformed root formation was similar for the various genotypes, the intensity of hairy root formation could vary widely.

Whole plants with hairy roots then were transferred to a fresh MS phytohormone-free medium. This step should be done since it was impossible to obtain a culture of isolated radish hairy roots directly from primary adventitious roots. Hairy roots intensively multiplied while the plants themselves grew slightly. After 2 - 3 months of hairy root cultivation in the parental axenic plants the establishment of isolated hairy root culture come to be possible. Hairy roots were isolated from the plants and these axenic hairy root cultures are cultivated on MS media without phytohormones for over a year.

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RATE OF OUTCROSSING IN INDIAN MUSTARD, *BRASSICA JUNCEA*

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The first yellow seeded Indian mustard , designated as Trombay Mustard-1 (TM-1) was evolved at this Research Centre(1). By hybridisation using TM-1 and an appressed pod mutant with black seeds, the yellow seeded mustard TM-5 with appressed pod character was obtained (2). The appressed pod and the yellow seed coat colour characters were governed by monogenic, recessive genes which were independent (3). A creamy-white flower type of mustard was evolved at Pulses and Oilseeds Research Station, Berhampur, designated as B-85 (4). Hybridisation of TM-5 and B-85, lead to a selection with three recessive markers namely white flower, appressed pod and yellow seed-coat colour.

The present report describes the rate of outcrossing for three consecutive years from 1988-1991, using flower colour as a marker character. Two lines each of 36 selections with white flowers, appressed pod and yellow seed characters were grown in 35 m x 4 m blocks with 2 m spacing between blocks, 30 cm between rows and 10 cm between plants, with cultures of yellow flowers on all the four sides. Seeds of three plants with yellow flowers from these lines were collected separately during 1990-91 crop season to confirm, whether they were the result of outcrossing. The segregation for flower colour in these lines was studied during 1991-92 crop season. The number and % of plants with white and yellow flowers due to outcrossing in 12 of these 36 lines are shown in Table-1. The rate of outcrossing for the three years from 1988-1991 in this study were, 6.5, 9.8 and 6.3 % respectively with a mean of 7.5 %. Eventhough Indian mustard is considered as self pollinated, a certain amount of outcrossing is reported due to insect pollinators. Chauhan et al (5) reported 11-24 % outcrossing by using seed coat colour and Ram Bhajan et al(6) reported 7-29 % using purple pigmentation of leaves. Three plants with yellow flowers tested in 1991-92 segregated for yellow and white flowers in a 3:1 ratio (Table-2), confirming that yellow flowers in the earlier generation in these lines were of F_1 plants resulted from outcrossing.

These lines with the three markers will be of use in genetic studies.

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Table-1. Outcrossing rates for flower colour

Line No	1988-89 Plants with white	yellow	1989-90 Plants with white	yellow	1990-91 plants with white	yellow
1	49	3	38	4	40	4
2	26	4	58	7	89	5
3	54	2	38	4	32	5
4	11	3	27	2	51	3
5	83	2	66	7	1oo	8
6	77	1	97	7	96	11
7	47	2	39	0	88	3
8	85	5	89	12	41	2
9	41	1	39	0	44	2
10	37	6	97	9	84	2
11	54	5	50	9	30	1
12	34	5	45	6	49	1
Total	598	39	683	67	744	47
Outcross %	6.52		9.81		6.32	

Table-2. segregation for yellow and white flowers in 1991-92

Plant No.	yellow	white	Tota l	X^2 (3:1)	P-value
1	54	14	68	1.41	0.2-0.3
2	44	12	56	0.76	0.3-0.5
3	29	9	38	0.76	o.3-0.5
Total	127	35	162	0.993	0.3-0.5

POLIMA CMS SYSTEM FOR PRODUCING F_1 HYBRIDS IN OILSEED RAPE

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A number of cytoplasmic male sterility systems available in *Brassica napus*, of these polima (*pol*) CMS is receiving much attention of *Brassica* breeders in India. Major limitation observed in this system in India include thermosensitivity of sterility expression and yield penalty associated with the system. Encouraged by the success of Chinese breeders, studies were undertaken to exploit this system for producing F_1 hybrids in oilseed rape which is a crop of significant economic importance in Punjab state of India. The results of the studies conducted during past five years are summarised in this communication.

1. Identification of sterility maintainers and stability of expression

Twenty five CMS lines (BC6) were evaluated for stability of sterility expression under field conditions throughout the crop season. Based on male sterility index, it was possible to identify as many as nine lines which showed stable sterility expression even under a maximum temperature range of 17.6 to 32.2°C. Notable among these were pMS 88-26, pMS 88-35, pMS 88-40, pMS 89-03, pMS 89-43, pMS 602, and pMS 706. It was also possible to categorize two sterile lines as low temperature sterile and one line as high temperature sterile. These results have demonstrated that the stability of *pol* CMS was determined by the nuclear background of the female parent and it is possible to develop stable CMS lines after careful screening. In order to further enlarge the scope of maintainer breeding, 53 elite pure lines (S_7-S_8) mostly bred at PAU (Banga, unpublished) were used to test cross a stable *pol* CMS line (pMS 602). As many as 17 combinations showed stable sterile reaction. The absence of temperature sensitive (TS) genes in identified 17 genotypes will await analysis of successive backcross generations as Chinese experience has shown that quite a few crosses produced fertile pollen with increased generation of backcrossing until BC4 (Fu *et al.*,1990). Stable sterile expression in some CMS lines was further confirmed by high temperature treatment (32°C/14°C) under controlled conditions.

2. Male fertility restoration

Screening of a large number of intervarietal crosses led to the identification of three perfect and stable restorers namely PFR 15-09, PFR 89-48 and PFR 89-53. The expression of RF gene(s) in hybrid combinations involving these restorers was not influenced by the genotype of the female parent. Based on the analysis of F_2 and testcross generations, the fertility restoration by these genotypes was found to be determined by single Mendelian gene. Test of allelism with previously documented source *B. napus* cv. Italy showed that the fertility restorer gene of PFR 89-48 was non-allelic to the *Rfp1* gene of *B. napus* cv. Italy.

3. Biological penalty associated with *pol* CMS

The biological penalty in our studies was determined by comparing the bioenergetic cost assessment of isonuclear alloplasmic and euplasmic lines. In at least four CMS lines, no yield penalty was associated with CMS conversion. The same was, however, not true for a majority of the CMS lines. Thus the *pol* CMS lines based on nuclear background of such nuclear donors as NL 706, HPN1, NL 89-00 and NL 89-03 are expected to result in realizing the heterotic potential of hybrids involving these lines.

4. Evaluation of F_1 hybrids

Eighteen F_1 hybrids derived from using stable CMS lines and two fertility restorers, PFR 89-48 and PFR 89-53 were compared with the commercial check, GSL-1 during winter 1992-93. Seven hybrids could outyield GSL 1, only released variety of this crop in Punjab, by margins varying from 7.5 to 19.6 per cent.

5. Conclusions

With the availability of genotypes, reported in this communication, the way is now clear to develop and commercialize F_1 hybrids based on *pol* CMS in Punjab.

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HYBRIDS IN OILSEED RAPE BASED ON *TOUR* CYTOPLASM

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At present several cytoplasmic male sterility systems are available in *Brassica napus* (Banga, 1993), but excepting *pol* CMS, no other system could be commercially exploited so far in the world. In the present communication we describe the successful development of F_1 hybrids based on *tournefortii* CMS system. The male sterility in the present case, was developed by substituting *B. napus* genome into *B. juncea* CMS line based on *tournefortii* cytoplasm. This male sterility was originally discovered in an unknown population of *B. juncea* (Rawat and Anand, 1979). The evidence from mt DNA profiles suggested that this CMS cytoplasm came from *B. tournefortii* (Pradhan *et al.*, 1991).

1. CMS lines and stability of the expression

As many as 63 CMS lines based on this system are currently being maintained at our University. The male sterility is characterized by the presence of rudimentary anthers with little or no pollen grains. The expression of sterility was not influenced by the temperature as well as day length conditions. In a majority of the nuclear backgrounds, the nectary size and female fertility was normal. In 9 lines out of the 63 CMS lines evaluated, impaired female fertility and reduced nectary size were recorded.

2. Male fertility restoration

Test crossing the CMS lines with a large number of *B. napus* genotypes, mostly developed through hybridization and pedigree selection (Banga, unpublished), led to the identification of five fertility restoring genotypes. These were TFR 88-26, TFR 88-51, TFR 89-53, TFR-9 and TFR 90. The pollen fertility and anther-stigma ratio in completely restored F_1 hybrids was similar to that in the euplasmic parent. The Rf gene source(s) associated with TFR 88-76, TFR 88-51 and TFR 9 restored male fertility in all the female genotypes used in crosses. The male fertility restoration ability of TFR 90 and TFR 89-53 was, however, influenced by the genotype of the maternal parent in the hybrid combination. Genetic analysis revealed monogenic dominant inheritance of fertility restoration by TFR 88-26, TFR 88-5

and TFR 9. On the other hand, involvement of two genes was indicated for TFR 90.

3. Biological cost of male sterilizing cytoplasm

Attempts were also made to study the biological penalty associated with *tour* CMS by comparing the bioenergetic cost assessment of isonuclear alloplasmic and euplasmic lines. In a majority of cases, the CMS conversion did not entail any in significant enhancement in photosynthetic requirement. This indicated very little yield penalty. There appears to be some indication of increased *alternaria* susceptibility of CMS lines as compared to respective maintainer parents.

4. Yield evaluation of F_1 hybrids

A large number of hybrids based on this CMS system were evaluated. Commercial yield heterosis upto 38.0 per cent was recorded in large scale yield trials during past three years. One such hybrid PGSH 51 was recommended by the Research Evaluation Committee of Punjab Agricultural University for farmer's field trials in the Punjab State of India during 1993-94. It is likely to be released for commercial cultivation during 1994. In the multilocation yield trials conducted over three years, this hybrid outyielded the only commercial check GSL-1 by about 20 per cent.

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Outcrossing in canola (*Brassica napus* L.) under greenhouse conditions

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The Canola and Rapeseed Breeding Program at the University of Idaho utilizes a greenhouse to advance material from the F_1 to the F_2 generation. Due to the time and expense involved, flowering racemes are generally not bagged to exclude foreign pollen. In addition to the practical difficulties of bagging, pollination bags reduce the amount of seed produced and seed is often small or shrivelled. Although *Brassica napus* is self compatible, it has a open-pollinated habit, which facilitates outcrossing (1). A study was carried out to assess the amount of cross pollination occurring between unprotected plants in a greenhouse environment without insect pollinators to determine if bagging of flowering plants is necessary in the greenhouse.

Single plants of four spring canola cultivars; 'Bounty', 'Hyola 401', 'Iris', and 'Legend', were planted 15 cm pots. Pots were placed in rows either 15 cm, 30 cm, or 45 cm on center from plants of the high erucic acid cultivar 'Hero' (Figure 1). Each of the three spacing treatments was replicated twice. The canola cultivars were chosen to represent a range of flowering times that would overlap with the flowering time of Hero. Bounty, Iris and Legend are inbred lines while Hyola 401 is an F_1 hybrid produced using a male sterility system.

Figure 1. Physical layout of greenhouse outcrossing study (one replication shown).

Treatments							
15cm			30cm		45cm		
						Key:	
X	H	X	I	X	L	X	X = Hero
X	L	X	B	X	B	X	B = Bounty
X	B	X	H	X	I	X	H = Hyola 401
X	I	X	L	X	H	X	I = Iris
							L = Legend

Erucic acid content of the seed produced during the study was used to indicate whether or not outcrossing had occurred. Erucic acid content in the seed is controlled by 2 loci with multiple alleles that act in an additive manner. Oil produced by the canola cultivars contains less than 2% erucic acid while that of Hero contains approximately 55% erucic acid. Any seed produced on the canola cultivars as a result of pollination by Hero, would contain approximately 25% erucic acid in its oil.

Each plant was tied to a bamboo stake to keep it upright and to allow easy access for watering. This procedure was used to duplicate the treatment of plants in the breeding program. After the plants had

matured, pods were harvested and threshed in an Agriculex single plant thresher following the same procedure that is used for breeding material. Ten seeds from each canola plant were individually analyzed to determine fatty acid composition.

During chemical analysis, one seed that contained 55% erucic acid was found in a package of seed harvested from a canola plant; this seed must have been a contaminant from the thresher, as outcrossing would result in seed with a erucic acid content of about 25%. Two seeds from Hyola 401 in the 30 cm treatment, one from each replicate, were found to contain approximately 25% erucic acid, indicating that outcrossing had occurred (Table 1).

Table 1. Number of outcrossed seed per sample of 20 seeds sampled.

CULTIVAR	15 cm spacing	30 cm spacing	45 cm spacing
Bounty	0	0	0
Hyola 401	0	2	0
Iris	0	0	0
Legend	0	0	0

The only cultivar that outcrossed to Hero was Hyola 401. This cultivar differs from the others in that it is an F_1 hybrid produced via male sterility. Perhaps Hyola 401 has a smaller pollen load, making it more susceptible to foreign pollen, Alternatively, perhaps outcrossing was detected only in Hyola 401 due to sampling error. We did not examined the pollen load or viability of Hyola 401. Both instances of outcrossing occurred in the treatment with intermediate spacing, suggesting that the 30 cm spacing was no better than when the pots were placed immediately adjacent to one another (15 cm). Combining the 15 and 30 cm treatments, the study resulted in 2.5% outcrossing when canola and Hero were grown on 30 cm centers or less, which is the standard arrangement used to advance generations in the breeding program.

The three pure line cultivars did not show any cross pollination, and we expect that the small amount that may occur in such lines would be inconsequential in our breeding program. When considering this fact in conjunction with the difficulties of bagging flowering racemes in the greenhouse, we feel that such bagging is unnecessary.

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The restorer gene of Brassica napus line Sv84-28053 located in the C-genome

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In a nap cytoplasmic male sterility system involving resynthesized Brassica napus lines (Chen et al. 1990a), the cultivated oilseed rape breeding line Sv84-28053 showed male-sterility-restoring ability conferred by a single dominant gene (Ms). However, it has not been established whether the restorer gene of this line is located in the A- or C-genome (Chen and Heneen 1990). In order to ascertain the genomic location of the restorer gene (Ms), Sv84-28053 (genomes: AACC, 2n=38) was crossed as female with B. campestris var. yellow sarson accession K-151 (AA, 2n=20) to produce a trigenomic hybrid (AAC, 2n=29). The restorer gene in this trigenomic hybrid will exhibit a disomic inheritance if carried by the A-genome (Chen et al. 1990b). On the other hand, the restorer gene will be in a hemizygous condition in the trigenomic hybrid if located on one chromosome of the C-genome, and its transmission to the aneuploid progeny after selfing will reflect the presence or loss of the C-genome chromosome carrying this gene (Chen et al. 1990b). The aneuploid seeds obtained after selfing of the trigenomic hybrid were visually classified into two groups of large and small seeds. Segregation patterns of male-fertile (mf) and male-sterile (ms) plants in the two groups have been presented in Fig. 1.

As seen in Fig. 1, the segregation patterns for mf/ms plants in the two groups of seeds do not fit well with a disomic inheritance ratio viz. 3 mf : 1 ms (data on chi-square test not presented), thus indicating that the restorer gene (Ms) is not located in the A-genome. A much higher frequency of mf plants was found among the large seeds (21mf : 2ms) than among the small seeds (10mf : 9ms). Comparing large and small seeds of a similar aneuploid progeny (AACC x AAC), MacKay (1977) indicated that the large seeds have retained more chromosomes of the C-genome than the small seeds. Accordingly, there may be a higher frequency of the C-genome chromosome carrying the restorer gene in the group of large seeds than in the group of small seeds in the present work, thereby giving rise to more mf plants. Therefore, we conclude that the restorer gene (Ms) of Sv84-28053 is located in the C-genome and can be designated as 'Ms_C'. Thus, the exhibition of 3mf : 1ms ratio, obtained when pooling the aneuploid seeds of different size in Fig. 1, is not evidence for supporting the location of the restorer gene in the A-genome.

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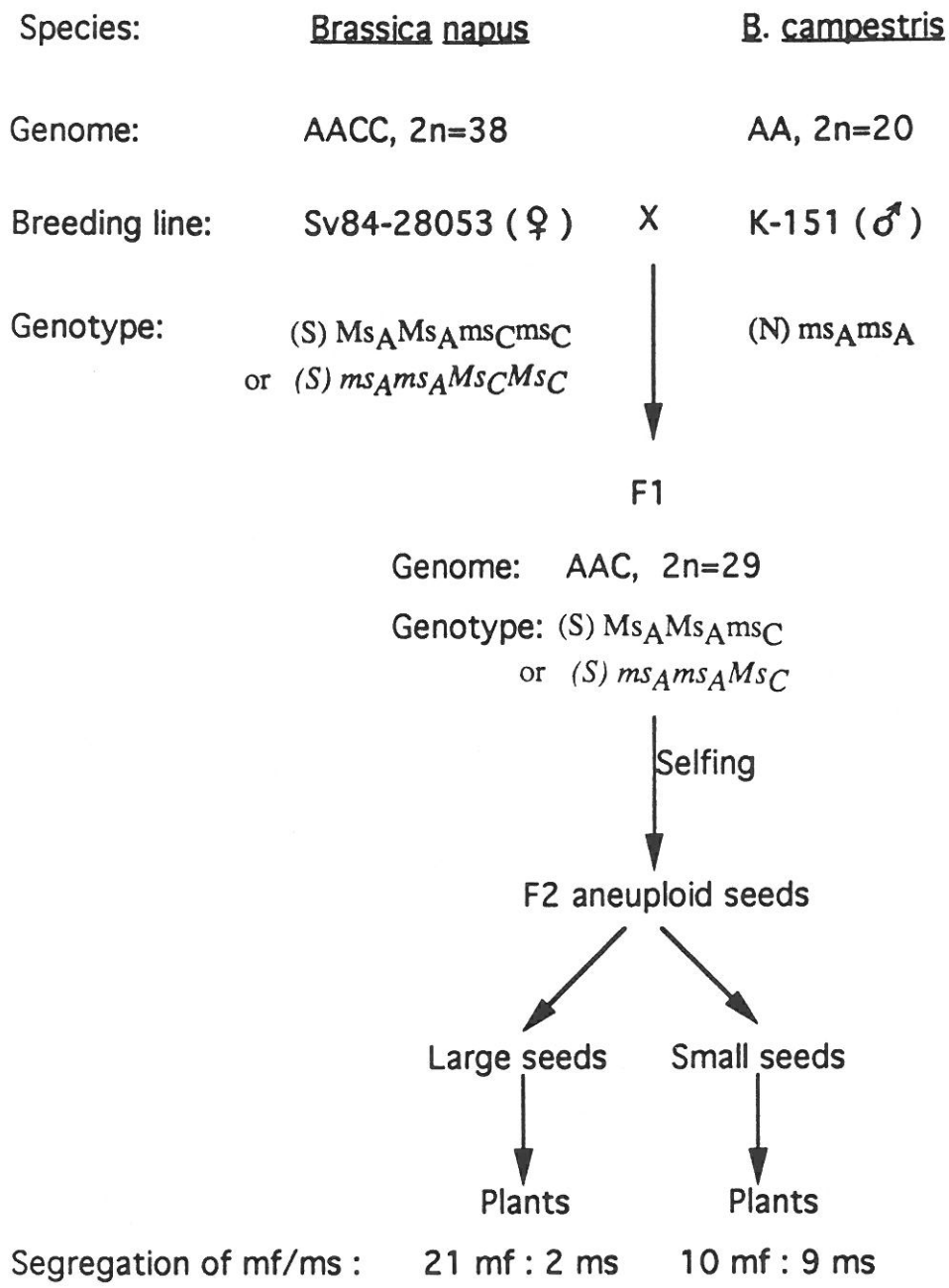

Fig. 1. Segregation of male-fertile (mf) and male-sterile (ms) plants in the two groups of seeds of different size and the pedigree of the material. Of the two alternative genotypes for Sv84-28053, the correct one is *italicized.*

Transfer of radish cytoplasmic male sterility from *Brassica napus* to *B.juncea* and *B.rapa*

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The cytoplasmic male sterility (CMS) originally found in radish (*Raphanus sativus* L.) by Ogura (1968) has been transferred to *Brassica oleracea* and *Brassica napus* by interspecific crosses (Bannerot et al., 1974). Resultant male sterility was highly stable but the male sterile lines showed chlorophyll deficiency and had low nectar secretion. Male sterile *B.napus* cybrids were produced through protoplast fusion (Pelletier et al., 1983) to generate male sterile lines without any defects (Pelletier et al., 1987). Fertility restorer alleles have been introduced from radish into rapeseed (Heyn, 1976) through intergeneric crosses between a CMS line of rapeseed and a *Raphanobrassica* (*R.sativus* x *B.napus* amphiploid). Fully restored plants with only one dominant restorer allele were selected on the best cybrids (Pelletier et al.,1987). However, the introduction of the restorer allele was accompanied by a large decrease in seed set (Pellan-Delourme and Renard, 1988). During the last years , improvement of the restorer material has been achieved and restorer lines with a good female fertility have been obtained (Delourme et al., 1991), thus leading to a workable cytoplasmic male sterility in *B.napus*.

