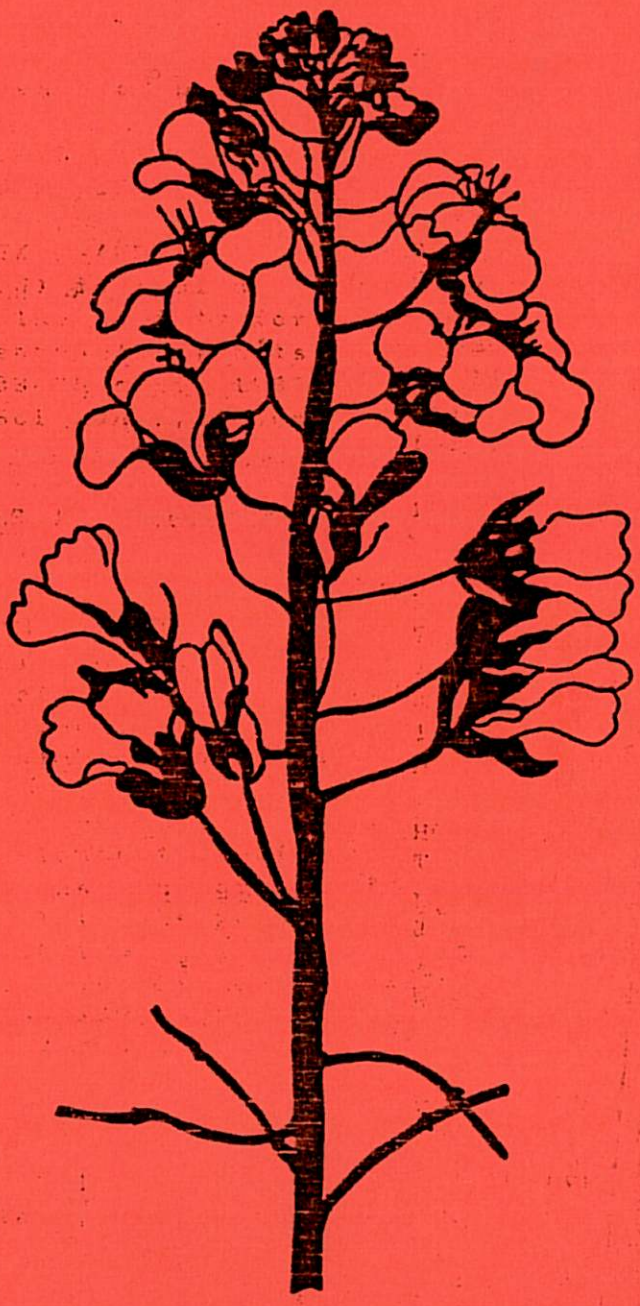
CRUCIFERAE NEWSLETTER No.11



November 1986

EUCARPIA

CONTENTS

Editorial

There was a satisfying response to the revised instructions for the preparation of scripts which were sent with the 1986 call for papers. As a result we have been able to accommodate 84 articles, including two longer review papers, without increasing the size of this issue over No. 10 which contained 74 articles. The total number of recipients has also increased and, as many will not now have a complete list of addresses, authors' addresses have been added at the foot of each title page.

In the past we have aimed to mail the Newsletter before the end of the year but we were unable to meet that target with either this issue or the previous one. We apologise to authors and readers for this situation and trust that you will bear with us.

Shortly we will be writing to commercial organisations to seek continuing financial support for the Newsletter, as we have now used all available funds. We request the support of readers in suggesting the names of companies in their own countries whom we could approach. In earlier issues we have stated the reasons for our reluctance to introduce a general charge for the Newsletter and we still wish to continue free distribution so long as our industry will support it.

Back numbers of all issues are still available. Please note that we do make a charge for these and that order forms are available from the editors.

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VEGETABLE GENE BANK

NATIONAL VEGETABLE RESEARCH STATION
WELLESBOURNE

D Astley

The Gene Bank is one of the genetic resource centres which the International Board for Plant Genetic Resources has designated as a base store for cruciferous crops.

Basic store responsibilities are:

Brassica oleracea
Brassica juncea)
Brassica napus) vegetable and fodder types
Brassica rapa)
Raphanus

We have been collecting material from national collections, plant breeders, commercial sources, expeditions and amateur growers. The collections now comprise:

		Total accessions	Available accessions
B. oleracea	- broccoli	175	141
	- Brussels sprout	941	326
	- cabbage	752	392
	- cauliflower	445	328
	- kale	109	92
	- kohl rabi	31	25
B. juncea		55	13
B. napus		215	127
B. rapa		281	154
Raphanus		500	404
	Total	3504	2002

The difference between columns indicates the numbers of accessions awaiting seed regeneration.

Seed from the active collections (2002) are available to research scientists worldwide.

Conversely if you hold material which you feel should be conserved long-term then please contact us.

Gene Bank,National Veg. Res. Stat.,Wellesbourne,Warwick CV35 9EF,UK.

CRUCIFER GENETICS COOPERATIVE

Paul H. Williams

The Crucifer Genetics Cooperative (CrGC) was established for the purpose of acquiring, maintaining, and distributing seed stocks and pollen of various crucifers and of crucifer-specific symbionts (pathogens and pests). Emphasis is on genetic, chromosomal and cytoplasmic variants useful for expediting a wide range of research in breeding, cytogenetics, genetic engineering, molecular biology, plant physiology, entomology, phytopathology, ecology and other biological studies. Seed, pollen, and symbiont cultures are received from those who have unique genetic stocks and who would like to share them with others interested in crucifer genetics. Annual summaries of CrGC activities are submitted through the EUCARPIA, CRUCIFERAE NEWSLETTER. All persons providing genes and stocks to the CrGC will be recognized as the source of those traits.

CRUCIFER GENETICS COOPERATIVE RESOURCE BOOK

The CrGC Resource Book provides a mechanism for supplying information among members of the CrGC. The Resource Book consists of sections representing various categories of information relating to crucifers. Information is stored on computer discs and printed as separate information documents (ID) which are coded as to the subject and originator and are dated to indicate the most current version of the document. Members are encouraged to submit information documents (IDs) that could be filed in the resource book. IDs would normally consist of summarized descriptive information or techniques that would be useful in research or teaching. IDs could also contain lists and descriptions of seed stocks, cell clones, gene libraries, etc., available from the CrGC member's laboratory or organization. IDs received from CrGC members will be codified, copied and sent to the CrGC membership for inclusion in their Resource Books. Originators of IDs should suggest new file categories for documents which would appropriately represent new areas of information. The system as constructed is open-ended and can accommodate new categories and subcategories. Contributors should revise their information documents as frequently as appropriate.

OPERATION OF THE CRUCIFER GENETICS COOPERATIVE

Members are encouraged to pay a subscription fee of $25.00 covering a three-year period. Subscriptions should be made out to Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin. Subscribing members will receive the CrGC Resource Book, current IDs and a mailing list of the members with their professional interests.

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ACTIVITIES OF THE CRUCIFER GENETICS COOPERATIVE 1985-1986

Membership in the CrGC exceeds 610, representing 24 countries with professional interests in genetics, plant breeding, molecular, cell, population and developmental biology, ecology, biochemistry, pathology and teaching. From August 1985 to September 1986, 653 seed requests were filled. Over 100 seed stocks are available upon request. As of September 1986, there were 210 subscribing members and 17 sustaining members of the CrGC. An Educational Materials Section of the CrGC was initiated to promote the use of CrGC stocks in teaching and learning at all levels of education from kindergarten through university. Educational materials being developed for teachers and students include, plant growing systems for the classroom, specialized seed stocks, and written, graphic, photographic, video and computer software to demonstrate various principles of biology

CRUCIFER GENETICS WORKSHOP III - 1986

On May 29-30, 1986, the CrGC together with the Plant Biotechnology Centre at the University of Guelph, Allelix and Agriculture Canada sponsored the Crucifer Genetics Workshop III at the University of Guelph, Canada. Two hundred and sixty people attended the Workshop which consisted of 50 poster presentations, 13 overview talks and discussion sessions organized around cell biology, molecular biology, diseases, and breeding cruciferous species. A seventy-one page proceedings of the Workshop is being sent to all subscribing members of the CrGC who did not attend the Workshop and is available to nonsubscribing members for $10.00 U. S. payable to the CrGC.