In order to transfer this CMS system from *B.napus* to *B.juncea* and *B.rapa*, interspecific crosses with these two species were realised. A spring male sterile line of *B.napus* cv 'Brutor' with cybrid 58 cytoplasm was crossed to *B.juncea* cv 'Varuna'. Then, successive backcrosses with 'Varuna' were made and a male sterile line of *B.juncea* (BC6) with 2n = 36 chromosomes has been obtained. The same scheme has been applied to *B.rapa*. The same male sterile line of *B.napus* was crossed and then backcrossed to *B.rapa* cv 'R500'. BC2 plants with 2n = 20 chromosomes have been produced. Resulting male sterility is stable in *B.juncea* as well as in *B.rapa* and the morphology of the male sterile flowers is normal. Female fertility of these male sterile lines will be established in field conditions in summer 1994.

The transfer of the restorer gene from *B.napus* to the other two species is also in progress. BC2 and BC1 plants carrying the restorer allele have been respectively produced with *B.juncea* and *B.rapa*. However, it has not been established whether the restorer gene in *B.napus* was introgressed into the A or C genome. If it is located in the C genome, its transfer to *B.juncea* and *B.rapa* will be more difficult. Study of the chromosome number and meiotic behaviour of the restored and male sterile plants in the successive backcrosses might be a way to answer this question.

This work will lead to a complete cytoplasmic male sterility system in *B.juncea* and *B.rapa*, which will be available to produce FI hybrids in countries growing these oilseed crops (Canada, China, India, Sweden...).

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Cold-tolerant Ogura CMS *Brassica* Vegetables for Horticultural Use

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Introduction

Use of the Ogura male sterile cytoplasm (CMS) for hybrid production in *Brassica* species has been limited because leaves of Ogura CMS plants show chlorosis at low temperatures (1). This chlorosis is related to the presence of *Rapanus sativus* chloroplasts in Ogura CMS *Brassica* materials (2). Protoplast fusion provides a way to replace the *Raphanus* chloroplasts with *Brassica* ones, while retaining the CMS associated with the *Raphanus* mitochondria. Through protoplast fusion and conventional breeding, we have produced cold-tolerant Ogura CMS *B. oleracea* vegetable lines with good horticultural characters. These lines are available for testing.

Materials and Methods

Two types of fusion experiments were done (see ref. 3 and 4 for details). The first (3) involved fusions of leaf protoplasts from an Ogura CMS cauliflower inbred (7642A) with leaf or hypocotyl protoplasts of male-fertile atrazine-resistant *B. rapa* (cv. Candle). Division of the regenerable cauliflower line was inhibited by treatment with iodoacetate. In some cases, the non-regenerable *B. rapa* protoplasts were treated with γ-irradiation prior to polyethylene glycol-induced fusion. Plants recovered were analyzed by flow cytometry to determine nuclear DNA content, cellulose acetate electrophoresis of isozymes, and molecular analysis of organellar composition using probes that distinguished the parental lines. Phenotypic characters related to the organelles (CMS, atrazine-resistance, cold tolerance) were also assessed. Plants that were diploid, CMS, and cold-tolerant were evaluated further in the greenhouse and the field. They were initially crossed with a white-flowered broccoli and then backcrossed to either broccoli or cauliflower. At each stage, plants were selected for good seed set and horticultural characters.

The second set of fusion experiments (4) was similar, except that the male-fertile partner was an atrazine-sensitive cauliflower line closely related to 7642A instead of *B. rapa.*

Results

Some 190 shoots were recovered from seven *oleracea* + *rapa* fusions. Plants from 3 calli were retained after selection in greenhouse and field trials (Table 1)

Table 1. Characteristics of selected plants recovered from fusions of Ogura CMS cauliflower and atrazine-resistant *B. rapa.*

				Organellar probes							
				cp[c]	mitochondrial					Male	Cold
Callus	KRad[a]	No. of Plants[b]	pg DNA/ nucleus	s8	p5.0	p5.2	p9.7	b2.3	D23	fertility	tolerance
	Cauliflower		1.24	*rph*[d]	*ogu*[d]	*ogu*	*ogu*	*ogu*	*ogu*	-	-
	B. rapa		1.03	*rapa*[d]	*ole*[d]	*ole*	*ole*	*ole*	*ole*	+	+
3142	0	13[d]	1.34	*rapa*	*ogu*	*ogu*	*ogu*	*ogu*	*ogu*	-	+
3105	66	5[d]	1.34	*rapa*	*ogu*	*ogu*	*ogu*	*ogu*	nd[e]	-	+
3125	66	10[d]	1.38	*rapa*	*ogu*	*ogu*	*rapa*	*rapa*	nd	-	+

[a] of γ–irradiation to the *B. rapa* protoplasts prior to fusion. [b] not all plants from each callus were tested in each assay. [c] cp:chloroplast. [d] *rph*: from *Raphanus*; *rapa*: from *B. rapa*; *ogu*: from *Raphanus; ole* : from cauliflower· [e] not determined, but presumably *ogu* since this region is correlated with Ogura CMS (5).

It is notable that diploid cybrid plants were recovered from callus 3142 although no irradiation had been applied to the *B. rapa* protoplasts.

Two plants from callus 3142 were backcrossed to heat-tolerant broccoli lines to produce lines designated NY7865, 7866, 7867, 7868 and 7869. When these were grown

in the field with natural bee pollination, seed set varied substantially, from poor to good (Table 2). When cuttings from the plants with good seed set were pollinated by flies in greenhouse cages, almost all set well.

Table 2. Seed set on cold-tolerant Ogura CMS broccoli in the field (first backcross)

Pedigree			Line no.[d]	No. of plants with seed set		
				Poor	Medium	Good
89-3142[a]-9	x WF[b]	x 2393[c]	NY7865	9	0	3
89-3142-11	x WF	x 2403[c]	NY7866	5	4	6
89-3142-9	x WF	x 2403	NY7867	6	0	6
89-3142-9	x WF	x 2403	NY7868	5	4	7
89-3142-9	x WF	x 2393	NY7869	7	1	8

[a] 89-3142-9 and 89-3142-11 are two plants from cauliflower cybrid callus 3142 (see Table 1). [b] A white flowered broccoli. [c] Heat-tolerant broccoli inbred lines. [d] Lines NY7865-7869 available for release are bulks of seed from plants that set well in both field and greenhouse cages and backcrossed to 2393, 2403, and 5415.

Backcrosses of the original cybrids or their progeny to various good cauliflower lines produced lines NY 7720 and 7760 (from plants 10 and 11 from callus 31242), line 7721 (from plant 5 of callus 3105), and NY 7719 (from plant 1 of callus 3125). Line 7719 has shown shown some erratic reversion to fertility in subsequent generations and is probably not suitable material for applied use.

Fusions of leaf protoplasts from Ogura CMS and fertile cauliflower also produced cold-tolerant Ogura CMS cybrids (Table 3). These showed only Ogura CMS hybridization patterns for the five mitochondrial probes used. Atrazine-sensitive cold-tolerant Ogura CMS lines of cauliflower (NY 8424, 8425, 8426, 8427) and broccoli (NY 8464, 8465) with good seed set were developed from these cybrids.

Table 3. Characteristics of selected plants from fusions of protoplasts from Ogura CMS cauliflower and atrazine-sensitive male-fertile cauliflower (modified from [4])

			Organellar probes							
			cp[b]	mitochondrial					Male	Cold
Callus	No. of Plants	pg DNA/ nucleus	s8	s10.1	p5.2	p5.0	b2.3	D23	fertility	tolerance
cms cauliflower		1.24±.03	*rph*[c]	*ogu*[c]	*ogu*	*ogu*	*ogu*	*ogu*	-	-
Fertile cauliflower[a]		1.24±.03	*ole*[c]	*ole*	*ole*	*ole*	*ole*	*ole*	+	+
DF2.1	2	1.25-1.37	*ole*	*ogu*	*ogu*	*ogu*	*ogu*	*ogu*	-	+
DF2.2	1	1.35	*ole*	*ogu*	*ogu*	*ogu*	*ogu*	*ogu*	-	+
DF2.3	1	1.26	*ole*	*ogu*	*ogu*	*ogu*	*ogu*	*ogu*	-	+

[a] protoplasts from the fertile line were treated with 20.2 Krad of γ-irradiation prior to fusion.
[b] cp:chloroplast. [c] *rph*: from *Raphanus*; *ole*: from *B. oleracea*; *ogu*: from *Raphanus*.

The improved lines described above are available for testing by signing a biological materials agreement. Licensing for commercial use in hybrid production is also possible.

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^{14}C-ASSIMILATES TRANSLOCATION IN EXCISED SILIQUAE OF DIFFERENT AGES IN INDIAN MUSTARD : THE EFFECT OF PHYTOHORMONES.

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Under optimal set of conditions, source and sink sizes are usually optimum in Indian musterd (Brassica juncea (L.) Czern & Coss) whereas translocation is usually a factor limiting productivity (Chhabra, 1986). Phytohormones are being used in crop production for different purposes as they bring about changes in metabolism, balance of growth and partitioning of assimilates as well as the quantity and quality of the desired economic product (Nickell, 1982; Nowak and Lawson, 1983). Though scattered information is available on the "hormone directed transport" in Phaseolus vulgaris, Solanum tuberosum, Glycine max, Zea mays etc. (Inanga and Kumura 1987; Rahman et al; 1989 andd Gundalia et al; 1990), not much information is available with respect to the role of hormones in promoting the rate and magnitude of translocation of ^{14}C assimilates in B.juncea and their field use in promoting seed yield and oil content. The present experiment was therefore conducted to examine the effect of various hormonal applications on the translocation of ^{14}C-Assimilates in the excised siliquae of different ages.

10 ml of the different hormone solutions alongwith one control containing 10ml distilled water were taken in petridishes. Siliquae of different maturity groups (15,30 and 45 days after anthesis) were placed separately in petridishes containing various hormones (IAA & GA 100 & 500 µM and Kinetin 10 and 100 µM) solutions so as to allow uptake for one hour. ^{14}C sucrose was then allowed to be taken up by the siliquae in scintillation vials containing 2ml of 10 µci ^{14}C-Sucrose for one hour in such a way that only the pedicels were immersed in the ^{14}C -Sucrose. Siliquae were then rinsed thoroughly in running tap water. The siliquae were cut into fine pieces. Tissue were extrected with 80% ethyl alcohol thrice at 65°C. The pooled supernatant was collected in scintilation vial and oven dried at 50°C till it evaporated. 15ml of Bray's liquid scintilation fluid was added to each vial and the cpm/g dry weight was recorded using liquid scintilation system model Beckman LS 100C. Data (Table-1) reveal the following observation :-

15 DAA siliquae : As compared to control, IAA 100 µM and KIN 100 µM increased the accumulation of ^{14}C- assimilates in these siliquae. IAA 100 µM caused an increase by about three times while KIN 100 µM brough about 62.27 per cent increase. On the contrary, GA 100 500 µM resulted in a reduction in the accumulation of ^{14}C-assimilates. IAA 500 µM and KIN 100 µM had no significant effect (Table -1).

30 DAA siliquae : IAA 100 µm increased the accumulation of ^{14}C-assimilates in these siliquae by 57.90 per cent and KIN 100 µM by 24.10 per cent (Table-1).

45 DAA siliquae : As compared to control, IAA 100 µM caused an increase in the incorporation of ^{14}C counts by 41 per cent whereas other hormonal concentrations had no significant effect.

Table : 1 **Effect of various hormonal concentrations on ^{4}C-translocation (cpm/g dry weight) in excised siliquae of different ages.**

Treatment	Age of siliquae			Mean
	15 DAA	30 DAA	45 DAA	
Control	14556 (120.51)	10172 (100.44)	6687 (81.76)	(100.90)
IAA100	40888 (202.18)	16062 (126.50)	9407 (96.39)	(141.69)
IAA500	26131 (130.72)	11556 (107.37)	8469 (91.94)	(110.01)
GA100	7267 (85.32)	7876 (90.54)	5709 (75.41)	(83.06)
GA500	9457 (97.17)	8122 (90.11)	8371 (91.34)	(92.87)
KIN10	12334 (110.94)	9566 (97.74)	8192 (90.48)	(99.72)
KIN100	23620 (153.68)	12624 (112.13)	4867 (70.61)	(111.81)
Mean	(128.63)	(103.17)	(85.42)	

C.D. (p=0.05) Hormone = (6.92); Component = (4.53);
Hormone X Component = (11.99)

Figures in parentheses are $\sqrt{n}$ transformations.

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PHOTOSYNTHETIC $^{14}CO_2$ UPTAKE IN INDIAN MUSTARD AS INFLUECED BY PHYTOHORMONES

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Phytohormones influence growth via increasing source or sink strength or by regulating the movement of substrates by increasing their translocation efficiency (Chhabra, 1986). The present paper reveals the possibility of IAA, GA and Kinetin in improving photosynthetic rate ($^{14}CO_2$ uptake) .

The experiment was conduced on Brassica juncea (L.) (Czern & Coss.) var. RH-30 crop raised in field. All recommended package of practices were followed to raise a healthy crop. 35 days after sowing 100 and 500 µm concentrations of Indole-3-acetic acid (IAA) and Gibberellic Acid (GA_3) whereas 10 & 100 µm of Kinetin (6 Furfuryl amino purine) were sprayed on the plant. The middle fully expanded leaf was chopped off 24 hours after hormonal application and three replicates of 1 g tissue each were examined for $^{14}CO_2$ uptake rate using assimilation tubes (Hooda and Dhawan, 1986) which were glasstubes of 3 cm diameter and 20 cm length with an additional 2.5 cm long and 0.5 cm wide side tube near the upper end. The samples were infiltered in these tubes for 30 seconds with 1 ml of $NaH^{14}CO_3$ (Specific activity 50 µci/ml) in phosphate buffer (pH. 7.6) containing 5 µci/ml of ^{14}C using a vacuum pump. The samples were then exposed to sunlight for 30 minutes, after which 2ml. of 2N-HCl was added to stop this reaction. tissue was extracted with 80% ethyl alcohol thrice at 65°C. The pooled supernatant was collected in scintillation vial and oven dried at 50°C till it evaporated. 15ml of Bray's liquid Scintillation fluid was added to each vial and the cpm/g fresh weight was recorded using liquid scientillation system Model Beckman LS 100C. Finally the counts were converted into cpm/g dry weight.

Data (Table-1) reveal that IAA 500, GA 100 and 500 and Kin 10 um caused a significant increase in the photosynthetic $^{14}CO_2$ uptake of the leaves whereas IAA 100 and Kin 100 µm casued no significant effect.

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Table : 1 **Effect of various hormonal concentrations on the photosynthetic rate of the leaves at the vegetative stage (35 DAS)**

Treatment (µm)	Cpm/m/g dry weight
Control	1170 (34.13)
IAA100	1228 (35.04)
IAA500	1650 (40.55)
GA100	1732 (41.61)
GA500	1634 (40.35)
KIN10	1626 (40.30)
KIN100	1162 (34.08)
C.D. (p=0.05)	(3.30)

Figures in parentheses are $\sqrt{n}$ transformations.

A CO_2/Water Vapour Mixture for use with Self-incompatible Brassicas

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Many attempts have been made to find a simple and reliable technique for overcoming self-incompatibility (SI) in Brassicas. However, three of the simplest substances have been found to overcome SI - CO_2 (Nakanishi and Hinata, 1975), high humidity (Carter and McNeilly, 1975) and salt (NaCl) solution (Fu et al, 1992).

High humidity is easily produced with hand-pollinations by covering the flowers with a polythene bag, but this may only work with weak SI. CO_2 can be combined with high humidity by injecting CO_2 into the polythene bag (Mithen and McGrath, 1992). A suitable CO_2/water vapour mixture can easily be produced without the need for any special equipment by simply blowing into the polythene bag.

The technique was tested using four plants of a highly SI swede line G, a plant of a slightly less SI line T and a plant of G x T. Two plants of kale and two of turnip were also used to check the method with other SI brassica species. On each plant, three sets of pollinations were covered with polythene bags; CO_2 was injected into one bag using a soda-syphon containing only gas; one bag was blown into to fully displace the air inside after inhaling slowly for about 20 seconds; the third was not enriched with CO_2. Control treatments were open- and bud-pollinations covered with glassine paper bags. Eight to ten flowers less than 2 days old were used for each set of pollinations, except for bud-pollinations, which were on buds 2 - 3 days before opening.

		Seed set per pollination				
		Paper bag	Bud poll -ination	Polythene bag	CO_2 injection	Blow-in-the-bag
Swede	G1	0.3	13.6	0.5	7.2	23.2
	G2	0.2	20.8	0.5	23.2	26.1
	G3	0.3	22.4	0.9	0.6	26.8
	G4	0.1	22.0	0.5	2.0	20.3
	T	1.0	11.3	1.9	5.4	21.3
	TxG	0.4	11.9	2.1	13.8	21.1
Kale	1	3.2	13.0	10.8	4.0	21.4
	2	3.4	22.9	6.6	6.0	19.0
Turnip	1	1.7	2.3	5.2	1.7	6.3
	2	2.7	5.8	9.0	8.4	17.8
Mean		1.3	14.6	3.8	7.4	20.2
Total Seed pollinations		128	1488	374	722	2054
		97	102	98	98	102

Bud-pollination gave good seed set, although not as good as blow-in-the-bag and it took much longer to carry out. CO_2 injection gave variable results, which may have been because control of injection was poor, and very high CO_2 concentrations may have been produced. Blow-in-the-bag gave consistent results, with initial CO_2 concentrations between 5.5 and 6.0%, and appears to create ideal conditions for overcoming SI.

A plant of a *B. napoleracea* line was also tested, and gave 0, 2.1 and 6.3 seed/pollination for open, bud and blow-in-the-bag, repectively. Fertility of this plant was not good, but it appeared considerably better when SI was overcome using the blow-in-the-bag technique. Any cytologist wishing to try chromosome counts on this material should please contact the author.

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GENIC AND CYTOPLASMIC MALE STERILITY (GCMS) IN RAPESEED

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Male sterility of rapeseed is an important way of heterosis breeding in rapeseed. Theoretially, there are two types of male sterility, i.e., cytoplasmic male sterility (CMS) and genic male sterility (GMS).

There are two sub-types of CMS. The first is the CMS system named as the alien CMS system, such as ogu CMS (Ogura, 1968, Bannerot et al, 1974), mur CMS (Hinata and Konno, 1979). The second is the CMS named as the native CMS system, such as nap CMS (Shiga, 1971, Thompson, 1972) and pol CMS (Fu, 1981), which are the spontaneous CMS in rapeseed.

Ogu CMS might be the most hopeful CMS among the alien CMS systems at present. The problem for utilizing ogu CMS in rapeseed heterosis breeding is lack of restorers with good enough restoring ability. And among the native (or spontaneous) CMS systems, pol CMS may be the most practical CMS system,which has male sterile lines with relatively stable male sterility and restorers with good enough restoring ability for F_1 seed production. The disadvantage of pol CMS is that its male sterile lines is sensitive to temperature. By comparing ogu CMS and pol CMS, we think pol CMS is more useful than ogu CMS in China.

There are also two subtypes of GMS in rapeseed.One is dominant GMS(DGMS) (mathias, 1985, Li and Zhang, 1983) and the other is recessive GMS (RGMS) (Takagi, 1970, Hou et al,1990) . The advantage of GMS in rapeseed heterosis breeding is their male sterile lines having stable male sterility and the disadvantage of GMS is lack of maintainers. Therefore, about 50% male fertile plants need to be removed in GMS male sterile lines before flowering time in F_1 seed production plots.

By utilizing the advantages of pol CMS and all GMS systems in rapeseed, Yang and Fu (1993) proposed a new way of heterosis breeding in rapeseed, i.e., genic and cytoplasmic male sterility (GCMS).

1) Theoretical hypothesis
According the report of Yang and Fu (1990), the genotypes of pol CMS three lines, i.e. male sterile line,maintainer and restorer, are S (rfcrfcTsTs……), N(rfcrfcTsTs……) and S or N(RfcRfcTsTs ……) , respectively. In them, S, N, rfc, Rfc and Ts mean pol cytoplasm, normal cytoplasm, maintaining gene, restoring gene and temperature sensitive gene, respectively.

According the study of Li and Zhang (1983), the genotypes of DGMS two lines, i.e., male sterile line and restorer, are $1/2N(MS_1MS_1rfnrfn) + 1/2N(MS_1MS_1Rfnrfn)$ or $1/2N(MS_1ms_1rfnrfn)+1/2N(ms_1ms_1rfnrfn)$ and $N(MS_1MS_1RfnRfn)$or $N(ms_1ms_1RfnRfn)$, respectively. In them, MS_1, ms_1, Rfn and rfn mean the male sterile gene, male fertile gene,restoring gene and maintaining gene, and among them, MS_1 is dominant over ms_1, Rfn over rfn, and Rfn epistatic MS_1. And according the reports of Takagi (1970) and Hou et al (1990), the genotypes of RGMS two lines, i. e. , male sterile line and restorer,are $1/2N(ms_2ms_2)+1/N(MS_2ms_2)$ and $1/2N(ms_3ms_3ms_4ms_4) +1/2N(MS_3ms_3ms_4ms_4)$or $1/2N(ms_3ms_3ms_4ms_4)+1/2\ N(ms_3ms_3MS_4ms_4)$, and $N(MS_2MS_2)$ and $N(MS_3MS_3MS_4MS_4)$,Respectively. In them, ms_2, ms_3 and

ms_4 are male sterile genes, and MS_2, MS_3 and MS_4 are restoring genes, respectively. Among them, MS_3 is dominant over ms_3, MS_3 over ms_3 and MS_4 over ms_4.

2) Possible system of GCMS in rapeseed.

1. Dominant genic and cytoplasmic male sterility (DGCMS)

The proposed DGCMS system of Brassica napus could be established by introducing the dominant male sterile gene MS_1 and its restoring gene Rfn of Brassica napus into the male steriles line and restorers of pol CMS, respectively.The genotypes of DGCMS three lines are as follows:

①male sterile lines

$1/2S(MS_1ms_1rfnrfnrfcrfcTsTs\cdots\cdots)+1/2S(ms_1ms_1rfnrfnrfcrfcTsTs\cdots\cdots)$

or $1/2S(MS_1MS_1rfnrfnrfcrfcTsTs\cdots\cdots)+1/2S(MS_1MS_1Rfnrfnrfcrfcrfcrfc\cdots\cdots)$

②maintainers

$N(ms_1ms_1rfnrfnrfcrfcTsTs\cdots\cdots)$

or $1/2N(MS_1MS_1rfnrfnrfcrfcTsTs\cdots\cdots)+1/2S(MS_1MS_1Rfnrfnrfcrfcrfcrfc\cdots\cdots)$

③restorers

S or $N(ms_1ms_1RfnRfnRfcRfcTsTs\cdots\cdots)$, or S or $N(MS_1MS_1RfnRfnRfcRfcTsTs\cdots\cdots)$

2. Recessive genic and male sterility (RGCMS)

By introducing the mono recessive male sterile gene ms_2 or the double recessive male sterile genes ms_3 and ms_4 found in Brassica napus(Takagi, 1970, Hou et al, 1990)into the male sterile lines of pol CMS, we could set up the mono recessive genic and cytoplasmic male sterility (MRGCMS) and the double recessive genic and cytoplasmic male sterility (DRGCMS) system.

①The genotypes of MRGCMS three lines

1) male sterile lines

$1/2S\ (ms_2ms_2rfcrfcTsTs\cdots\cdots)+1/2S(MS_2ms_2rfcrfcTsTs\cdots\cdots)$

2) maintainers

$1/2N(ms_2ms_2rfcrfcTsTs\cdots\cdots)+1/2N(MS_2ms_2rfcrfcTsTs\cdots\cdots)$

3) restorers

S or N $(MS_2MS_2RfcRfcTsTs\cdots\cdots)$

②The genotypes of DRGCMS

1) male sterile lines

$1/2S(ms_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)+1/2S(MS_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)$

or $1/2S(ms_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)+1/2S(ms_3ms_3MS_4ms_4rfcrfcTsTs\cdots\cdots)$

2) maintainers

$1/2N(ms_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)+1/2N(MS_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)$

or $1/2N(ms_3ms_3ms_4ms_4rfcrfcTsTs\cdots\cdots)+1/2N(ms_3ms_3MS_4ms_4rfcrfcTsTs\cdots\cdots)$

3) restorers

S or N $(MS_3MS_3MS_4MS_4RfcRfcTsTs\cdots\cdots)$ or S or $N(MS_3MS_3ms_4ms_4RfcRfcTsTs\cdots\cdots)$

or S or N $(ms_3ms_3MS_4MS_4RfcRfcTsTs\cdots\cdots)$

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Evaluation of cytoplasmic male steriles for female fertility in *Brassica juncea* L. Czern and Coss

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The female fertility of the cms line is an important factor for the success of any hybrid programme. With the availability of different stable cms systems like *B. tournefortii*, *B. carinata*, *B. oxyrrhina* and others, extensive efforts are in progress to search for the restorer in the germplasm. However, the success in the latter area has been limited in the case of *B. juncea*. Simultaneously, efforts have also been devoted towrads the evaluation of cms systems at hand for their fertility using the cms and the B lines.

Data for female fertility in the case of 8 cms lines of *B. tournefortii*, one of *B. carinata* and one of *B. oxyrrhina* (available in *B. juncea* backgrounds) are presented in Table-1. The data (of another experiment) in relation to the ratio of female to male lines necessary for the optimum seed production on one of the cms lines under isolation, are presented in Table-2.

Data (in Table-1) on the number of seeds produced per siliqua on the male fertile line and male sterile line under open pollination and the average seeds produced on the A line expressed as percent of the 'B' line reveal interesting results. Seeds per siliqua on the cms line expressed as percent of 'B' line ranged from 20.9 to 56.2 percent in *B. tournefortii*, 16.3 percent in *B. carinata* and 91.6 percent in *B. oxyrrhina* based cytoplasms (all these are available in *B. juncea* background). Therefore, it appears that the cms lines developed by the incorporation of the *B. oxyrrhina* cms in *B. juncea* background would be ideal for their exploitation in the hybrid programme.

In an isolation plot, the experiment on the ratio of female to male rows necessary for optimising the seed production using PCMs 106 revealed, that six lines of cms (female) and 2 lines of MF (Male) would produce the maximum seed as compared to 3CMS:1MF and 4CMS:2MF combination of female to male rows.

Authors wish to sincerely thank Shri Narender Singh for assistance in collection of field data.

Table 1. Female fertility in different CMS lines of Mustard (Brassica juncea). Number of seeds per siliqua on A line expressed as per cent of that on B line, IARI, Delhi 1992-93 rabi

CMS system/	Number of seeds Per siliqua (Average)		Seeds/Siliqua (Average) on A line (per cent of B line)
	MF (B. line)	CMS (A line)	
B. tournefortii			
Pusa Bold	15.3	7.6	49.7
Pusa Barani	14.5	7.8	53.9
Prakash	14.4	8.1	56.2
RLM198	13.1	6.2	47.3
RH. 30	13.4	6.3	47.0
Varuna	11.6	2.4	20.9
PCMS-71	13.0	5.3	40.8
PCMS-106	13.2	7.2	54.5
B. carinata			
PCMS-10	13.5	2.2	16.3
B. oxyrrhina	14.6	13.1	91.6

Table 2. Coordinated Isolation Experiment on Mustard PCMS 106 A & B, IARI Delhi; 1992-93. Seed yield per plot (gms) produced on A line and B line

Combination of B : A		A line (gm)	B line (gm)	Seed yield per row on A line (gm)	B line (gm)	Seed on 'A' line expressed as % of that on 'B' line
B1	A3	425.0 (36)	475.0 (12)	11.80	39.58	29.8
B2	A4	235.0 (36)	460.0 (20)	6.52	23.0	28.3
B2	A6	390.0 (36)	565.0 (24)	10.83	23.54	46.0

A new petaloid-type male sterility in alloplasmic *Brassica campestris* L.

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Introduction

Petaloid type MS is a kind of male sterility which stamens are fully or partially transformed into petal or petal like structure. In full petaloid type it is impossible to produce any pollen, on the contrary some pollen may be formed in partial one (Kaul 1988).

For the F_1 seed production in *B. campestris* L., there is now a problem about the uniformity of F_1 hybrid. The F_1 seed production based on self incompatibility (SI) is not stable to environmental factors. Therefore, generated F_1 hybrid seeds are mixture of sib-crossed and successful F_1 seeds. So far as we know, petaloid type MS is more successful in many plant species for F_1 hybrid seed production when compare to other types of male sterility because of its stability when the female fertility is sustained at the promising level.

Materials and Methods

In 1986, intergeneric hybrids were produced between *Eruca sativa* and *B. campestris* by ovary culture [culture method was developed by Inomata (1977) and Matsuzawa (1978)], when *E. sativa* and *B. campestris* were used as female and male, respectively. From a F_1 plant, 4 F_2 seeds were obtained by open pollination. The genomic constitution of some F_2 plants was ascertained to be sesquidiploid (EAA, when E and A were used in place of *E. sativa* and *B. campestris* genome, respectively) by cytological studies (young anthers were squashed in 1% acetocarmine). Only one F_2 plant could produce 21 open pollinated seeds. In F_3, some plants showed malformed anthers and could not produce any fertile pollen, they were known as male sterility, and obtained F_4 seeds by open pollination. Also in F_4 and F_5 generation, male sterile plants could produce some open pollinated seeds. In F_6 , male sterile plants were crossed with 4 cultivars of *B. campestris* as pollen donor by hand pollination. The morphological traits of floral organs in F_7 progenies were observed and recorded. The breeding diagram is shown in Fig. 1.

In the case of open pollination, progenies were cultivated side by side with many sub-species of *B. campestris* in vinyl house. So we assume that all seed was set by crossing with *B. campestris*.

Results and Discussion

Observing the morphological characteristics of floral organs in F_7 male sterile plants that were obtained in 1993, following 4 types of male sterility were recognized:

1. Full-petaloid type MS.
2. Partial-petaloid type MS.
3. Antherless type MS.
4. Brown-anther type MS.

The percentage of each male sterile type is shown in Fig. 2. We expected these male sterile plants were alloplasmic ones carrying *E. sativa* cytoplasm. For full-petaloid type MS all of 6 stamens have completely converted to petal, having 10 petals and no stamen in flowers. On the basis of the observation, full-petaloid and antherless type could not produce any pollen throughout the flowering season but partial-petaloid and brown-anther type produced some pollen at the end of flowering season. To confirm the alloplasmic nature of this male sterility, mitochondrial DNA of this MS line will be analyzed along with *E. sativa* and *B. campestris*.

For the occurring of this male sterility, it is assumed that this trait may be under control of mitochondrial DNA or both of mitochondrial DNA and nuclear DNA. Moreover, translocation between E and A genome chromosome in the course of back crossing resulted the rearrangement of new male sterile genes. To ascertain these 3 hypotheses, we will do in further study.

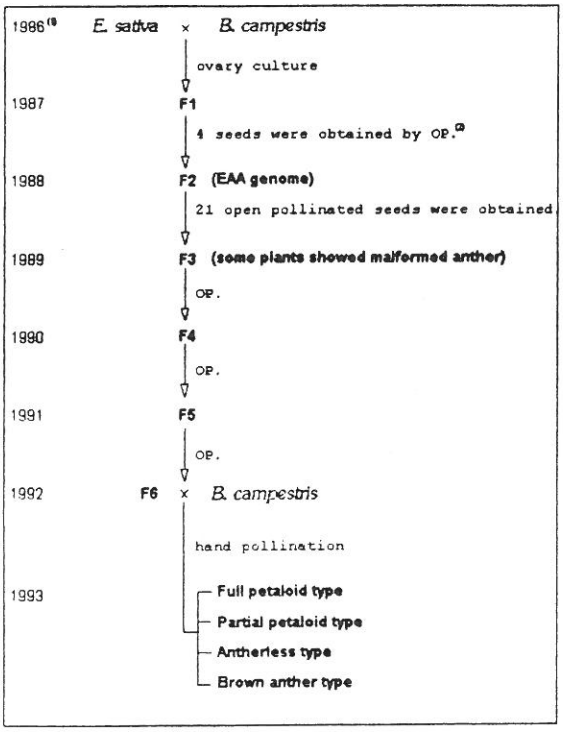

Fig. 1 Breeding diagram of petaloid type MS in *B. campestris*.

(1) year

(2) OP.=open pollination

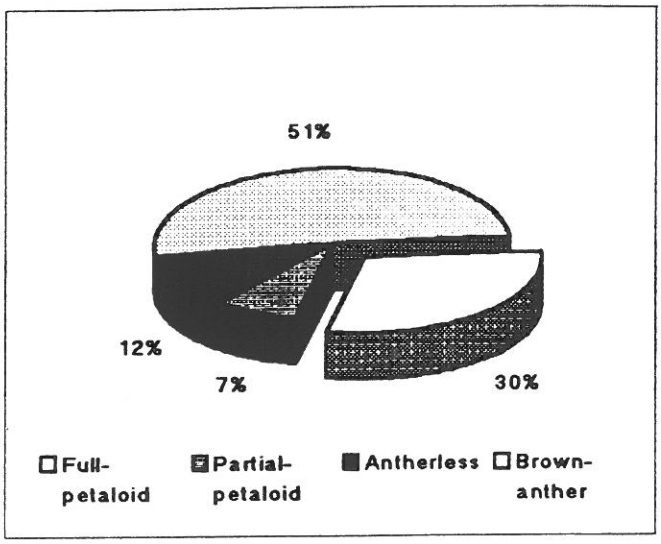

Fig. 2 The percentage of male sterile type in F_7.

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Genetic Components of Seedling Characters Over the Environments in Indian Mustard

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Rapeseed and mustard are generally grown in dry land conditions which are manifested with soil salinity and alkalinity problems. Irrigated land and salinization continues to increase worldwide. The breeding for salt tolerance in many crops has progressed slowly (Blum, 1988 and Johanson et al., 1992) particularly due to limited sources of genes for salinity tolerance, lack of efficient procedures and poor understanding of genes involved in controlling salt stress problems. The early seedling characters like speed of germination and seedling vigour have been identified as important parameters for selecting salt tolerant genotypes at the early seedling stage. Three relatively salt tolerant viz., RH 7859, RH 7846, RH 781 and three susceptible namely RH 8315, RWH 1 and RH 8113 genotypes were selected from the germplasm. These were mated in diallel fashion to derive genetic informations.

Three replications with 10 seeds per replication for six parents and their 15 F_1^s were placed on the top of filter paper in petriplates containing 0, 125 and 175 meq/l chloride dominated salt solutions. The data on germination was recorded daily to calculate speed of germination by the method as per Maquire (1962). Seedling vigour index was calculated as (root length + shoot length) X seedling dry weight.

Additive component (D) was significant only under 125 meq/l salt stress for speed of germination and 175 meq/l for seedling vigour whereas dominant components (H_1, H_2 & h^2) were significant for both the characters in all the three environments except speed of germination in E_3 (Table-1).

The dominant component (H_1) was greater than the additive component (D) for both the traits. This was also revealed by the ratio (H_1/D)^2 , which was greater than one in all the analyses. The important ratio h^2/H_2 showed atleast one group of genes with dominance effect in all three environments for speed of germination and two to three gene groups for seedling vigour were responsible to control these characters and exhibit dominance. Heritability in narrow sense for speed of germination was low in all the three environments whereas for seedling vigour it was low in E_1 and E_2 and moderate in E_3.

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Table 1 : Estimates of genetic components of variance for seedling characters under three environments in Indian mustard

Components of variation	Speed of germination			Seedling vigour		
	E_1	E_2	E_3	E_1	E_2	E_3
D	0.032 ±0.038	0.042* ±0.038	0.012 ±0.008	365.3 ±314.3	629.9 ±442.0	582.4* ±86.4
H_1	0.384* ±0.097	0.223* ±0.042	0.081* ±0.021	5683.8* ±797.7	4936.0* ±1121.9	1316.7* ±219.3
H_2	0.320* ±0.086	0.192* ±0.037	0.069* ±0.019	5456.2* ±712.6	4783.3* ±1002.3	1256.5* ±195.9
h^2	0.158* ±0.058	0.055* ±0.025	0.019 ±0.013	13927.1* ±479.7	8768.4* ±674.6	3454.3* ±131.8
F	0.049 ±0.093	0.008 ±0.040	0.014 ±0.020	365.3 ±314.3	106.2 ±1079.7	252.1 ±211.0
E	0.001 ±0.014	0.002 ±0.006	0.001 ±0.003	0.01 ±118.8	0.03 ±167.0	0.02 ±32.7
Degree of dominance	3.464	2.304	2.598	3.945	2.799	1.504
Symmetry of genes	0.203	0.215	0.213	0.240	0.242	0.239
Proportion of dominance and recessive genes	1.561	1.086	1.880	1.290	1.062	1.336
Groups of genes exhibiting dominance effects	0.494	0.286	0.275	2.553	1.833	2.749
h^2 (n.s.)	0.221	0.398	0.240	0.077	0.182	0.383
t^2	10.230*	0.217	0.801	4.981	1.204	0.628

* Significant at P = 0.05 ; E_1, E_2, E_3= 0, 125 meq/l and 175 meq/l salinity

Investigation of Heterosis in Spring Canola

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Numerous crop species have shown heterotic yield increases when compared to inbred cultivars, and several studies have suggested that rapeseed (*Brassica napus* L.) is a crop with high heterotic advantage (Sernyk and Stefansson 1983; Grant and Beversdorf 1985). Indeed, hybrid spring canola cultivars have been developed, and seed is currently being produced and sold to growers. However, it has yet to be conclusively proven that hybrid canola cultivars offer any agronomic advantages over traditional inbred lines. The goals of this study were to determine the levels of high parent heterosis in spring canola grown in the Pacific Nortyhwest region of the USA, to investigate the genetic basis of this heterosis and to determine which morphological characters influence yield response.

Materials and Methods

Four diverse inbreds ("Westar;" "Helios;" "SN.91.33.1" and "Star") were selected as parental lines. These were chosen because research has shown that rapeseed crosses of disparate origin exhibit greater levels of heterosis than crosses between more genotypically similar lines (Lefort-Buson, et al. 1987). These four homozygous parents were hand-crossed in a half-diallel design to produce six cross combinations, and the parents were allowed to self-pollinate in order to maintain the lines through the next generation. A portion of the seed from each of the F_1 families and the four homozygous parents was then grown in a four-replicate randomized complete block design under greenhouse conditions, and the plants were evaluated for dates of flower start and flower end, date of maturity, height at maturity and seed yield. The remainder of the F_1 seed was planted at one field site (Genesee, ID) with two replicates, and F_2 seed obtained from the greenhouse trial was planted at two field sites (Genesee and Moscow, ID) with four replicates at each site. The F_2 generation was also used to investigate heterosis because heterozygosity only decreases by 50% each generation; thus, any heterosis observed in the F_1 generation is present at 50% in the F_2 generation. Randomized complete block designs were used for all trials. The same morphological characters examined in the greenhouse trial were recorded in the field, with the addition of a lodging score at maturity.

Hayman and Jinks analyses were carried out on each trial to examine the inheritance of recorded characters. In these analyses, differences between progeny effects were partitioned into additive effects, directional dominance, non-directional dominance and other effects, such as epistasis or linkage. Correlation

analyses were also carried out to investigate relationships between morphological characters observed and yield.

Results

Hybrids in the greenhouse had 12.99% higher yield than the inbreds. Corresponding yield increases in the field F_1's were 7.89% and in the field F_2's were 20.15% and 5.47% at Moscow and Genesee, respectively. The Genesee trials experienced a severe diamondback moth (*Plutella xylostella* L.) infestation during the growing season, and this could have decreased the heterosis observed at that site.

Hayman and Jinks analyses revealed that additive genetic variance predominated in both greenhouse and field F_1's. A more complex inheritance pattern was observed at the F_2 stage, probably due to allelic segregation. Additive and dominance effects were found, along with other genetic factors.

Among the morphological characters observed, plant height, lodging and date of flower start influenced yield significantly in the greenhouse F_1's, field F_1's and Genesee F_2's, respectively.

Conclusions

At this time, it is premature to recommend the commercial production of hybrid canola, due to the inconsistent yield increases at the various sites, especially considering the elevated cost of hybrid seed. Furthermore, one must remember that the hybrids used in this study were produced by hand-pollination, whereas commercial hybrid production via such methods as cytoplasmic male sterility (CMS) or self-incompatibility (SI) can result in less than 100% hybrid seed. Thus, the heterosis observed at a commercial level may be even less than this study suggests. Additionally, commercial hybrid seed production methods can have adverse effects if complete fertility/compatibility is not restored in the hybrid seed. Further investigation is required before the yield advantage of hybrid spring canola can be confirmed.

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INCORPORATING ATRAZINE RESISTANCE IN INDIAN OILSEED RAPE

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Weed control is an important crop management strategy. Higher yields of oilseed rape (Brassica napus L.) can be achieved by effective weed control using triazine herbicides. Current commercial cultivars of B.napus are suceptible to three herbicides, but a weed biotype of B.campestris having a very high level of triazine tolerance was first found in Canada (Maltais and Bouchard, 1978). This cytoplasm resistance was transferred, via B. napus cv. Triton, seeds of which were kindly supplied by Prof. Beversdorf, to 15 elite B.napus lines at this University through backcross subtitution. In this communication we report the evaluation of these lines to analyse the biological cost of the atrazine resistant cytoplasm and to develop agronomic practice for commercializing atrazine based weed control.