CRUCIFER GENETICS WORKSHOP IV - 1987

The Crucifer Genetics Workshop IV - 1987 will be held at the University of Wisconsin-Madison, October 12-14 1987, under the umbrella organization the Crucifer Improvement Cooperative (CrIC). Also meeting with the CrIC will be the NCR-100 committee on seed borne diseases of crucifers and the Crucifer Crop Advisory Committee (CrCAC) of the United States National Plant Germplasm System. The format of the Crucifer Genetics Workshop and the Crucifer Improvement will include poster presentations, discussion groups, review and overview sessions. There will be an opportunity for specialized subject matter workshops to be given (eg. disease resistance screening, uses of brassicas in education, cell biology, molecular mapping etc). Persons interested in participating in the CrGC or in attending the 1987 Crucifer Genetics Workshop should write, Paul Williams, Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706, (608) 262-6496.

REFERENCES

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INTERNATIONAL CLUBROOT WORKING GROUP

G R DIXON

A meeting was held as part of the 22nd International Horticultural Congress, Davis, California, USA on 12 August 1986. Twenty-one clubrooters were present from Belgium, Denmark, Federal Republic of Germany, German Democratic Republic, Japan, Norway, Poland, South Africa, UK and USA. Dr G R Dixon took the Chair.

Recent changes in the International Clubroot Working Group were reviewed. A postal ballot had shown most members favoured abandoning an independent Newsletter. It is now recommended that results and other information relating to clubroot should be submitted to the Cruciferae Newsletter. The quantity of information coming forward for an independent Newsletter has reached very small proportions. It was unlikely that any form of sponsorship could be obtained for a Newsletter with so little content. Data collected using the European Clubroot Differential (ECD) Series had been analysed and summarised by Ir H Toxopeus, Dr G R Dixon and Dr P Mattusch. Summary and publication of data was requested by a meeting of the International Clubroot Working Group held at Wageningen, The Netherlands in 1979. The publication had been accepted by the Transactions of the British Mycological Society with the title "Physiological specialisation in Plasmodiophora brassicae: an analysis by international experimentation". Salient features of the paper were described by the Chairman. Data used in this paper have been lodged with The Biological Data Collection in the General Library of British Museum (Natural History) London.

A general discussion of the world importance of clubroot ensued. Existence of this problem was originally seen in South Africa in the 1930's. Thereafter no further reports appeared until recently, now it is becoming of great importance. Reasons for this are a lack of effective rotations and increasing cabbage production. A comment was made that in California, USA, a similarly explosive development of Plasmodiophora brassicae in The Salinas Valley had been controlled by liming. The American soils are however naturally calcareous whereas those in South Africa have pH 4.5-5.5 which greatly encourages P. brassicae. Experience in the German Democratic Republic had shown particularly with sandy soils liming by itself was insufficient a control measure for P. brassicae. Calcium cyanamide had been tested and found to be as effective as lime. Some use of Quintozene (= pentachloronitrobenzene, PCNB) was permitted, the mode of action was suggested to be through an inhibition of the primary stages of root invasion. Considerable crop losses take place in Poland caused by P. brassicae. Control methods include using a combination of thiophanate and carbendazim as a root dip. Additionally liming is also recommended. Greatest losses take place in late season cabbage. Resistance was reported from South Africa in cultivars of white cabbage and chinese cabbage obtained from Japan. A plant breeder from Japan stated that a single dominant gene for resistance had been transferred from European turnip into chinese cabbage.