MATERIAL AND METHODS

The experimental material comprised fifteen alloplasmic B. napus strains and their euplasmic donor parents. All these genotype were assayed for various yield components (including harvest index) and seed biochemical parameters like protein, oil, total sugars, crude fibre, and mineral content. The biochemical components were estimated using standard analytical procedures. For developing agronomic weed control recommendation a trial was laid down in a split plot design with two varieties (GSL-1 as commercial check and GSL-2, an atrazine resistant line) as main plots and weed control treatments as sub plots for two years 1991-92 and 1992-93. Atrazine was apllied @ 300g/ha, 400g/ha and 500g/ha as pre or post-emergence treatment. These treatments were compared with unweeded control and with the normal practice of two hand hoeings. Pre-emergence application was made immediatly after sowing, while post emergence application was made on one month old crop.

RESULTS AND DISCUSSION

A comparisom of isonuclear euplasmic and alloplasmic lines indicated the deterimental effect associated with the atr cytoplasm. This was evident from a general depression in the values for oil content, protein content and total sugar, in alloplasmic lines as compared to those recorded for euplasmic nuclear donor parents.Based on bioenergetic cost conversion of seed biochemical components, a yield penalty of 0.9 to 4.5 percent as found to be associated with atr cytoplasm. The agronomic investigations indicated that both pre as well as post- emergence application of atrazine was effective in weed control as compared to unweeded control plots. Dry weight of weeds at maturity in unweeded plots of GSL-2 was 247kg / ha.

Compared to this almost complete weed control was achieved following post-emergence application of atrazine @ 500g/ha. Except for pre-emergence application of atrazine @ 300g/ha, the dry weight of weeds in various treatment plots ranged from 13.0 kg/ha 75.0 kg/ha. An intresting observation was the yield superiority of GSL-2 over GSL-1 at all doses of application (including mechanical weed control) and comparatively a lower competitive ability of GSL-2 against weeds as compared to GSL-1. This was evident from lower yield of GSL-2 (1303 kg /ha) in the unweeded control as compared to GSL-1 (1390kg /ha) - as shown in table 1.

Table 1: Effect of atrazine application on the *gobhi sarson* GSL-2

Treatment	Yield (kg/ha)	Gross income (Rs/ha)	Cost of culti. (Rs/ha)	Cost Benefit Ratio (Rs/ha)
Unweeded	1303	11076	4621	1.40
Hand hoeing	1929	16397	5221	2.14
Atrazine 300 gm/ha Pre-emergence	1866	15861	4854	2.27
Atrazine 400 gm/ha Pre-emergence	2010	17085	4911	2.48
Atrazine 500 gm/ha Pre-emergence	1948	16558	4969	2.33
Atrazine 300 gm/ha Post emergence	1885	16023	4854	2.30
Atrazine 400 gm/ha Post emergence	1957	16635	4911	2.39
Atrazine 500 gm/ha Post emergence	1875	15938	4969	2.21

Maximum cost benefit ratio of 2.48 was achieved when weeds were controlled through atrazine (400g /ha) application. This was significantly superior than the corresponding cost-benefit ratios achieved at for unweeded control (1.40) and mechanical weed control (2.14). Pre-release farmer field trials are currently underway in the Punjab state to evaluate the performance of GSL-2 at the pre-emergence application of atrazine @ 400g /ha.

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A PURPLE MUTANT IN CULTIVATED RADISH (*RAPHANUS SATIVUS* L.)

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In 1992, a radish plant (*Raphanus sativus* L.) with purple root spontaneously occured in a male-sterile line corresponding to a third back-cross with the recurrent sterility maintaining line. This one was originated from a population of plants all belonging to lines of small European more or less red radishes. This population was adapted to field cultivation with half-long red and white roots and white flowers.

The mutant plant was quite similar to the others except for the purple colour of all the organs : primary cortex of the hypocotyl axis, root cortex, root flesh, petiole edges, insertion basis of leaves, and stem.

This plant was backcrossed in 1993 to the corresponding sterility maintaining line. On 350 observed progeny, the same number of red and purple plants was exactly obtained ; there was no significant difference between the two categories with regard to leaf and root measurements.

This 1:1 segregation is in agreement with that obtained by backcrossing following hybridation between red and white radishes. According to MAKAROVA and IGNATOVA (1981), root colour is controlled by two genes, one conditioning presence/absence of colour (*Aa*) and the other conditioning type of colour (*Bb*). This determinism leads to the same results than those of HOSHI *et al.* (1963) for whom the *R* gene controls the red colour and genes *R* + *E* control the purple colour.

This suggests that our spontaneous mutation concerns the *E* (or *B*) gene which controls the transformation of pelargonidin to cyanidin by hydroxylation (HARBORNE and PAXMAN, 1964). We have identified these two pigments, pelargonidin in red plants and cyanidin in purple plants, by their reaction to $FeCl^3$ and by their spectral analysis according to MARKHAM (1982).

This mutant line is available free of charge from the author*.

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Effects of Swathing on Yield and Quality of Spring Canola

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Farmers in the Pacific Northwest region of the U.S.A. have traditionally grown between 1,000 and 2,000 hectares of winter rapeseed (*Brassica napus* L.) annually. Since 1990 however, the amount of spring-planted, low erucic acid rapeseed, or canola, has increased from nearly none to an estimated 35,500 hectares in 1993. Spring *Brassica* crops, including spring canola, are new to Pacific Northwest growers, and they have adopted cultural practices developed in the western Canadian prairies where spring canola is grown extensively. The short growing season in Canada requires that canola be swathed prior to threshing to hasten maturity, avoid frost damage and reduce chlorophyll content (1, 2). Because of the longer growing season in the Pacific Northwest, compared to western Canada, swathing of spring canola may not be required. In order to investigate the need for swathing, a series of trials were initiated in 1992 at the University of Idaho.

A trial containing five cultivars of B. napus was planted at Moscow and Genesee, Idaho in 1992 and 1993, respectively, to compare the yield and quality of swathed and directly combined crops. The double low cultivars 'Global,' 'HC120,' 'Legend,' 'Vanguard' and 'Westar' were planted both years. In the preliminary trial grown during 1992, plots measured 1.07 by 4.9 meters, while in 1993 larger plots, 2.14 by 11 meters, were used. Four replications were used in each trial. To simulate swathing, plots were cut prior to harvest with a sickle-bar mower. Following Canadian recommendations, plots were swathed when approximately 40% of the seed on the main raceme had turned brown (1). Once the plants had sufficiently dried, the windrows were picked up and threshed with a Hege 125B plot combine. Directly combined treatments were harvested with the same plot combine when the plots were ripe. Indices measured included yield, 1000–seed weight, percent oil, and fatty acid composition. In 1993, two replicates of each treatment were also assessed for chlorophyll content.

Results

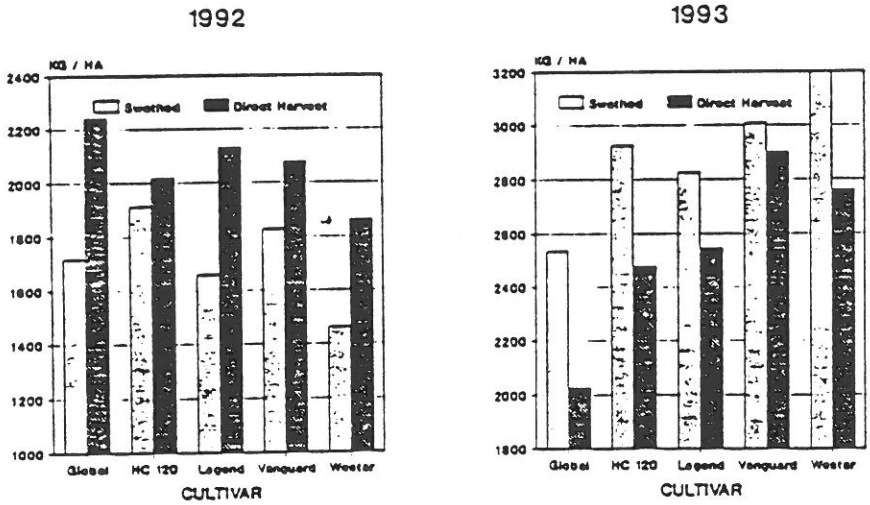
In 1992, swathing reduced yield across all five cultivars tested. The mean seed yield of the swathed treatment was 1,716 kg/ha, and the mean yield of the directly combined treatment was 2,065 kg/ha, both at 4% moisture (Figure 1). After threshing, seed from swathed plots contained a mean of 4.7 percent moisture and directly harvested seed contained 7.7 percent moisture. Weight of 1,000 seeds, oil content (percent) and fatty acid composition were not affected.

Swathed plots produced the highest yields in 1993. The mean yield of swathed plots was 2,899 kg/ha and the mean yield of directly harvested plots was 2,541 kg/ha (Figure 1). Percent moisture was 10.5% in swathed

plots, and 13.4% in directly harvested plots. Seed weight was lower in swathed plots, 3.27 g/1,000 seed, compared to 3.39 g/1,000 seed in directly harvested plots. Oil content and fatty acid composition was again unaffected. Mean chlorophyll content in the seeds from swathed plots was 7.3 ppm. When directly harvested all cultivars except Global contained less than 1% chlorophyll in the seed. Global contained 12.0 ppm and 4.8 ppm chlorophyll in swathed and directly harvested plots, respectively.

When compared across both years, yields for swathed and directly harvested plots were practically equal. Swathing did not produce a reduction in chlorophyll content as expected; conversely, chlorophyll content was actually higher in the swathed treatment. Yield losses encountered with the direct harvest treatment during the second year were most likely due to shattering losses. Timely harvesting could allow growers to minimize such losses. If swathing cost $20 per hectare and canola was 22 cents per kg , a grower could afford to lose about 90 kg/ha if he did not swath and still receive the same income if he had swathed. The contrasting results of this study suggest that the decision to swath or directly harvest should be made on a field-by-field and year-by-year basis. This study is currently being repeated on a near field scale basis with three cultivars and three swathing times.

Figure 1. Yield (kg/ha) of five canola cultivars from swathed and non-swathed plots grown in 1992 and 1993.



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Selection for fatty acid composition of *Brassica napus* using microspore culture

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Microspore culture has been accepted as an effective tool in the production of new varieties of *Brassica napus* (Swanson, 1990). The microspore system utilizes the haploid cell (the developing pollen grain), from which homozygous plants can be regenerated. Success in the production of microspore derived plants is influenced by a strong genotypic effect (Choung, *et al.*, 1988). Most work using the microspore system to produce new cultivars of *B. napus* has been aimed at the production of canola quality cultivars (*i.e.*, those with low erucic acid and low glucosinolate content). At the University of Idaho, we are concerned with producing both canola quality cultivars and industrial cultivars which produce oil with high levels of erucic acid.

The inheritance of erucic acid in *B. napus* is controlled by multiple alleles at 2 loci (Downey *et al.*, 1971). The gene action is additive, with each high erucic allele contributing about 12-13% erucic acid to the oil produced in the seed. If four high erucic acid alleles are present, then the oil will contain approximately 50% erucic acid.

Progeny of a cross between the high erucic acid cultivar 'Hero' and the low erucic acid cultivar 'Jaguar' produced intriguing results when the fatty acid profiles of the seed derived through microspore culture were compared to the profiles of F_3 seed collected from single plant selections produced by traditional breeding methods. The fatty acid composition of the seed from this cross was determined for 20 F_3 single plant selections from field grown F_2 plots and for 20 plants derived from microspore culture of the F_1 pollen.

Plants grown in the field showed a distribution of percent erucic acid similar to expected values (Table 1). Fifteen of the microspore derived plants were low in erucic acid and five were intermediate, a skewed distribution when compared to the expected ratio. The slightly skewed distribution and higher frequency of intermediate erucic acid types in the F_3 plants can be explained by a low frequency of outcrossing within the segregating population found in each field plot.

Plants from microspore culture with two copies of the high erucic allele at one loci and two copies of the low erucic acid allele at the other would produce seed with 24% erucic acid. Those homozygous for the high allele at both loci would have at least 48% erucic acid, and those homozygous for the low allele at both loci would contain less than 5%

erucic acid. The plants regenerated from microspores would only have alleles in the homozygous state and therefore could not be 12% or 36% types.

Table 1. The number of plants observed according to percentage of erucic acid in the oil extracted from the seed.

Source	<5%	12%	24%	36%	>48%
Expected ratio of F_3	1.25	5	7.5	5	1.25
F_3 observed frequency	3	5	9	3	1
Expected haploids	5	0	10	0	5
F_1 Microspore derived	15	0	5	0	0

The ratios of the plants observed produced via microspore culture were contrastingly different from the expected frequencies, with 15 plants low in erucic acid, 5 plants intermediate and no plants high in erucic acid. At least two explanations can be presented to account for this skewed distribution. First, selection pressure that acts against the allele for synthesis of erucic acid could exist as the microspores pass through the culture system. Alternatively, Hero may contain a gene or genes linked to one of the erucic acid loci that make it less receptive to the embryogenesis technique than Jaguar.

More detailed investigations of this characteristic are currently being carried out over a wider range of cross combinations.

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A new genetic stock for low erucic acid in Indian mustard (*Brassica juncea* (L.) Coss.)

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Mustard oil is considered to be inferior in quality to most other vegetable oils as it contains very high amounts of undesirable long chain fatty acids, like erucic acid (40-55%). *B. juncea* plants having genetic block in biosynthetic pathway towards erucic acid were intially identified in 1981. Since then these sources, named as Zem 1 and Zem 2, were utilized for improving the oil quality of Indian mustard. Experience at this University has shown that the low erucic acid character in Zem 1 is associated closely with several undesirable traits like yield penalty, low oil content, and susceptibility to downey mildew.

Concerted efforts were thus intiated to develop an alternate donor with in Indian accessions of *B. juncea*. Large scale screening of germplasm lines/segregating generations resulted in identification of a plant with intermediate erucic acid level (30 percent). Analysis of the subsequent self generations of this plant resulted in lower generation mean values for erucic acid i.e. 27.8q3.2, 25.5q1.4, 19.5q0.3, and 18.6q0.8 indicating lower overall mean and reduced varience following repeated selfing. It was possible to identify the genotypes having less than 10 percent erucic acid. One plant (S_7) even had 5 percent erucic acid. The analysis of fatty acid profile of the segregants with less than 10 percent erucic acid has indicated that the reduced erucic acid level was in general associated with higher amount of oleic acid (27%), linoleic acid (32%), linolenic acid (12%) and have no adverse associations like small seed size, low yield, lower oil content and susceptibility to downey mildew. While the backcrossing programme has been intiated to transfer this charcter to commercially important cultivars, the yield evaluation of low erucic acid derivants is underway. One such derivant, RLE 7 (8% erucic acid) has performed creditably in both local and all India cordinated yield trials. It surpassed both the commercial high erucic acid checks Varuna and Kranti by margins of 14.5 and 26.3 percent respectively. Half seed analysis of the individual seeds from plant with 5% erucic acid may help in identification of plants with '0' erucic acid. Efforts have also been initiated to do allelism tests of newly identified source with the previously available zero erucic acid source Zem 1.

Table : Mean values and range observed for various fatty acids in different self generations.

Population		Fatty acids				
		18.1	18.2	18.3	20.1	22.1
Base		23.6	8.5	14.6	-	82.4
S_2	M	19.6± 1.1	17.7± 1.1	11.3± 1.3	12.5± 0.7	27.8± 3.2
	R	12.8-25.3	11.1-22.9	3.1-18.2	7.3-15.9	13.1-45.4
S_3	M	21.7± 0.7	20.8± 0.8	10.9± 0.4	12.0± 0.3	25.6± 1.4
	R	12.8-34.6	7.5-26.6	7.4-18.4	6.1-15.7	12.2-44.8
S_4	M	26.3± 0.6	21.9± 0.4	11.1± 0.2	15.6± 0.4	19.5± 0.3
	R	19.0-29.8	20.1-25.4	9.7-12.3	13.3-18.5	16.9-21.4
S_5	M	20.5± 0.4	22.7± 0.3	14.3± 0.4	13.5± 0.2	24.2± 0.8
	R	12.5-27.3	16.6-27.8	10.1-29.0	7.9-16.8	15.8-41.5
S_6 (B)	M	20.6± 0.6	26.3± 0.5	13.2± 0.3	15.4± 0.4	20.3± 1.1
	R	12.3-28.5	18.7-37.8	9.5-19.0	7.7-21.2	10.1-43.8
S_6 (Y)	M	21.4± 0.7	27.2± 0.4	13.1± 0.4	15.7± 0.3	18.6± 0.8
	R	9.4-30.3	21.1-32.9	8.6-17.7	11.7-20.0	12.0-37.0

M = Mean; R = Range

Anthocyanin and chlorophyll content in Purple heading broccoli

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Introduction

Purple heading broccoli have edible inflorescences comprising immature flowerbuds (Gray 1982). Anthocyanins within the sepals give rise to the characteristic purple colouration which is best expressed under cool field conditions. Shading, as for example by leaves inhibits its development. Cultivation under warm glasshouse conditions also results in much of the purple colour being lost (Benoit and Ceustermans, 1987). Thus unlike green-heading calabrese cultivars, purple cultivars are less suited to production using protected cultivation methods.

Broccoli heads including green and purple forms are more tolerant to frost than are white-curded cauliflowers (Grout et al., 1982). Both forms contain chlorophyll, although in the purple heads it is masked by anthocyanin.

Of interest to us was the relative anthocyanin and chlorophyll content in purple heading broccoli, compared with calabrese, and how this might relate to both frost hardiness and to visual colour assessment.

Materials and Methods

Half-sib progenies of early, and partially inbred lines of late autumn-maturing purple heading broccolis, produced as part of a MAFF funded breeding programme, were sown in a glasshouse on 5 and 22 June respectively. Seedlings were pricked out into peat blocks to give approximately sixty plants per progeny or line. They were transplanted at Wellesbourne on 3 and 20 July into sandy loam at 0.6 m x 0.6 m spacing.

Single broccoli heads were harvested at marketable maturity on 25 September and 4 October from the early progenies (the early group) and on 16 and 23 November from the later-maturing lines (the late group). The colour of the heads was recorded in natural daylight in front of a north-facing window using a Horticultural Colour Chart (Anon, 1966).

Chlorophyll and anthocyanin content were measured separately on four replicate 50 - 200 mg fresh weight samples taken from each mature head. Chlorophyll was extracted in 3 ml absolute methanol for 24 h in the dark and the absorbance of the extract was measured at 650 and 665 nm. Total chlorophyll was estimated using Holden's equation (Holden 1965) and the results are expressed as μg chlorophyll per g fresh weight. Anthocyanin was extracted in 5 ml of a mixture of propan-2-ol, hydrochloric acid and water (81:1:81 v/v) for 24 h in the dark. Anthocyanin content was estimated by subtracting the absorbence at 650 nm from that at 535 nm, and to correct for weight differences between samples the results are shown as this figure divided by the fresh weight of the sample.

Results and Discussion

The early group showed wider variation in pigment concentration than the late group (Table 1). This was not unexpected since the late group had been selected over more generations and were also partially inbred. Nevertheless there were wide fluctuations in anthocyanin content in apparently similar broccoli heads suggesting some environmental effects. Chlorophyll levels as shown by their coefficients of variation, showed less fluctuation than anthocyanin but were lower in the early group compared to the late group. As expected, from the paler colour of the green parts in purple heads there was less chlorophyll in the purple broccolis than in the calabrese line sampled, ranging from 22% (PHB 5/89-7/3E) to 65% (PHB-4/89-13/3).

The overall differences in chlorophyll levels between the early and late purple heading broccolis may relate to their genetic origins and the conditions under which they were bred. The late group was derived from crosses involving autumn-maturing Purple Sicilian broccoli, spring-maturing (overwintering) purple Cape broccoli and a winter-heading Portuguese broccoli 'Roxo de Cabeça' (Crisp and Gray, 1984). The early group was subsequently derived from crosses between the late group above and cv. 'Rosalind', a late summer broccoli, itself developed from Purple Sicilian broccoli, 'Flora Blanca' cauliflower and calabrese (Crisp and Gray, 1984). Both groups have since been maintained as distinct populations over several generations. The late group was selected under comparatively cool conditions in October-November in which frosts occurred. It is thus possible that selection for frost tolerance could have resulted in enhanced photosynthetic activity and higher chlorophyll content as correlated responses.

The use of the Horticultural Colour Chart (Anon, 1966) broadly classified the colour of the broccoli heads and provided a constant reference. It was less useful for discriminating minor colour differences arising from surface appearance (texture) due to bud size and regularity, and degree of waxing (bloom). Perceived colour differences also bore little relation to the anthocyanin content, although it may be of interest that the only head (PHB 4/89-64/1) classed as 'Royal purple' was one of two in both groups that had by far the lowest chlorophyll content.

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Table 1. Assessmentsof chlorophyll and anthocyanin content in individual heads taken from purple heading broccoli and calabrese.

Individuals sampled	Horticultural Colour † Chart assessment	Anthocyanin (absorbance g^{-1} fwt)	Chlorophyll g^{-1} fwt
Purple Heading Broccoli (Early)			
PHB 5/89 - 4/1E	931	12.6	222
5/1E	934	6.1	137
5/2E	934	5.8	162
6/2E	931	14.1	161
7/1E	931	6.9	144
7/3E	934	2.9	108
20/1L	934	17.6	182
Mean		9.4	159.4
Variance		28.65	1303.33
Coefficient of variation		56.9%	22.6%
Purple Heading Broccoli (Late)			
PHB 4/89 - 3/1	934	4.9	155
4/1	934	11.8	310
11/1	934	10.0	227
13/1	934	14.2	142
13/3	931	11.1	319
15/1	934	13.0	151
30/1	not assessed	4.1	198
42/1	934	4.8	189
45/1	934	8.7	278
48/1	934	9.3	254
48/3	931	12.0	197
53/1	934	7.1	242
63/1	934	9.2	170
64/1	834	9.1	111
66/5	934	16.0	209
69/3	934	4.6	290
71/1	934	3.0	180
Mean		9.0	213
Variance		14.49	3761.31
Coefficient of variation		42.3%	28.8%
Calabrese			
GHB 5/89 - 31/1	0906	0	490

† 931 = dahlia purple; 934 = plum purple; 834 = royal purple; 0906 = spinach green

VARIATION OF SEED GLUCOSINOLATES CONTENTS IN JAPANESE WINTER RAPE

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Since 1930, 47 cultivars of rape (*Brassica napus* L.) have been breeding in Japan. However, glucosinolates contents of these cultivars were not determined until now. From some years ago, we have been screening to breed the rapeseed having low glucosinolates contents and excellent agronomic characters in Tohoku Nat. Agr. Exp. Station. In this study, we report the differences of seed glucosinolates contents on Japanese rape cultivars.

Analysis of total amount of seed glucosinolates was performed on 50 cultivars (contained 3 local varieties) of Japanese rape and 12 cultivars of Canadian and European rape using Palladium-glucosinolate complex method. Seed glucosinolates composition was analysed using HPLC method.

The average of total amount of glucosinolates of Japanese cultivars varied from 60.5μmol/g of Norin 18 to 161.4μmol/g of Norin 12 (Fig.1). In 50 Japanese cultivers, 2 cultivers of Norin 16 (65.4μmol/g) and Norin 18 (60.5μmol/g) showed low glucosinolates contents.

HPLC analysis of seed glucosinolates revealed that rapeseed had 10 main peaks (Fig.2). Of these peaks, peak area of No.1, 6, 7 in chromatograms had more than 80% of all glucosinolates peaks area, and they were identified as Progoitrin, Gluconapin, 4-Hydroxy-glucobrassicin, respectively.

Norin 16 and Norin 18 have been bred by the breeding program of "*B.napus* X *B.campestris*" about 45 years ago. In this program, fermale parent was same variety group of "Wase-chousen". Now, investigation of origin of such low glucosinolates traits is currently carrying out.

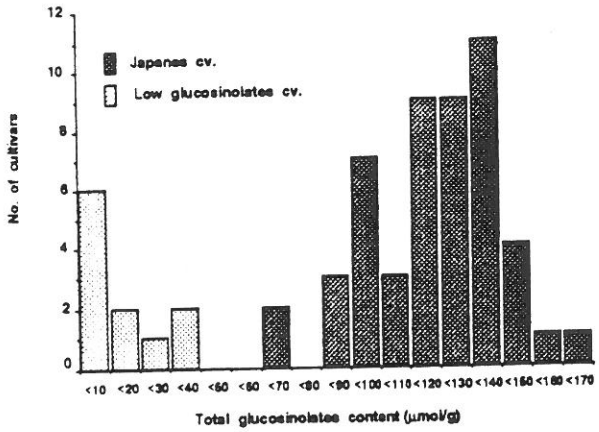

Fig. 1 Distribution of cultivars for total glucosinolates content in rape seeds.

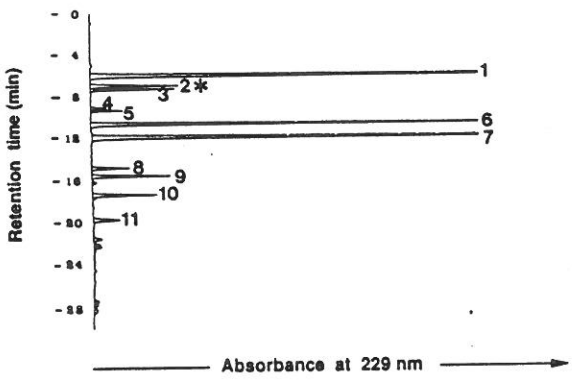

Fig.2 HPLC chromatograms of desulphoglucosinolates of cv. Norin 16 of *B. napus*.
✻: Internal standard (Sinigrin ; 0.12 μmol/ml)

EFFECT OF PARTHENIUM EXTRACTS ON HETEROCHROMATIN IN RADISH

U.C.Mehta, K.C.Mahato & J.N.Singh

In radish heterochromatin have been studied quite extensively and the genetic system responsible for the maintenance of an optimum level of heterochromatin (also known as chromocentres) and its distribution is understood to certain extent (Dayal, 1975, Dayal et al 1982, 83, 85).

There are only few studies on the effect of chemicals on radish. It is known that crucifers in general and radish in particular are highly resistant to chemicals (Zon & Rapoport 1971, I.Panicker et. al. 1985, Sarita Kumari 1988).

Only two varietal populations of radish, namely Bombay Red (BR) & Japanese White (JW) were used in the present investigation. 100-200 seeds were treated with different concentrations (10%, 20%, 30% & 40%) of Parthenium alkaloids for 6 hrs, 12 hrs, 24 hrs & 48 hrs. The seeds were sown simultaneously in identical field condition and plants were raised. Methods of cytological analysis are the same as used earlier (Dayal, 1975).

It is known that Parthenium alkaloid is highly carcinogenic & causes cancer.So, in the present study, the two varietal populations of radish varied significantly in the number and distribution of chromocentres per nucleus. Interestingly, the variety JW had significantly higher C.fr than the variety B.R. used of alkaloid, had a marked on Cfr.and its distribution pattern, significantly reducing the mean Cfr.in both the varieties at all the doses of chemicals at different hours (Table I). At this stage we do not exactly know as to what factors are actually responsible for the differential behaviour of different population of radish to alkaloids of Parthenium. Chromocentres have been considered as an adaptive characters. Besides, the role of genome size, genetic factors & DNA content may not also be overruled. Parthenium alkaloid probably breaks up the heterozygosity and the genetic balance and the buffering properties of the varietal populations of radish in different ways. Here it is shown that Parthenium alkaloid reduces the mean Cfr. and its distribution pattern in radish.

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Table I . The number and distribution of chromocentres/nucleus in two varietal population of radish at different concentrations and different hours of treatment.

Materials	No. of chromocentres in nuclei										Chromocentres/Nucleus Mean ± SE	CV(%)
	7	8	9	10	11	12	13	14	15	16		
JW(K)					6	16	18	54	80	26	14.3 ± 0.10	10.4
JW(10 % 6 hrs)				28	40	40	50	34	45	05	12.7 ± 0.05	0.45
JW(20% 12 hrs)				40	30	45	43	29	18	25	12.2 ± 0.48	0.49
JW(30% 24 hrs)				35	40	33	39	47	24	27	12.3 ± 0.25	0.32
JW(40% 48 hrs)				36	45	39	43	19	14	01	12.1 ± 0.35	0.30
BR(K)		48	35	34	24	26					10.4 ± 0.12	13.8
BR(10% 6 hrs)		35	39	45	15	29					10.0 ± 0.45	12.6
BR(20% 12 hrs)		40	17	44	65	64					9.8 ± 0.45	11.5
BR(30% 24 hrs)		30	55	27	44	46					9.4 ± 0.35	10.9
BR(40% 48 hrs)		44	40	17	35	34					9.1 ± 0.30	9.9

BRASSICA CULTIVARS AS SOURCES OF PLANT FIBRE

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INTRODUCTION

The Brassicas are a genus of the Cruciferae family and the economically more important members are used as human and animal food sources. There is relatively little information in the literature on the composition and use of forage rape stems, especially for industrial uses. Results are presented of a comparison between forage Brassica cultivars for yield and composition of the fibre fraction as well as preliminary results on the quality of paper from forage rape stems.

MATERIALS AND METHODS

Eight cultivars of forage rape, most with known parentage, were compared. To assess differences, each cultivar was separated into an apical and a basal sample and analysed separately. The general analytical techniques used were accepted methods for cell wall (and fibre) analysis.

RESULTS

Yield comparisons between cultivars

The mean height, fresh weight and dry matter of five cultivars are shown in Table 1. There are considerable variations in height not only between the different cultivars but also within some of the individual cultivars. Large differences in yield were observed both between cultivars and between the apical and basal stem portions for each cultivar. For example, the average fresh weight of the basal sample from Bonar was 98.8g while the average fresh weight of the apical sample was 29.8g. There was less difference in the values for Winifred, namely 70.5g and 57.1g, respectively. The average dry matter yields per plant ranged from 36.4g for Crack to 20.1g for Emerald. Therefore, the choice of cultivar will have a major effect on the yield of dry matter.

TABLE 1. Mean height, yield and DM content of some of the Brassica cultivars.

Cultivar	Mean Height (m)	Yield g/plant		Dry Matter (%)		Yield g/plant
		Apical	Basal	Apical	Basal	
Crack	0.74 ± 0.11	78.3	115.3	16.1	20.6	36.36
Bonar	0.71 ± 0.06	49.8	98.8	15.2	16.6	23.97
Winifred	0.33 ± 0.09	57.1	70.5	17.4	19.1	23.41
Arran	0.87 ± 0.18	39.3	88.2	24.7	17.7	25.32
Emerald	0.59 ± 0.31	35.1	67.0	17.6	20.8	20.12

Fibre comparisons between cultivars

The yields of fibre and the major components present in the fibres, cellulose, NCP and lignin, are shown in Table 2. For all cultivars, the apical sample had a lower total fibre content and lower individual components of fibre than the basal sample but the difference between apical and basal

material varied considerably between cultivars. Hence, there could almost be a two-fold difference in fibre yield depending on the cultivar selected.
The basal samples of each cultivar also had higher cellulose, NCP and lignin contents, as percentages of the dry matter, than the apical samples. However, when the results for cellulose, NCP and lignin were calculated as percentages of the fibre content, the differences between apical and basal samples became far smaller. It was only the lignin content which was always higher in the basal sample than the apical sample. The carbohydrate composition in the stem fibre is relatively consistent: There is a gradual reduction in the lignin content on progressing from base to apex. Since cellulose is the most important constituent for industrial uses, differences of almost 100% in its yield could be obtained, assuming all the cultivars tested can be grown at the same stocking rate.

TABLE 2. Fibre, cellulose, non-cellulosic polysaccharides and lignin content of Brassica cultivars (% dry matter). The figures in parenthesis are the fibre components as a percentage of the fibre.

Cultivar		Fibre	Cellulose	NCP*	Lignin
Crack	Apical	62.7	36.1*(37.6)*	18.4*(29.3)*	7.2*(11.5)*
	Basal	80.3	45.2*(56.3)*	24.1*(30.0)*	9.5*(11.8)*
Bonar	Apical	65.9	37.8*(57.4)*	22.4*(34.0)*	5.9*(9.0)*
	Basal	75.0	40.4*(53.9)*	22.9*(30.5)*	10.0*(13.3)*
Winifred	Apical	69.2	39.3*(56.8)*	21.6*(31.2)*	6.7*(9.7)*
	Basal	81.3	44.0*(54.1)*	25.1*(30.9)*	9.7*(11.9)*
Arran	Apical	68.5	33.3*(48.6)*	17.4*(25.4)*	6.2*(9.1)*
	Basal	78.4	42.5*(54.2)*	26.1*(33.3)*	8.6*(11.0)*
Emerald	Apical	62.6	35.8*(57.2)*	19.3*(30.8)*	7.5*(12.0)*
	Basal	74.0	43.2*(58.4)*	20.2*(27.3)*	10.1*(13.6)*

*NCP Non-cellulosic polysaccharides

Paper quality comparisons between cultivars
Finally, pulps were prepared from selected cultivars and the quality of paper handsheets compared with a standard eucalyptus and a standard mechanical pulp. Although the pulping method was not optimised, it is clear that acceptable paper can be produced and warrants further investigations.

Table 6. Physical properties of paper from forage rape pulps (with two standards for comparison)

Cultivar	Burst Index $kPa.m^2g^{-1}$	Tear Index $mN.m^2g^{-1}$	Tensile Strength Index $N.mg^{-1}$	Density $g.cm^{-3}$
Crack	3.15	6.7	59.2	0.66
Bonar	2.49	4.3	41.3	0.56
Winifred	2.45	2.3	41.0	0.71
Eucalyptus	6.80	11.8	89.0	0.70
Mechanical	1.22	4.1	24.5	0.46

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Induction of chlorophyll-deficient mutants in *Brassica napus*

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Introduction

Nucleus or chloroplast encoded chlorophyll deficiency can be used as visible marker in protoplast fusion. Depending on the inheritance of the mutation nuclear and/or cytoplasmic hybrids can be detected. Although this technique has proven to be successful (e.g. in the *Solanaceae*) it was not applied for protoplast fusion in the genus *Brassica*. An efficient mutagenesis-system is one prerequesite for this task. It should enable the induction of a large number of mutants (preferably from seeds) in a reasonable time frame from any desired genotype.Therefore we tried to establish a seed mutagenesis treatment for *B. napus* using N-nitroso-N-ethylurea (NEU) which is known to induce mutations in the chloroplast genome (Hagemann 1982).

Material and Methods

Seeds of three *B. napus* cultivars were used: a. "Brutor", b. "Loras", (both spring-rapeseed) c. "Ceres" (winter-rapeseed). A greenhouse method as well as an in vitro technique was applied. The protocol for seed mutagenesis is given in Fig. 1.

Fig. 1 Protocol for induction of chlorophyll-deficient mutants in *B. napus* with Nitroso-ethyl-urea (NEU)

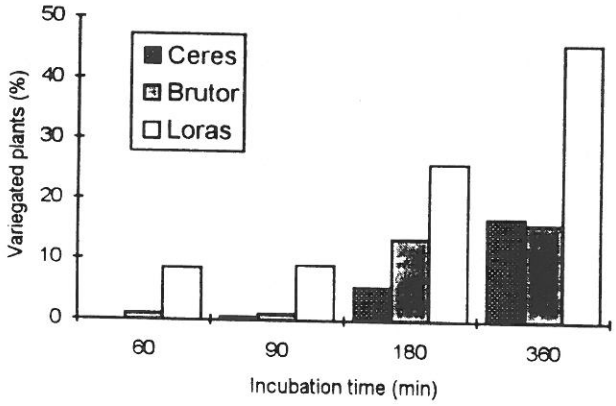
In vitro		Greenhouse
Sterilize seeds ↓ Incubate in 10 mM NEU Wash 3 times in H_2O ↓ Transfer on hormone-free MS-medium or water-agar for germination ↓ When plants expand cotyledons, transfer to MS-medium (2mg/l BAP, 30 g/l sucrose)		Incubate in 10 mM NEU Wash 3 times in H_2O ↓ Transplant to soil and transfer to greenhouse
↓ Select for variegated plants/sectors	→ Transfer of chimaeric plants to greenhouse	↓ Select plants with variegated sectors
↓ Select and subculture on the same medium until pure lines are obtained	← Sterilize variegated sectors and transfer in vitro	↓ Promote growth of variegated sectors by cutting unaffected shoots.
↓ Maintain pure lines as sterile shoot cultures on MS-medium (0,1mg/l NAA, 30g sucrose)		↓ Selfing or reciprocal backcross with the original line

Incubation times of 30, 60, 90, 180 and 360 minutes for the in-vitro technique and 90, 180 and 360 minutes for the greenhouse method were applied. 200 seeds/genotype were treated for every incubation time.

Results and discussion

In both experiments the mutagenic treatment resulted in a delayed germination of the seeds. The over all germination rate was in the range of the untreated control (95-99%) even at an incubation time of 360 min. Variegation (white or yellowish sectors) was scored on the first true leaves (Fig. 2).

Fig.2 Percentage of variegated plants after in vitro mutagenesis with 10mM NEU



By selection of sideshoots variegated sectors could be enriched and pure white lines were obtained from all genotypes (cv. "Loras" 60,90,180,360 min, cv. "Brutor and "Ceres" 90,180,360 min). Therefore this protocol enables the production of chlorophyll-deficient mutants directly from the M_1-generation. Many of these mutants show retarded growth habit, altered leaf morphology and are unable to form roots. Viable protoplasts can be isolated from some mutants. They divide, form calli, but do not regenerate plants.

Whereas the percentage of variegated plants in the greenhouse experiment was comparable to the in-vitro procedure only small parts of the leaves were affected. Completely white side shoots were only obtained from cv. "Loras" at an incubation time of 360 min. Walters et al. (1990) were able to induce variegation in *B. campestris* by seed mutagenesis with much lower concentrations of N-Nitroso-N-methyl-urea (NMU) (25µg/ml compared to 1100 µg/ml NEU in this investigation).

The transfer of plants into the greenhouse after enrichment of variegated sectors in vitro enabled the induction of pure white/yellowish flowering sideshoots. Most of these side shoots were female sterile and showed reduced pollen ferility. First investigations on a limited number of plants showed, that the mutation is maternally inherited.

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INHERITANCE OF SEED COAT COLOUR IN INDIAN MUSTARD [BRASSICA JUNCEA (L.) Czern & Coss.]

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The de-oiled cake of rapeseed and mustard is a good source of protein and used as a major cattle feed. But a major portion of which is not utilised due to high content of glucosinolates and reddish to blakish brown colour of seed coat resulting brown seed specks of seed meal. Besids, brown seed cultivers of mustard yield low oil content together with dull coloured oil rated poor than the bright yellow oil with high oil content produced by light seed colour, Stringam *et al.*,1974. Due to good clarity of oil,yellow sarson is preferred over brown sarson.Thus the present investigation was carried out to study the nature of inheritance of seed coat colour in Indian mustard.

The yellow seeded varities vij.;DIRA 313, DIRA 326 and RW 3/86 were crossed with brown seeded varities namely, RCC 15,Kranti and Krishna of Indian mustard. F1 plants alongwith their parents from each cross were grown in field during rabi 1988-89. At flowering each F1 plant was emasculated and back crossed as female to yellow and brown seeded one and self pollinated by bud pollination to get seeds for F2. A fresh crosses were also made between parents to get F1 seeds. Thus seeds of three families consisted of six generations each (P1,P2,F1,F2,BC1 and BC2) were grown in randomised block design with three replications during rabi 1989-90. In each replication two rows of parents and each F1's,three rows of back crosses and six rows of F2's were sown in 3m long rows keeping 45cm distance between rows and 15cm between plants within rows.

Open pollinated field grown back crosses and F2 plants were classified for seed colour after they had fully matured. Two broad classes of seed colour were identified and all seed was classified as either brown or yellow. Data were analysed using chi square method.

The seed coat colour of all F1 plants of each cross were found brown seeded which indicate that brown seed colour is completely dominant to yellow. The observed number of brown and yellow seeded plants in F2 and back cross progenies with their respective ratios of segregation and Chi square values is given in Table-1. The plants of F2 generation segregated into 15 brown: 1 yellow in all cross combination idividually as well as over all. The BC2 generation of

all the crosses gave a segregation ratio of 3 brown: 1 yellow. The F2 and the test cross behaviour indicated the digenic nature of seed coat colour inherited with duplicate type gene action. The findings are in agreement with the conclusion of Stringam et al.(1974),Vera et al.(1979) and Chauhan and Kumar (1987) but differ from that of Nayar and George (1970), who reported monogenic control for this character in rapeseed and mustard.

Table 1. F_1,F_2 and back cross progeny classification for reddish brown Vs yellow seed coat colour in Indian mustard

Crosses	F1	F2 plants Brown	F2 plants Yellow	Ratio	B1	B2 ratio Brown : Yellow	Value (χ^2)
RCC15 x DIRA313	Brown	343	17	15:1	Brown	3 : 1	1.42
Kranti x DIRA326	Brown	340	20	15:1	Brown	3 : 1	0.28
Krishna x RW3/86	Brown	340	18	15:1	Brown	3 : 1	0.96

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BREEDING APPROACHES FOR SALT TOLERANCE IN RAPSEED AND MUSTARD

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In arid and semi arid regions the problem of salinity in ground waters and irrigated soils is very common. Moreover, these areas receive scant and erratic rainfall and are characterized mainly by inherent salinity problems.

Accounting Problems : Salt concentration in the root zones proves deterimental for plants, imposing effects on growth stages and ultimately very adverse effects on yield (Bernstein, 1964; Mass and Hoffman, 1977; Kumar et al., 1988). The saline area is increasing with the increasing irrigated areas. Thus to combat this problem of salinity, there are two different approaches (i) Reclamation, drainage and improved irrigation practices. But these practices are costly hence, prohibitive for poor farmers to adopt them, (ii) Breeding for salt tolerance which may provide a relatively cost effective short term solution.