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Attempted transfer into Brassica oleracea types had been unsuccessful. Resistance developed by Dr Weisaeth in cabbage was referred to and it was learned that work continued in Norway to develop his material.

Professor Bochow (German Democratic Republic) delivered a paper "New Aspects in the control of Plasmodiophora brassicae". Since fungicides have not provided satisfactory control of P. brassicae consideration is now moving towards improving methods of biological control. Factors such as soil temperature, water content, pH and structure, will affect the rate at which resting spores germinate. Studies have been made into the influence of "non hosts" on resting spore germination. For this work turnip and tomato plants have been used. Root exudates from both stimulated resting spore germination. Greatest stimulation was achieved with extracts from turnip roots and the effects could be altered by changing pH. Since these effects could be obtained from both hosts and non-hosts it was concluded that stimulation of spore germination was a non-specific effect. Macerated root extracts from a range of plants such as rape, onion, rye, ryegrass and tomato stimulated infection. There was a tendency however for greater stimulation to be associated with root extracts from "host" compared with "non-host" plants. Rape extracts tended to depress the numbers of successful infections once invasion had started.

Studies of liming in the field indicated direct correlation between pH and disease index. In one experiment full and empty resting spores were assessed after soil had been treated with calcium carbonate. Only where treatment concentrations reached 1.6 g calcium carbonate/100 g soil was any reduction in the numbers of empty resting spores noticed. In further experiments it was shown that land sown to ryegrass which was cut and then the land fallowed resulted in significant reductions in P. brassicae infestation.

In a discussion of this paper it was emphasised that the general soil environment must per se have a considerable effect on P. brassicae inoculum levels. Elsewhere it has been demonstrated that the concentration of organisms in the rhizosphere around black mustard roots was higher than that around those of rape. This could help prevent P. brassicae infection of mustard. Consideration was given to other topics such as the effects of calcium concentration on both host and pathogen, potential for extended rotation with non-host plants; potential trigger substances in root exudates which might be exploited in order to encourage spore germination. Experiments in Belgium using soil fumigation were reported. Dichloropropene produced an effective control but repeated applications were required every 2-3 years. Control was reported from Taiwan using a mixture of ricehulls, calcium carbonate and ammonia. This combination is mixed into the soil prior to transplanting and is thought to protect the tap root from infection.

The International Clubroot Group acknowledges with gratitude assistance given by the International Society for Horticultural Science in providing the facilities and advertising which enabled this meeting to take place.

GENETIC RESOURCES OF CRUCIFEROUS CROPS COLLECTED IN NEPAL BY IBPGR

H. Yamagishi, S. Yui and H. Yoshikawa

IBPGR(International Board for Plant Genetic Resources) sent the exploration parties twice to Nepal as one of the projects for its 10th anniversary in 1984 and 1985(The leader was Dr. M. Iizuka). The purpose of the exploration was to collect indigenous genetic resources and their wild relatives in grain crops, fruits and vegetables. In Nepal, heavy genetic erosion is rapidly spreading and many kinds of classic varieties should be explored as soon as possible.

The seeds of local varieties were collected mainly in the hilly and mountaneous areas of east, central and west Nepal. The senior author joined in the first party in 1984.

The cruciferous crops collected by the two explorations were shown in Table 1. The samples collected in 1984 were evaluated in 1985 autumn.

In Brassica campestris main samples were identified as 'toria'. 'Toria' was very early to bolt, taking less than one month to flower. 'Sarson', especially 'yellow sarson', had larger plant size than 'toria' and was later to bolt. Nine out of twelve 'sarson' samples included small amounts of 'toria' seeds as contamination.

Generally B. juncea had very wide intra-strain variations in leaf shape and color, but several samples had superior yield and high quality.

The yield and quality of R. sativus was extremely low compared to Japanese varieties.

The evaluations of 1985 collections and propagations of seeds are now undergoing.