Adaptation of Brassica species : Among various species of Brassica, two species namely B. juncea and B. carinata have shown wide adaptation due to their high yield. The amphidiploids have been found to possess much higher tolerance to salinity as compare to their diploid species (Chhabra and Singh, 1994).

Early screening suggests, genetic variability for the parameters which confer tolerance to salinity :

It is well established that soil salinity/alkalinity conditions affect almost all the plant stages in one way or other. In fact, a general depression in growth by these adaphic stresses have been known since long but the mechanism of these adverse effects are not yet fully understood (Stapels and Toennissen, 1984). Soil salinity may affect germination (i) by increasing the osmotic pressure of soil solution to a point that will restrict the intake of water or by (ii) causing toxicity to the embryo. Both factors retard/prevent the germination process, resulting in poor stand of the crop. Devine (1982) concluded that among the vegetative growth phase, the seedling stage was the most efficient stage for screening of large number of genotypes for salt tolerance.

Systematic research programme has been initiated to generate the basic information on the genetic variability in the germplasm lines of Brassica juncea and Brassica carinata, identification of source material, gathering genetic information for breeding and to identify salinity tolerant genotypes systematically in B. juncea and B. carinata. The researches are being conducted at seedling level and adult plant basis under laboratory and varification under field conditions. The efforts are being made to identify some characters of seedling stage itself.

The germplasm lines of B. juncea and B. carinata has been screened in laboratory condition in petriplates under various levels of salinity. It was observed that salinity affected the rate of germination in all the genotypes of Indian and Ethiopian mustard but had little or no effect on the final count of seed germination. Bernstein and Hayward, 1958 also found that salinity generally reduces the germination with little or no effect on the final number of seedlings which emerge. Weisel (1972) also reported such a behaviour of halophytes and glycophytes in saline media is not unusual. When varieties show similar levels of germination, speed of germination is helpful in bringing the differences in varietal responses (Singh and Rana, 1989). It was observed that speed of germination, root length, shoot length and seedlings dry matter are important seedling parameters for selecting salt tolerant genotypes in rapeseed and mustard. On the basis of these parameters RH 7859, RH 7846, RH 781 of B. juncea and BC 2, HC 7, HC 15/were found to be relatively salt tolerant whereas RH 8315, RWH 1, RH 8113 of B. juncea and HC 2, C6YS7B and CAR 5/were identified as salt susceptible genotypes. These results can be confirmed by conducting further experiemnts. After screening

/ of B. carinata

the genotypes for salinity, these can be utilized for generating further genetic information. Efforts are on to strengthen research work on this aspect in future.

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FIELD EVALUATION OF MUSTARD GERMPLASM RESISTANT TO WHITE RUST

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Rapeseed-mustard are the important **RABI** oil seed crops of northern india . White rust caused by **ALBUGO CRUCIFERARUM** (S. F. Gray) is an endemic problem and causes significant yield losses. The cultivation of resistant varieties is the best and cheapest method for getting ensured production. Considering the economic importance of the crop and the disease, 700 germplasm of rapeseed-mustard were screened during **RABI** 1989-91 under artificial epiphytotic condition. The experiments were conducted at Agricultural Research Station. Rajasthan Agricultural University, Sri Ganganagar. Several single plants which remained either free or showed highly resistant reaction were selected from 1989-91 sown materials. These single plants genotypes were sown in RBD with three replicates in a plot size of 2 X 2.5 m during two consecutive years. A highly susceptible cultivar "**VARUNA**" was used as a check. To ensure epiphytotics condition of the disease oosporic inocula was applied along the seed. Sufficient disease pressure was also created by artificial inoculation as foliar spray. Observations on white rust, malformation and seed yield were recorded.

The results of the two years rigrous testing showed that eight single plant genotypes selected from WRR -3-1, RH-8545, Gulivar and SV7739035 X RH30-12-15 exhibited< 5% disease and having 14-18 Q/ha seed yield. These genotypes have minimum number with smallest size of pustules and thus proved to be as highly resistant. Four genotypes viz. PC-5, SV7739035 x RH30-10-3, SV7739035 X RH30-10-6 and SV7739035 X RH30-2-17 contracted <8% disease with 12.4-18 Q/ ha seed yield recognised as resistant genotypes. Cultivar "**VARUNA**" popularly grown for commercial production in Rajasthan showed maximum number as well as larger size of pustules having 57.83 % white rust intensity and 42.26 % malformation. These resistant sources were used in rapeseed - mustard hybridization programme and the resultant crosses as expected are showing superiority both in terms of disease index and seed yield.

White Mold or Sclerotinia sclerotiorum Resistance in B. oleracea

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White Mold (WM) is a world wide disease which infects over 360 species of plants. WM causes losses to cabbage both in the field and in storage. It is damaging to cauliflower and broccoli especially during the reproductive stages, causing losses in seed production.

WM resistance is being developed in a number of crops and most of the progress in breeding has been in the past 10-15 years. The fungus needs a source of energy for the spores to germinate and initiate infection. In most crops this source is a flower. In some crops such as cabbage, researchers have not yet determined the source of energy or the site of initial infection, although weed flowers and seeds and insect frass have been suggested. For our trials we have used agar discs infected with WM. In 1991 and 1992 we placed two discs on each plant under the wrapper leaves, when the cabbage heads were partially to fully developed. On cauliflower and broccoli the discs were placed in leaf axils. The plants were then irrigated three times a day for 5 minutes to provide adequate moisture for the development of the disease. In 1992, in spite of careful application of the discs we felt there probably was some variation due to the applicator. In 1993 we applied 4 discs, one on each quadrant of the plant and each was applied by a different person. This inoculation resulted in a severe and uniform test where all of the susceptible cabbage and cauliflower checks died

We tested six commercial cabbage varieties previously identified as very susceptible (3) and more tolerant (3) especially one line. Every plant of all 6 varieties died, but the plants of the most tolerant line survived longer than other lines. There seemed to be some tolerance or possibility of escape among the broccoli lines. However, all plants were individually scored on a scale of 0-5 for degree of disease about 18 days after inoculation with the agar discs, at which time the majority of the most susceptible plants had collapsed. We also removed a petiole + leaf or large leaf of a cabbage from each plant. The petioles were cut to 15cm length and the cabbage mid rib removed to provide a 15 cm rib. An agar disk was placed on the stem end of the petiole and they were placed in a crisper (plastic box) with 0.5cm of water in the bottom and covered with a screen on which the petiole were placed. 10 days later the length of infection of the petiole was measured as well as a score

given for the degree of mycelial growth. The results with the crispers were somewhat erratic and the most reliable result was from the field score of each plant for disease level.

The results indicated resistance was recessive. All F1s died and crosses of cabbage x a resistant collard indicated a major recessive gene for resistance. Crosses of a tolerant savoy cabbage x cabbage indicated recessive resistance, but possibly more than one gene might be involved.

Plants which survived in the field with little of no disease were dug and saved for seed production. The progeny will be tested in 1994. However, in view of the uniform lethality of the test on the check cauliflower and cabbage, the plants which survived must have had a level of resistance. It was also apparent that the collard had good resistance, although there is no such thing as immunity to WM in any crop tested so far. A few plants which had no sign of infection may have been escapes, but with four inoculation sites on each plant that is hard to believe.

It appears we can breed for resistance to WM in B. oleracea. Seed is not available from this program in 1994, but hopefully will be in 1995.

Table 1. Segregation for White mold Resistance. Collard x Cabbage Cross

Pedigree	No. Plants in Disease Classes*								
	0	1	2	3	4	5	R:S	Expected	P
8204 x 8063F2	0	8	7	5	14	36	15:55	17.5:52.5	.50
(8204 x 8063)8204	1	16	25	10	2	16	41:30	35.5:35.5	.25
(8204 x 8063)8063	0	0	0	1	3	68	0:72	0:7	1.00
8204 collard	18	19	13	9	3	13	50:25	75:0**	- -
8063 cabbage	1	0	0	0	0	34	1:35	0:36	1.00
Cabbage x Cabbage Cross									
8246 x R UpF1	0	0	1	0	5	12	1:17	0:18	
8246 X R UpF2	2	0	10	3	9	47	12:59		
(8246 x R Up)R Up	2	0	6	2	4	58	8:64	0:72	
(8246 x R Up) 8246	1	0	4	4	9	53	5:66		
R. UP	0	0	1	1	1	15	1:17	0:18	
8246	10	1	3	2	7	30	13:39	52:0**	

*, 0 = No disease, 1 = slight infection, 5 which indicates the plant is dead or totally collapsed.
**. We have never had 100% survival in these tests even with the collard which has the highest level of resistance. However, both lines consistently do much better than any other lines among the over 400 tested.

Transfer of Disease Resistance to *Brassica oleracea* L. by Protoplast Fusion

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Introduction

Black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* and black leaf spot caused by the fungus *Alternaria* spp. are two of the most important diseases of *Brassica oleracea* vegetable crops. Sources of resistance to the diseases within *B. oleracea* are insufficient, and available control means are limited. Therefore, development of breeding lines with high stable resistance will be extremely valuable.

Sufficient resistances are not found in species that readily cross sexually with *B. oleracea*. Certain lines of *B. napus* contain high resistance to *X. c.* pv. *campestris* (Guo et al. 1991), and *Sinapis alba* possesses good resistance to *Alternaria* spp. in addition to other desirable characters such as drought resistance and possibly resistance to the cabbage maggot as well. Since crossing these species to *B. oleracea* is very difficult, an alternate way of gene transfer must be used. This is offered by protoplast fusion, which may overcome the interspecific and intergeneric crossing barriers.

Materials and Methods

Rapid cycling *Brassica oleracea* L. (CrGC 3-1) was fused with either a *B. napus* L. line highly resistant to *X. c.* pv. *campestris* or a genotype of *S. alba* L. selected for good cell division in protoplast culture. Protoplasts were isolated enzymatically from newly expanded leaves of in vitro grown plants. The *B. oleracea* fusion partner was inactivated by treating the protoplast solution with iodoacetate prior to processing to prevent division of unfused protoplasts. Since *B. napus* and *S. alba* have poor regenerability, no pretreatment was needed for these species. The protoplast fusion was done according to Thomzik and Hain (1988), with minor modifications. The protoplasts were plated on feeder layer plates (Walters and Earle 1990) in a concentration of 8×10^4-1×10^5 intact protoplasts per ml. The protoplasts were cultured on the series of media developed by Pelletier et al. (1983) as previously described for rapid cycling *B. oleracea* (Hansen and Earle in press).

Hybrid identity was confirmed by morphological characters and by estimation of nuclear DNA content using flow cytometry according to Arumuganathan and Earle (1991). The *B. oleracea* (+) *B. napus* fusion products were further analyzed by RAPD PCR markers, and the *B. oleracea* (+) *S. alba* fusion products were examined by isozyme analysis using cellulose acetate electrophoresis.

The *B. oleracea* (+) *B. napus* hybrids were tested for resistance to *X. c.* pv. *campestris* by pricking leaves with a pin dipped in cultures of the pathogen followed by incubation for 7 days at 30°C. *B. oleracea* (+) *S. alba* fusion products were screened for resistance by spraying the plants with a suspension of *A. brassicae* conidiospores (5×10^5 spores/ml) and incubated under mist at 20°C for 5 days.

Resistant *B. oleracea* (+) *B. napus* hybrid plants were self pollinated and crossed reciprocally to a bridge line, 'line 15' (Quazi 1988), and directly to various genotypes of *B. oleracea* followed by embryo rescue attempts. Offspring have been analyzed like the somatic hybrids.

Results

Brassica oleracea (+) *B. napus*

Out of a total of 460 isolated calli, shoots regenerated from 6 calli. Ten plants were obtained and grown to maturity.

Flow cytometry showed that four plants had a DNA content close to the sum of the two parents, one plant had twice that amount of DNA, and five plants were *B. napus* escapes. RAPD analysis has confirmed hybrid identity. Morphologically the hybrids resemble *B. napus*, although they are larger and more branched. The plant with doubled DNA is abnormal with sterile flowers.

Inoculation with *X. c.* pv. *campestris* showed no or very little symptom development in the four normal fusion products, nor in the five *B. napus* escapes. The abnormal plant with high DNA content showed severe symptoms.

Offspring have been obtained from the crosses between the resistant somatic hybrids and 'line 15' as well as from self-pollination of the hybrid plants. All analyzed plants from crosses to 'line 15' were susceptible, while almost all plants from self pollination had good resistance. Out of 169 direct crosses between the somatic hybrids and *B. oleracea* followed by ovary culture, one embryo was used successfully as an explant for shoot morphogenesis. Four normal plants were obtained. These plants have shown DNA contents intermediate to the two parents and high resistance to the pathogen. Pollen tube growth in crosses between these plants and various *B. oleracea* genotypes is being studied by fluorescence microscopy.

Brassica oleracea (+) *Sinapis alba*

Shoots developed from 8 out of 1527 isolated calli, generating a total of 25 plants. All plants show intermediate morphology, with partially divided leaves and some trichomes on stems and leaves. Plant height is 1-1.5 m. The flowers have reduced anthers with little pollen production.

Flow cytometry as well as the banding patterns of both the enzymes LAP and PGI confirmed the hybrid status of the regenerated plants.

Plants from cuttings from the somatic hybrids have shown variable degrees of resistance to *A. brassicae*, some having very good resistance. Similarly obtained plants are being screened for resistance to the cabbage maggot.

Backcrosses using *B. oleracea* as the pollen parent are being attempted. Pollen tube growth is being studied by fluorescence microscopy

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Evaluation of TuMV-resistance in f monosomic addition line of radish (2n=19)

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Introduction

Monosomic addition lines of radish with single kale chromosome (a~g types, 2n=19, KANEKO et. al. 1987) are valuable for the genetic analysis of each kale chromosome and for the introgression of some genes to radish. On the artificial inoculation tests of turnip mosaic virus (TuMV) in seven monosomic addition lines of radish (a~g types), resistant gene(s) have been suggested to locate on the added f kale chromosome (KANEKO 1989).

In this paper, the degree of resistance to TuMV was measured by the enzyme linked immunosorbent assay (ELISA) in the original f type of monosomic addition line and f type of monosomic addition line that was derived from crossing between the original f type and four Japanese radish cultivars.

Materials and Methods

The original f type of monosomic addition line of radish cv.'Shogoin' was used as female parent and crossed to four cultivars of Japanese radish (Kuroba-Minowase, Miura, Miyashige-Sobutori and Horyo). After the second backcrossing, f type of monosomic addition line of four Japanese radish (2n=19) and the revertants (2n=18) were obtianed. The original f and these monosomic addition lines, the revertants, *Raphanobrassica* Rb_{63} (2n=36, MATSUZAWA et al. 1985) and radishes (Miura F_1 hybrid and pure-bred Shogoin, Kuroba-Minowase, Miyashige-Sobutori and Horyo) were also used in this study. Seeds were sown in the middle of September and plants were grown in vinyl-house.

For inoculation test, diseased leaves were homogenized with a buffer which was a mixture of 1/15 M Na_2HPO_4 and 1/15 M KH_2PO_4 in the ratio of 7:3 and then 0.1% 2-Mercaptoethanol was added. The suspension was inoculated on each two young leaves of one-month-old seedlings sprinkled with the 600 mesh carborundum.

The ELISA was adopted according to CLARK & ADAMS (1977). From three weeks after the inoculation, the observation and scoring of visible disease symptoms were performed on alternate weeks.

Results and Discussion

The symptoms caused by TuMV in the revertants (2n=18) and radishes were observed at two week after the inoculation.

The symptom and ELISA value at the third and fifth week were shown in Table 1. In the third week, all of the f type of monosomic addition lines did not show the detectable symptom, and ELISA value was from 0.135 to 0.185 (av.0.154). The value of Rb_{63} was 0.185. On the other hand, revertants (2n=18) and radishes were susceptive to this diseases, resulting in the mean ELISA value of 1.259 to 0.774, repectively. The value of the revertants derived from f type of 'Shogoin' and cv.'Shogoin' were lower, although the leaves exhibited some degree of the symptom. It was consequenly suggested that cv. 'Shogoin' have the fluctuating resistant gene(s) that was different from the one(s) locating on the f chromosome.

In electromicroscopical analysis, no virus particles were detected in any leaves of the f type and Rb_{63} plants.

Above mentioned, f type of monosomic addition lines of radish may have remarkable resistance to TuMV, because the suppression of the virus multiplication was observed. Now, we are analyzing this gene(s) and introgressing to radish.

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Table 1. Score of visible disease symtom and ELISA value of TuMV on the youngest leaf of five f type of monosomic addition lines (2n=19), Rb_{63}, the revertants (2n=18) and radishes at each observed week .

			The third week		The fifth week	
Strains	Type[1]	No. of Plants observed	Score[2]	Elisa value	Score	Elisa value
f × Shogoin	f	5	-	0.185 ± 0.085	-	0.185 ± 0.105
f × Kuroba-Minowase	f	5	-	0.151 ± 0.071	-	0.190 ± 0.110
f × Miura	f	2	-	0.135 ± 0.009	-	0.134 ± 0.023
f × Miyashige-Sobutori	f	5	-	0.154 ± 0.074	-	0.136 ± 0.035
f × Horyo	f	5	-	0.144 ± 0.021	-	0.145 ± 0.022
Mean				0.154		0.158
Rb_{63}	RC	3	-	0.185 ± 0.096	-	0.171 ± 0.032
f × Shogoin	R	3	+,++	0.423 ± 0.358	-,+	0.333 ± 0.150
f × Kuroba-Minowase	R	3	++	1.655 ± 0.488	-	0.248 ± 0.061
f × Miura	R	3	++	1.577 ± 0.200	+,++	0.620 ± 0.268
f × Miyashige-Sobutori	R	2	+,++	1.160 ± 0.232	+	0.645 ± 0.368
f × Horyo	R	3	++	1.478 ± 0.495	-	0.205 ± 0.041
Mean				1.259		0.410
Shogoin	R	3	+,++	0.206 ± 0.034	-,+	0.283 ± 0.100
Kuroba-Minowase	R	3	-,+	0.697 ± 0.500	-,+	0.199 ± 0.074
Miura	R	3	+,++	0.606 ± 0.486	-,+	0.211 ± 0.056
Miyashige-Sobutori	R	3	+,++	1.151 ± 0.372	-,++	0.349 ± 0.077
Horyo	R	3	+,++	1.211 ± 0.260	-,+	0.350 ± 0.242
Mean				0.774		0.278

[1]Type : f - f type monosomic addition lines (2n=19), RC - Genome symbol of *Raphanobrassica* (2n=36), and R - Genome symbol of the revertants (2n=18) and Radish.

[2]Score : - (Non symptom), + (Symptom in the youngest leaf after infection), ++ (Serious symptom in it).

Identification of Resistance to *Alternaria brassicicola* in *Brassica oleracea*

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Introduction

Alternaria leaf spot of crucifers can be caused by three different pathogens, but the most common pathogen causing disease on vegetables appears to be *A. brassicicola*. There have been some reports of host plant resistance to this disease, but we could find no reports describing the genetics involved in resistance to this potentially severe disease. We were able to identify sources of partial resistance to *A. brassicicola*, optimize the screening conditions for detecting resistance among these sources, and determine the genetics involved in resistance.

Materials and Methods

More than 600 crucifers (410 accessions of *B. oleracea*) were screened for resistance to *A. brassicicola* in the growth chamber; selections were transplanted to the field, and reinoculated at maturity. Plants for the growth chamber inoculation (6 plants/accession) were grown to the 3-4 true leaf stage in 72 cell speedling trays, inoculated by spraying a 50,000 spores/ml suspension plus 1 drop per L Tween-20 to run-off, and placed in plastic bags. The temperature was maintained at 20° C, and a 12 h photoperiod was supplied by F96T12/VHO fluorescent lamps. Plants were scored three days following inoculation using a visual rating system based on a scale of 1 to 10, where 1 = immune, 2 to 3 = small flecks, but no large lesions, 4 = one or two small lesions, 5 to 9 = increasing numbers and size of lesions, and 10 = dead. For selection purposes, plants with scores of 3 or less were classified as resistant and scores of 4 were considered intermediate. Scores of 5 and above indicate varying degrees of susceptiblity. Selections from the seedling test were transplanted to the field and reinoculated with the pathogen as the plants approached maturity. The field was irrigated briefly every morning and evening following inoculation to maintain a high RH. The plants in the field were then given a visual rating 14 days following inoculation, after the susceptible checks showed a high level of infection.

Selected accessions and susceptible checks were then used to evaluate the screening procedures, where we examined the effects of spore concentration and temperature on disease development. Spore concentrations ranged from 1000 to 50,000 spores per ml. The incubation temperatures included 15°, 21°, 30°, and a 20° day/15° night incubation temperature regime. All experiments were conducted on an intermittent-mist bench in a growth chamber. The mist was calibrated to maintain 100% RH, but not to the point of causing run-off on the leaf surface.

Nine sources of resistance were included in a twelve parent diallel that also included a susceptible cauliflower, broccoli, and cabbage parent. Crosses were made between all twelve parents, and the selfed parents and F_1's were evaluated using the seedling inoculation procedure, which was repeated three times. The data were used to generate a mean squares analysis, to determine the significance of additive gene effects and dominance.

Heritability estimates were obtained from the cross PI 291998 (resistant red cabbage) X NVRS 02,006704 (susceptible green Chinese kale), using the variance components of 96 F_2's and 48 plants of each backcross to determine the broad-sense and narrow-sense heritability.

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Results and Discussion

Fifteen accessions of *B. oleracea* were identified as having varying degrees of resistance to *A. brassicicola* (Table 1). The selections were not based solely on scores, but also on notes taken in the field. The cauliflower PI 291565 was selected because the curd remained free of disease, even though the leaves can sometimes become severely infected. The sprouting broccoli NVRS 04,004712 reacted to the disease by dropping its leaves as soon as they became infected, and is probably a good mechanism for this plant to limit the spread of the disease since this accession is extremely branching with many leaves. The cabbage PI 291998 was consistently among the most resistant accessions in this study.

Table 1. Summary of selected accessions response to inoculation with *A. brassicicola*.

B. oleracea ssp.	Accession number	GC[a]	Fld[a]
capitata	PI 291998	2.3	2.6
acephala	NVRS02,004887	2.8	2.8
capitata	PI 245000	4.5	2.9
acephala	'Dwarf Blue Vates'	5.1	3.0
selensia	NVRS02,003597	4.5	3.8
gemmifera	PI 243050	4.3	3.2
italica	IHRGRU04,003571	3.5	4.8
alboglabra	NVRS02,006707	3.8	3.4
capitata	PI 244988	4.6	4.0
italica	IHRGRU04,004712	2.8	2.9
gemmifera	PI 343670	4.8	3.4
acephala	IHRGRU04,005416	4.3	3.4
botrytis	PI 291565	3.8	4.1
gongylodes	NVRS07,003475	4.8	3.8
botrytis	PI 441510	5.2	3.8
botrytis	'Imperial 10-6'[b]	6.9	5.7
capitata	'Round-Up'[b]	8.0	5.8
$LSD_{0.05}$		1.0	1.7

[a]Mean scores from the growth chamber (GC) and field (Fld) inoculation procedures. Scores based on a scale of 1 to 10, where 1 = immune, and 10 = dead.
[b]Susceptible checks.

The results of the various screening conditions revealed no significant differences in level of infection for spore concentrations from 23,000 to 50,000 spores/ml, but the level of disease was reduced at 10,000 spores/ml or less (data not shown). The effect of the temperature following inoculation revealed that at very high temperatures (30° C) the level of resistance exhibited by these selections was reduced. The lower temperature (15° C) required a longer incubation time (7 d), compared to the 20° C temperature (3 d). While there were statistically no significant differences between disease development at 15° or 20°, there appeared to be a better separation between the most resistant accessions and susceptible checks at the lower temperature. The fluctuating temperatures (20° day/15° night) gave essentially the same separation as the constant 15° temperature, but the disease developed at the same rate as the constant 20° temperature (3 d). There was a highly significant correlation of r = 0.67 between the growth chamber and field inoculation methods.

The genetic investigation of the resistance in these selections showed that both additive gene action and dominance have a role in resistance, with partial dominance apparently the most important. We observed no transgressive segregation in any of the hybrids created from these sources of resistance, even after two cycles of recurrent selection. The level of dominance exhibited by these selections suggests that it should be possible to create hybrid varieties with resistance to *A. brassicicola* using one parent carrying resistance; while the hybrid is not immune to the disease, it has been shown to be more resistant than any varieties we have examined under these severe inoculation procedures.

The heritability estimate of the cross between the red cabbage PI 291998 and a susceptible Chinese kale was $H^2 = 86\%$ and $h^2 = 53\%$. Segregation data on the pigmentation of the F_2 showed no correlation between plant color (red or green) and resistance.

Our recent efforts have been to combine the resistance to *A. brassicicola* in these accessions with horticulturally acceptable green cabbage, cauliflower, and broccoli. We currently have a limited amount of BC_1F_2 seed available for distribution.

DIVYA MUSTARD : A UNIQUE PLANT TYPE AND ITS DEVELOPMENTAL TRAITS IN DISEASE MANAGEMENT

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Three years (1990-1992) field experimentation at the University of Pantnagar revealed for the first time development of an early dwarf (85-96 cm), high yielding (upto 2200 kg ha^{-1}), compact plant type mustard (Brassica juncea) maturing in 95-100 days duration as against traditionally grown conventional tall type (122-186 cm), late maturing (135 days) mustard cv 'PR 18' yielding upto 2300 kg ha^{-1}; and short duration (95-100 days), short-stature (75-110 cm) rapeseed (B. campestris) cv 'PT303' yielding upto 1800 kg ha^{-1}. The new early dwarf mustard has been named as 'Divya' mustard. Three other outstanding characteristics of the 'Divya' mustard are : (i) its very high degree of tolerance to black spot disease (BSD) by Alternaria brassicae; (ii) its resistance to lodging is a major assest ensuring stability of its yield performance and ease of spray application of pesticides (if need be) and ease of harvesting and threshing operations, and (iii) its more suitability to multiple and intercropping systems with sugarcane and potato in comparison to traditionally grown mustard cv 'PR 18' and rapeseed cv 'PT 303'.

When sown in the first fortnight of October, 'Divya' mustard gave significantly higher harvest index (HI) of 27 to 28 per cent as against HI of 21 to 22 per cent in the case of rapeseed cv 'PT 303' and 15 to 20 per cent HI in the case of long duration tall mustard cv 'PR 18'. In this sowing, 'Divya' mustard also gave highest yield, that is, 16.93 kg day^{-1} ha^{-1} as compared to 10.40 kg day^{-1} ha^{-1} in the case of mustard cv 'PR 18' and 14.28 kg day^{-1} ha^{-1} in the case of rapeseed cv 'PT 303'.

When sown upto mid-November, 'Divya' mustard escaped the severe development of staghead phase of the white rust (WR) caused by Albugo candida associated with downy mildew (DM) caused by Peronospora parasitica; whereas rapeseed cv 'PT 303' showed very high degree of susceptibility to the staghead phase under similar conditions. However, beyond mid-November, 'Divya' mustard also showed susceptibility to the staghead phase, but it always gave highest seed yield day^{-1} ha^{-1} in all the sowing dates. Development of such a plant type as 'Divya' mustard is considered to be an ideal ideotype in mustard improvement work under normal sowing (October) conditions in mustard growing countries in the Asian region.

Table 1 Some contrasting characteristics of mustard 'Divya' in comparison to rapeseed cv PT 303 and mustard cv PR 18

Characteristic feature	Rapeseed cv PT 303	Mustard cv Divya	Mustard cv PR 18
Total pod bearing branches	15	31	10
Primary branches	5	4	3
Secondary branches	7	12	5
Tertiary branches	4	11	14
Growth habit	Spreading to semi-spreading and bushy	Erect bushy bunch type	Tall-spreading
Growth period	Short(95-10 days)	Short(95-100 days)	Long(130-135 days)
Height (cm)	75=110	85=96	122-186
Foliage	Medium to heavy foliage growth; the 3rd or 4th leaf 20 cm above ground has about 147 cm^2 area, leaves being sessile closely clasp the stem; no whorl at the leaf axil	Less foliage growth the 3rd or 4th leaf 20 cm above ground has about only 84 cm area; petiolate leaves and there exists very distinct whorl around the leaf axil which serves as mark of identification	Heavy foliage growth; the 3rd or 4th leaf 20 cm above ground has about 380 cm^2 area; petiolate leaves without the presence of distinct whorl around the leaf axil
Initiation of flowering(days after sowing)	25-30	20-29	44-46
Days taken to full flowering	60-64	60-64	83-87
Yield/10 plants(g)	52	84	71
Seed pod^{-1}	18-19	16-18	18-19
Pod length (cm)	4.2	4.0	4.3
1000 seed weight(g)	3.5	3.2	4.0
Oil content(%)	40-40.5	38-39.5	38-40
Seed size	Medium bold	Small to medium	Bold
Seed dormany	None	Moderate	Little to moderate
Harvest index (%)	21-22	27-28	15-20
Av.yield $day^{-1} ha^{-1}$(kg)	14	17	10
Yield range(kg ha^{-1})	1200-1800	1500-2200	1700-2300
Reaction to black spot disease (*A. brassicae*)	Susceptible	Highly tolerant to resistant	Moderately susceptible to tolerant
Reaction to white rust (*A.candida*) + downy mildew (*P.parasitica*) complex	Can escape if sown in last week of September but shows susceptibility under late sown conditions in October	Can escape if sown even upto mid-November but shows susceptibility if sown beyond mid-November and onwards	Can escape if sown in first week of Octobe but shows suscept bility when sown in mid-October and onwa

RELATIVE SENSITIVITY OF THE DIPLOID AND AUTOTETRAPLOID FORMS OF BRASSICA VARIETIES AND THEIR F_1 HYBRIDS TO MUSTARD APHID

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Incorporation of genetic resistance to mustard aphid (Lipaphis erysimi Kalt) in cultivated varieties is now considered necessary. Some studies on the varietal reactions to this pest in Cruciferous oilseed crops have been reported. 3,4,5,6,8,10. This paper reports the relative susceptibility of the diploid and the colchicine induced autotetraploid forms of rapeseed and mustard varieties and their F_1 hybrids.

The experimental materials comprised of one toria variety (PT 30), two sarson selections (DYS 18 and DYS 26) and their three hybrids at the diploid as well as their induced autotetraploids alongwith a check mustard variety 'Varuna'. All the materials were grown during Rabi 1990-91 at the Crop Research Centre of the Institute in a randomised block design with three replications. Four meter long rows of each entry were planted at 45 cm apart; plants spaced at 15 cm. The crop was planted rather late to favour heavy infestation of aphids under field conditions. Observations on aphid infestation was recorded on 10 competitive plants in each replication at full flowering stage on 0 to 5 scale, where 0 is free from aphid and 5 represents the most heavily infested plants. 1,2. These ratings were based on the quantification of no aphids in grade 0, 1 to 20 in 1, 21 to 100 in 2, 101 to 250 in 3, 251 to 500 in 4 and more than 5oo in 5. Later on, these values averaged out, which represented the aphid rating of the variety/hybrid concerned and the relative comparisons were made based on this value.

The toria variety 'PT 30', which has aphid infestation rating of 3.7 (table1), supported comparatively less aphid population than that of the sarson selections 'DYS 18(4.5) and 'DYS 26'(5.0). Since toria is basically an early maturing material than sarson, it might have escaped the aphid infestation, even though it might have the same level of susceptibility. So far the genetic resistance between toria, sarson varieties and their hybrids is concerned, there is little to choose between them. Rather, mustard (B. juncea) varieties show comparatively better tolerance than those of the B. campestris selections. 8,9. In the present study also, the mustard variety 'Varuna' has shown relatively better tolerance than either toria or the sarson selections at both the ploidy levels.

In general, except the 'PT 30' and the hybrids 'PT 30'x 'DYS 26' and 'DYS 18' x 'DYS 26', the autotetraploids supported lower populations of aphids as comapred to their diploid counterparts. The two hybrids, however, did not show any difference in the aphid scores at the two ploidy levels except 'PT 30' x 'DYS 18'. If the relative ratings 1 to 2.5 as resistance (R), 2.6 to 3.5 as moderately resistant (MR) and 3.6 to 5.0 as susceptible (S) are considered, then the autotetraploid forms of the hybrid (PT 30 x DYS 18) could be considered to be resistant,

TABLE 1. COMPARATIVE APHID INFESTATION SCORES OF DIFFERENT PARENTAL POPULATIONS OF RAPESEED MUSTARD AND THEIR HYBRIDS

Brassica Types	Parents/ Hybrids	Average aphid infestation scores (on 0 to 5 scale)		Level of Resistance *	
		Diploid	Autotetraploid	Diploid	Autotetraploid
Rapeseed					
Toria	PT 30	3.7	4.0	S	S
Sarson	DYS 18	4.5	3.5	S	MR
Sarson	DYS 26	5.0	4.0	S	S
Average		4.4	3.8	S	S
Mustard -	Varuna	3.5	3.0	MR	MR
Hybrids					
PT 30 x DYS 18		3.0	2.5	MR	R
DYS 18 x DYS 26		4.0	4.0	S	S
PT 30 x DYS 26		3.5	3.5	MR	MR
Average		3.5	3.3	MR	MR

* S= Susceptible, MR = Moderately Susceptible and R = Resistant

even though both parents 'PT 30' and'DYS 18' were susceptible. Some heterozygote advantage for better aphid tolerance could be expected. A better genic balance for tolerance or antibiosis may be accomplished in the hybrids by complementation from the parental populations. It is likely that because of early maturity of the hybrids than its parents, they could have developed a possible escape mechanism, which supported less aphid. The heterozygote advantage for tolerance to frost injury has been noted in tomato hybrids. 7. It is possible that if carefully chosen, the development of commercial hybrids in oilseed Brassicas, besides giving superior yield, may also provide an useful defence mechanism for reducing the menace of the aphids.

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PRODUCTION OF SINGLE SPORE ISOLATES OF *PLASMODIOPHORA BRASSICAE*

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INTRODUCTION

Populations of *Plasmodiophora brassicae* Woron., a pathogen causing the clubroot disease of crucifers, consist of a mixture of pathotypes which have differential pathogenicity. Moreover, the presence of spores of one pathotype can affect infection by spores of another (Jones *et al.*, 1982). The heterogeneity of populations and the interaction between pathotypes within a population have important implications when screening for resistance or in studies for pathogenicity on differential hosts : results may be biased when populations of *P. brassicae* are used. Therefore, in our laboratory, we have started to build up a collection of single spore isolates as a useful material to tackle the study of both host-pathogen interactions and genetics of resistance in *Brassica oleracea*.

This paper describes a method for obtaining single-spore isolates based on a modified Tinggal & Webster (1981) technique and it was used to study the influence of various factors on germination of single resting spores.

MATERIAL AND METHODS

Spore suspensions were prepared from clubs of three populations (K92, SJ92 and Pb137) of different geographical and host origin, maintained in the glasshouse on Chinese cabbage (*Brassica campestris* spp *pekinensis* cv Granaat). Galls were homogenized in distilled water and then filtered through three sieves of 500, 250 and 100 µm respectively. The spores were washed four times by centrifugation at 2500 g for 5 min at 4°C. The final pellet was resuspended in distilled water and stored at 4°C for 2 days. A drop of a spore suspension containing about 10^3 spores/ml was spread over 1% purified water agarose poured on a slide. After a few minutes drying, the agarose was sliced to get cubes of approximately 1 mm^2. The slide was scanned under microscope to locate single, well separated spores. Only the larger resting spores were selected. The agarose cube containing one single spore was removed with a needle and transferred on the root hairs of a (3-6 days old) Chinese cabbage seedling. Each inoculated seedling was placed in a Petri dish in the dark. Three factors were simultaneously analyzed : a) contact time between the agar piece and the root (24 h or 48 h) before the seedling was transplanted, b) temperature (19°C or 25°C) and c) solution used for moistening the root hairs after inoculation (a drop of distilled water or a drop of a EDTA 10 µM solution). EDTA 10 µM was used according Yano *et al.* (1991) who have shown that spore germination of *P. brassicae* was enhanced in its presence.

After these treatments, seedlings were transplanted into 8-cm-pots containing a steam sterilized mixture of soil and peat (1:1, v:v). The pots were placed in a growth chamber at 19-22°C (temperature night-

day respectively) and a 16 h photoperiod with frequent watering in order to maintain a high relative humidity. Eight to fourteen weeks after inoculation, plants were examined for symptoms.

RESULTS

Four hundred twenty three inoculations have been performed with single resting spores of *P. brassicae*. Fifty-seven inoculations resulted in gall development (13.5 % success rate).

The effect of different treatments on percentage of infection is shown in Table 1. Significant differences were only observed for the factor « contact time ». The percentage of infection obtained after 48 h was higher than that obtained after 24 h (20.8% and 10.2% respectively). No interaction was observed between these factors.

Regardless of analysed factors, there were significant differences in the success rate of infection by single resting spore according to the field population origin : 9.4% for K92, 8% for SJ92 and 21.6% for Pb137.

With this delayed transplantation, most of the inoculated seedlings were strong enough and the death seedling percentage was very low (3.8 %)

Experiments to characterize isolates derived from single resting spores and to examine interaction between them are in progress in our laboratory.

Table 1. Percentage of infection by single resting spores coming from three field populations

Contact time (h)	Temperature (°C)	Moistening solution	Nb of inoculated plants	Nb of infected plants	% of infection
24	19	DW	85	11	12.94
24	25	DW	76	4	5.26
24	19	EDTA	76	6	7.89
24	25	EDTA	56	9	16.07
48	19	DW	54	14	25.92
48	25	DW	20	5	25
48	19	EDTA	36	6	16.66
48	25	EDTA	20	2	10

DW: distilled water; EDTA 10 µM

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MBC FUNGICIDES - ARE THEY STILL EFFECTIVE AT CONTROLLING LIGHT LEAF SPOT IN WINTER OILSEED RAPE?

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Light Leaf Spot (*Cylindrosporium concentricum* anamorph *Pyrenopeziza brassicae*) is the most important foliar disease of winter oilseed rape in Scotland. Cool, wet conditions during the late autumn and winter months are conducive to disease development. The disease, although not visible in the first few months of growth, is regularly detected on susceptible varieties from December onwards (after incubation in a damp chamber). The typical white spore droplets can be found on leaf tissue which is visually green and apparently healthy. Significant early disease infection predisposes plants to winter kill when hard frosts occur.

Work carried out by the Scottish Agricultural College in the 1980s showed the use of MBC and/or prochloraz fungicides in the autumn to control early infections of Light Leaf Spot was essential, reducing winter kill and significantly increasing yield (Wale *et al*, 1990). However, in 1990/91 growers started reporting that MBC fungicides were not controlling Light Leaf Spot as well as in previous years. Questions were asked whether MBC resistance was occurring in *P. brassicae* as had occurred with the eyespot fungus in cereals.

Trial results show disease pressure from *P. brassicae* in 1986-89 was on average low, with MBC and prochloraz fungicides giving similar reductions in infection levels (Fig. 1). Disease pressure in 1990-93 was much higher and MBC fungicides failed to reduce levels of Light Leaf Spot to any extent. In these more recent trials it was advantageous to have prochloraz as an autumn fungicide treatment.

This poses the question whether there is resistance to MBCs in the Light Leaf Spot fungus or whether MBC is inherently less effective against *P. brassicae* , with high disease pressure making this more apparent. Ilott *et al* (1987) failed to find resistance to MBCs in field isolates of the Light leaf Spot fungus. Work is currently being carried out in Aberdeen to investigate this question.

References

Ilott, T.W; Ingram, D.S; Rawlinson, C.J (1987) Studies of fungicide resistance in *Pyrenopeziza brassicae*, cause of light leaf spot disease of oilseed rape and other brassicas. Transactions British Mycological Society 88 (4), 515-523.

Wale, S.J; Brokenshire, T; Oxley, S; Sutherland, K; Munro, J.M (1990) Fungicidal control of light leaf spot (Pyrenopeziza brassicae) in oilseed rape in Scotland. Proceedings Crop Protection in Northern Britain 1990, pp 289-294

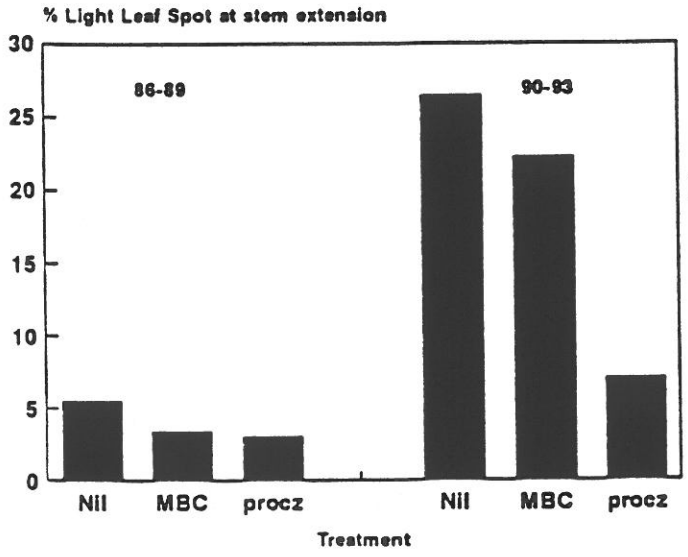

Fig. 1 Effect of MBC fungicides on Light Leaf Spot control, 1986-93

Oilseed Rape Varietal Mixtures

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Introduction

When the first double low varieties were grown in the north of Britain, they achieved greater height than in the south and were more susceptible to lodging (Fisher and Walker, 1992). It has been found that lodging encourages spread of fungal diseases and feeding by small birds (Daniels *et al.*, 1982) and may be associated with large seed losses at harvest (Travis *et al.*, 1993). Shorter varieties which are now available are associated with lower yields (Anon, 1993), so cultivation of the tall varieties is still attractive to maximise yield.

Some farmers in Scotland have been mixing varieties in commercial winter oilseed rape crops for a number of years and several hundred hectares are grown in this way. Mixtures of varieties with certain different characteristics may be grown in order to reduce lodging of a high yielding but weak strawed variety and also to reduce the risk from disease of the crop by perhaps creating diversification of disease susceptibility.

The purpose of the present trial was to assess the effectiveness in reducing lodging of mixing a weak strawed variety which yielded well in favourable conditions, with a lower yielding variety which had stiff straw. An objective was to assess the optimum mixture of the 2 varieties, considering that it was desirable to maximise the amount of higher yielding variety in the mixture.

Materials and Methods

Varieties were selected on the basis of yields and stem stiffness assessments made over 5 years in Scotland (Anon, 1993). The variety Envol was selected as the high yielding variety with a yield of 109% of the control varieties (where the controls yielded 3.67 t ha^{-1}). Envol is also a weak strawed variety, scoring 4 on the stem stiffness assessment scale of 1-9. Rocket was selected as a stiff stemmed variety with a stem stiffness score of 8, but with the lower yield of 103% of control.

The trial was sown on 26 August 1992 at Tillycorthie Farm, Aberdeenshire, Scotland. The plot size sown was 22m x 1.88m which was trimmed back to 20m x 1.88m by harvest. Plots of pure stands of Rocket and Envol were sown at 6 kg ha^{-1}. The 2 varieties were mixed by seed weight to give mixtures of 50:50, 25:75 and 10:90 Rocket;Envol, and each mixture was sown at 6 kg ha^{-1}. The mixtures and pure stands were randomised, and replicated 3 times.

All plots received 18N:90P:90K kg ha^{-1} at sowing and 180 kg N ha^{-1} in the spring split equally into applications on 17 February and 22 March. Elemental sulphur (Thiovit) was applied at 10 kg S ha^{-1} and 5 kg S ha^{-1} on 11 May. Diseases were controlled to low levels by applications of standard fungicides at permitted rates.

The plots were assessed for lodging before flowering on 3 May and for late lodging on 14 July.

Results

No lodging was seen pre-flowering in any of the varietal mixtures of pure stands. Lodging occurred later in the season as harvest approached. An angular transformation of the lodging scores was undertaken before statistical analysis. Data are presented as scores and statistical significance is discussed in the text.

Results from the lodging assessment showed clearly that Rocket was significantly less susceptible to lodging than Envol, receiving a score of 7.7 on a scale of 1-9 compared to 3.7 for Envol (Figure 1). Lodging in the mixtures was significantly less than for Envol alone and significantly more than for Rocket grown alone. Mixtures containing 10, 25 and 50% Rocket were associated with progressively less lodging than Envol grown alone, but differences between the mixtures were not significant. Inclusion of both 10 and 25% Rocket in the variety

mixture resulted in less lodging than would have been expected from the weighted arithmetic mean of the lodging performance of these varieties alone.

Discussion

The results indicate that the mixing of a stiff strawed variety with a weak strawed variety was effective in reducing the lodging of the resultant crop stand. Inclusion of a low rate of Rocket (less than 50%) had a disproportionate effect on improving the standing ability of the crop, and may be worthwhile if reducing lodging is an important objective. Further work is necessary to evaluate the actual proportions of varieties required to optimise performance.

Mixing varieties may offer an alternative to restricting the plant population, which can reduce lodging but is associated with unacceptable yield depressions in seasons when no lodging is observed (Fisher and Walker, 1992), although development of varieties which combine these characteristics must be the long term aim. Mixing varieties to combine the standing ability of one variety with the high yield of another may in practice be a useful management tool, which has the benefits of not incurring extra costs and being environmentally friendly, as no additional agrochemicals are involved. Further work is in progress to assess the value of mixing varieties of different qualities in terms of lodging, disease resistance and yield.

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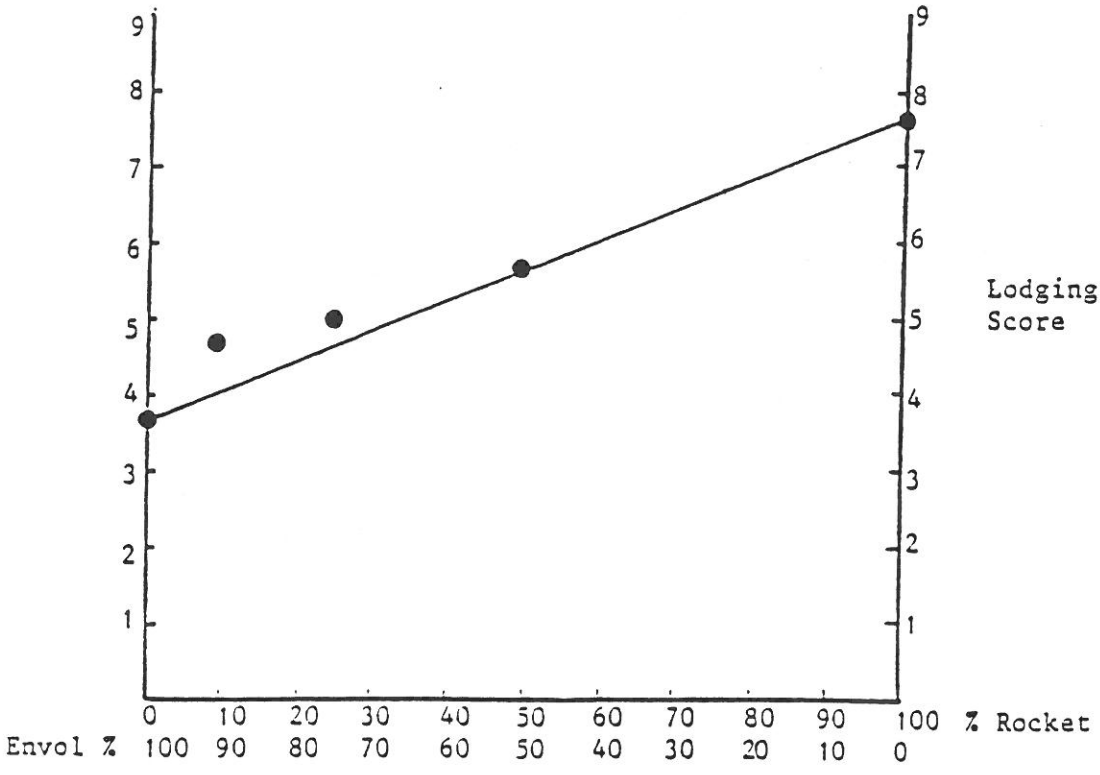

Figure 1. Lodging scores for variety mixtures.

ISHS SYMPOSIUM ON BRASSICAS
NINTH CRUCIFER GENETICS WORKSHOP

GENERAL INFORMATION

The section Vegetables of the International Society for Horticultural Science (ISHS), The Crucifer Genetics Cooperative (CrGC), and the Portuguese Horticultural Association (APH) invite scientists with interests in crucifers to participate in and present papers at the joint ISHS Symposium on Brassicas and Ninth Crucifer Genetics Worhshop.

This scientific meeting will be the first ISHS Symposium devoted exclusively to brassicas and the first Crucifer Genetics Workshop to be held outside North America. It will provide a golden opportunity for cooperation and exchange of information among scientists from throughout the World.

Cole crops are of great economic importance and have a long tradition of cultivation in Portugal ; they constitute an important component of the Portuguese diet. November is the beginning of the mild-winter harvesting season for the majority of brassica vegetable crops and is therefore an excellent time to visit the brassica production areas near Lisbon while enjoying the pleasant weather at this time of the year.

The Symposium/Workshop will cover all scientific disciplines of relevance to crucifers in general and brassicas in particular. The primary emphasis will be on genetics, breeding, biotechnology, crop production, and crop protection.

INVITED PAPERS

Cabbages, cauflower and turnips on Flemish/Dutch paintings from the16th-18th century.
A.C. ZEVEN
Agriculture University, Wageningen, The Nederlands.

Asymmetric fusion between *Arabidopsis thaliana* and *Brassica nigra*
J. SIEMENS
Berlin Free University, Germany.

Application of molecular markers and cytogenetic stocks to Brassica genetics, breeding and evolution.
CARLOS F. QUIROS
University of California, Davis, USA.

Genome variation and evolution of *Brassica* amphidiploids.
KEMING SONG
Indiana University, USA.

The role of brassicas in cancer chemoprotection.
JED FAHEY
Johns Hopkins University, Baltimore, USA.

Recent progress in understanding the biology of *Plasmodiophora brassicae* - new solutions to an old problem ?
HUTOKSHI BUHAROWALLA, RUTH MAGRATH and RICHARD MITHEN
Cambridge Laboratory, John Innes Centre, Norwich, United Kingdom.

Viruses of Chinese cabbage in China.
LIU HSUPING
Heilongjiang Academy of Agriculture Sciences, China.

in root hairs which migrate directly into the stele forming secondary plasmodia and resting spores. The pathogen moves intracellularly and within ten days of infection the spores begin to replicate; infection is normally associated with high concentrations of lipids. Molecular studies include the development of restriction maps and sequence analysis of rDNA (ITS region), chromosome and karyotype specificity, RAPD markers aiming to study genetic diversity and horizontal DNA transfer and the uptake of host DNA by *P.brassicae.*

Typing of *P,brassicae* isolates was described by Afra Neuvel, The Netherlands, the problems of working with mixed isolates was discussed and the need for standardised methods of specifying physiological races which would relate results from the European Clubroot Differential Series (ECD) with DNA typing identified. Also discussed were the problems encountered with vital stains, tetrazolium chloride had given difficulties and there was a preference for fluorescein diacetate.

The potential for biological and cultural control was highlighted by Helmut Bochow, Germany, catch crops and bacteria may be used to reduce inoculum potential. A 90% reduction in inoculum potential was achieved with Chinese cabbage, used with bacteria obtained from suppressive soils reduced the size of resting spore masses by chitinolytic activities. Inhibition of secondary root infections by colonising bacteria which cause nutrient competition was discussed, drenches with *Pseudomonnas fluorescens* and 2% Neem oil substantially reduced disease.

Solarisation has proved to be effective in Australia as descibed by Ian Porter, but the treatment is expensive and will not provide year round control. Placement of dazomet (< 100kg/ha) is used by growers on sandy soils but is ineffective on clay soils which have higher inoculum concentrations. Metham sodium is still used in Victoria but public attitudes to agrochemicals are forcing growers to seek alternative sytems and less noxious chemicals.

In general discussion participants agreed there were needs to :-

1. reassess the alternative hosts for *P.brassicae* and define their role in the carryover of disease, particularly identifying their influence on primary and secondary invasion,
2. understand more fully the modes of effect of soil components such as calcium, pH and boron,
3. identify the complete life cycle and the role of the root hair stage, especially the importance of primary zoospores,
4. understand how the organism moves within the host,
5. develop an understanding of the role of movement in free soil water and the mechanisms of survival in the absence of *Brassica* hosts
6. standardise methods of typing and provide more rapid and accurate assays for detection
7. circulate information concerning the importance of clubroot to crop production around the world and views of current research, a particular lack of knowledge of work in Japan was identified.

This meeting was attended by 30 workers drawn from 12 countries.

The organisers and participants wish to express their gratitude to their Canadian colleagues for permitting this meeting to accompany the 6th International Congress of Plant Pathology and noted the affliation of ICWG with the International Society for Plant Pathology. Particular thanks go to Dr Michele Heath, University of Toronto, who expertly organised the timing and facilities for this meeting.

Meetings in 1994 will be held in association with the International Horticultural Congress,Kyoto,Japan and Crucifer Genetics Symposium, Lisbon, Portugal.

INTERNATIONAL CLUBROOT WORKING GROUP

REPORT OF A MEETING HELD ON 6th AUGUST 1993, MONTREAL, CANADA

by I. Porter*, G.R.Dixon** and T. Price***
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**Department of Horticulture, SAC/University of Strathclyde, Scotland
*** Department of Agriculture, LaTrobe University, Victoria, Australia

The Chairman, Geoff Dixon, indicated that ICWG meetings are informal gatherings open to all scientists interested in *Plasmodiophora brassicae*, related organisms and the diseases which they cause.Each participant gave a brief account of their work and interests.A summary of current chemical control indicated that in the UK, no effective fungicides are available for commercial crops - thiophanate methyl is still registered but of variable efficacy while calcium cyanamide has been marketed on a limited scale compared with Germany and Japan, in comparison PCNB is still registered as a pre-plant treatment in Australia, fluazinam is being registered and metham sodium is applied by some growers and chlorthalonil is used effectively in Belgium. Other participants noted that seed companies in Japan are developing resistant cultivars and there is support from the Biotechnology Research Centre (RIKEN) to formulate fungicides. In the Phillipines there is a joint programme with the German Technical Co-operation Program since clubroot is of major significance in the Highlands, in Canada substantial losses occur in Nova Scotia while a group from Syria indicated that clubroot is of commercial significance. Several participants noted that research into *Polymyxa betae* provides valuable parallel information for clubroot workers.

A review of research over 15 years into the influence and interaction of pH, calcium and boron and their effects on the *P.brassicae* life cycle was given by Geoff Dixon. Improved methods of studying the root hair using Rapid Cycling Brassicas (ex Paul Williams, Wisconsin, USA) allow detailed monitoring of infection and sporogenesis. Calcium and pH reduce the rate of sporangial development while boron has a similar effect but also retards morphogenesis in cortical cells. Boron must be present at levels in excess of 15ppm in order to achieve a continuous influence on pathogen growth. Improved methods of sustaining these concentrations are being investigated. Each of these effects is moderated by inoculum potential.

Work at Horticulture Research International, UK, was discussed by Roy Kennedy, where the surfactant Agral provides substantial disease control. This material is most effective when applied as a drench at or before transplanting, it is suggested that the mode of action may result from changes to pathogen adhesion to the root hair surface. Other workers at HRI are developing immunological methods for identifying *P.brassicae* in small soil samples (<5g). Effective stains for resting spores were noted as aniline blue and acetocarmine.

Genetic studies at the John Innes Centre for Plant Science,UK, were described by Richard Mithen, the main thrust is to transfer resistance genes (single genes from *B.rapa*) into *B.oleracea* and RFLP mapping of resistance genes, much work results from mutants of *Arabidopsis* due to difficulties with hairy root culture methods. A co-worker Robert Vrieland has described two pathways for penetration by *P.brassicae* after penetration, one via primary plasmodia, zoosporangia and secondary zoospores and the second via amoeba

BEHAVIOUR OF SOME CAULIFLOWER GENOTYPES TO CURD DEFECTS UNDER MID-HILLS OF WESTERN HIMALAYAS

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Department of Vegetable Science , HP Agricultural University, PALAMPUR 176 062, INDIA

The quality of curds is of great importance in cauliflower as it has bearing on the marketability of the produce. There are various distinct features characteristic of poor cosmetic quality . The most prevalent and disfiguring defects are bracting, pinking and riceyness (Crisp and Gray, 1979) . Of late, Indian consumers have also become cautious about the quality of vegetable product. The present study was , therefore , undertaken to locate defect free genotype (s) which can be included in quality breeding.

Eighty genotypes of cauliflower obtained from the then NVRS, Wellesbourne (UK) were scored for bracting, pinking and riceyness at marketable stage at the Vegetable Research Farm , Himachal Pradesh Agricultural University, Palampur (India) during *Rabi* season of 1987-88 using a four - point scale (0=absent, 3= severe).

The field observations revealed that genotypes cc 81050, 82308 and 831169 were completely free from all the three defects studied. CC 83064, 831168, 80717 ,83004 and 79039 did not show any sign of riceyness and pinking. Whereas cc 83005, 82160,80720, 80723,79004, 80732 and 79031 were found to be free from bracting and pinking. Those genotypes in which curd defects were absent (cc 81050,82308 and 831169) can be used to produce potential new cauliflower cultivars with ricey - free, bract - free, non - pink curds.

Acknowledgement
Thanks are due to Horticultural Research International, Wellesbourne (UK) for providing the cauliflower germplasm.

Reference
Crisp,P.and A.R.Cray ,1979. Hort. Res.,19;49-53.

Tab. 1. Mean performance ($\bar{x}$) and structure of cauliflower crop of parents components and orange curd F_1 hybrids.

Parents components	Earliness days*	Curd weight	Curd dimension	Good commercial quality curd	Curds with ricegness and bracting	loose curds	carotene
		g	cm	%	%	%	µg/100
Maternal F_1							
90	43	290	19.2	63.3	0.0	36.7	20
91	42	210	20.2	69.8	0.0	30.2	20
95	47	210	19.1	74.5	1.3	24.2	20
96	51	408	25.5	79.3	0.0	20.7	20
Paternal orange curd inbred line	34	56	6.9	0.0	100	–	1060
Orange curd F_1 hybrids							
90x orange inbred line	36	390	23.1	57.1	28.6	–	139.7
91x orange inbred line	36	390	23.2	38.5	30.8	–	70.6
95x orange inbred line	37	350	23.4	78.6	14.3	–	293.4
96x orange inbred line	36	320	22.5	93.3	0.0	6.7	155.2

* from planting in the field to maturity

References:

1. Czapski J., Saniewski M.(1985) Effect of Methyl Jasmonate on carotenoids in tomato fruits; Gartenbauwissenschaft,Band 50, Heft 1, pages 35-37.

2.Dickson M.H.(1988) Orange-curd high carotene cauliflower inbreds NY 156, NY 163, and NY 165. HortSci. Vol 23(4) 778.

3. Hoser-Krauze J.(1989) The comparison of self-incompatibility and male sterility (CMS) sources with respect to their suitability for breeding of F_1 cauliflower hybrids. Vortrage fur Pflanzenzuchtung, Heft 15-II, Book of posters Abstr. part II, XII Eucarpia Congress, Göttingen, Germany F.R.

Orange curd high carotene cauliflower F_1 hybrids

J. Hoser-Krauze, H. Michalik, Research Institute of Vegetable Crops ul. Konstytucji 3-Maja 1/3, 96-100 Skierniewice, Poland

Orange curd cauliflower doesn't need the curd protection from light as white curd cauliflower cultivars, which usually turn cream or brown depending on light intensity, because they don't have high level of beta-carotene which orange curd contains.

Materials and methods

Maternal components of orange-curd F_1 cauliflower hybrids were four early white curd self-incompatible F_1 of two isogenic lines differing by incompatibility alleles (3). Paternal component was high carotene organe curd inbred line of Dickson breeding material (2).

The horticultural value of early parents was compared in 1991 for spring - summer crop of cauliflower grown in the field in random block design with three replication each, each plot had 20 plants.

The commercial value of orange cauliflower curd F_1 hybrids was observed on 15 individuals grown on 5 m^2 plots in a field-cultivation at the Outdoor Vegetables Dept. of the Institute of the Vegetable Crops, for middle sumemr crop of curds.

Beta - carotene content was determined according to the column chromatography method described by Czapski and Saniewski (1).

Results

The high horticultural value of orange curd F_1 hybrids depended of the high value of the mother's components because father orange curd line produced uncommercial small curds with very strong green and violet ricengness and bracting (tab.1).

After hand pollination under greenhouse conditions only single white curd plants of mother component were observed amount orange F_1 hybrids plants.

Depending on temperature climatic conditions in the field growing seeds production and bee pollination percentage of white-curd plants can be higher. For this reason male sterile lines should be preferred to self-incompatible lines as a mother component of orange curd F_1 hybrids.

The organe-curd cauliflower hybrids contained 70.6 to 293.4 micrograms of beta-carotene per 100 g of fresh tissue. In means that hybrid with highest content of beta-carotene contained about 3.5 times less the ingredient than father line (1060 µg/100 g).

Obtained results confirm Dickson's observations (2) of the orange inbred lines and F_1 hybrids having as a mother component cytoplasmic male sterile lines and various white-curd cauliflower cultivars.

EUCARPIA CRUCIFERAE NEWSLETTER Nr.17

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1994 - 1995

Next Cruciferae Newsletter will be produced and edited in 1995. A group of INRA Rennes scientists will take charge but as previously, it will be produced by direct photocopying the material you submit. Therefore, we would be grateful if you would, please, follow instructions below, from which will depend the quality of your script.

1 - Contributions should be in English.

2 - The scripts will not be retyped and must be produced with the best typing quality. Laser printing is expected with a clear black character.

3 - Contributions must be, as previously, concise and must not exceed two (2) pages, including tables and figures. Whole pages are preferred. (eventually reduce the format through computer printing). Consecutive pages must be numbered in pencil.

4 - The heading of the paper must include the title (1 line), followed by the authors names (line below) and adresses. Please use only two lines for this information (authors and adresses).

5 - Single spacing is required. If not, it will be published at the editors discretion.
The A 4 format is required with margins (not less than 3 cm on the left, 2 cm above and below). No other format will be accepted.

6 - Tables and figures must be included in, or at the end of the text. Photographs cannot be included and reproduced.

7 - Please do not fold the script and send two copies of each script protected by card. All invoices to :

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