Table 1 Cruciferous crops collected by the IBPGR projects in Nepal

Species	Number of samples	
	1984	1985
Brassica campestris		
(toria)	50	} 123
(sarson)	12	
(turnip)	2	-
Brassica juncea	28	27
Brassica oleracea	2	1
Brassica (unknown)	-	33
Raphanus sativus	15	37
Lepidium sativum	3	-

Veg. & Ornamental Crops Res. Stat.,Ano,Mie 514-23,Japan.

STUDIES ON HORTICULTURAL CLASSIFICATION OF NON-HEADING CHINESE CABBAGE

Cao Shouchun

Non-heading Chinese cabbage (Brassica campestris ssp. chinensis (L) Makino) originated in China. There have been rich germplasm resources of these vegetables with characteristics of nourishing quality, wide adaptation and rapid growth. Being major favourable vegetables in meeting the market requirement they play an important role in supplying vegetables all the year round in southern China.

Since 1954, more than 190 stocks of these vegetables have been systematically observed and identified. Their morphological, biological and genetical characteristics have been studied in our research and a systematic horticultural classification can be offered based on extensive accumulated data.

1. Criteria of classification

Three levels of criteria have been used as follows: The first grade is based on the taxonomic position of the cultivars; the second grade is based on their main biological and cultural characteristics, and the third is in accordance with their major morphological characteristics.

2. Horticultural classification in non-heading Chinese cabbage

I. Pu Tung Pe Tsai (Pak choi) variety (var. Communis Tsen et Lee, var. erecta Mao). Plant growth habit erect or spreading, varying in height and leaf shape, generally high yielding, good quality and wide adaptation. There are many cultivars which are the most important and common varieties in non-heading Chinese cabbage, the appropriate combination of which could be cultivated to meet the market demands all the year round. Three types can be identified according to the period of maturity, bolting and cultivation.

A. Autumn-Winter type: mature early, sown in autumn and bolting in the following February along the middle and lower reaches of the Yangtze river valley. Growth habit erect, it may be further divided into two forms based upon the colours of petioles.

B. White-petioled form: leaves green or dark green, leaf margins entire or dissected with white petioles.

C. Long-petioled group - plant 45-60 cm in height, the ratio of petiole length and leaf blade >1, petiole much developed and fibrous, coarse texture, suitable for pickling, eg Nanjing Kan Chiae Pe Tsäi (flat petiole). Yangchow Hwa Ye Dai Tsäi (round petiole), Changchow Chang Pe Ken (half round petiole) etc.

CC. Short-petioled group - plant 25-30 cm in height, ratio of petiole length and leap blade <1, petiole thick, generally crisp, tender in texture, suitable for fresh consumption, eg Nanjing Ai Chiao Hwang (flat petiole), Changchow Tuan Pe Ken (half round petiole).

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CCC. Middle-petioled group - plant intermediate in height, petiole as long as leaf blade, suitable for fresh vegetable or pickling, eg Nanjing Er Pe (flat petiole), Wingnan Suon Tao Pe (half round petiole) etc.

BB. Green-petioled form: botanical characteristics and use same as white form, except with green or light green petioles.

C. Long-petioled group - plant above 45 cm in height, round or half round petiole, eg Taichow Ching Ken Dai Tsai, etc.

CC. Short-petioled group - Plant about 30 cm in height, flat or half round petiole, eg Shanghai Ai Gi Pe Tsai. Suchow Ching etc.

CCC. Middle-petioled group - Plant intermediate in height, flat or half round petiole, eg Kweichow Piao Er Pe, Hangchow Yu Tung Er etc.

AA. Spring type: intermediate to late in maturity. Sowing in late autumn and bolting in March to April next year, along the middle and lower reaches of Yangtze River Valley. Growth habit generally spreading, middle or dwarf in height. Suitable for fresh consumption. Two forms could be identified according to the period of bolting and maturity.

B. Early spring form: matures in early spring, bolting and harvest in March, divided into two groups based upon the colour of petioles.

C. White petioled group - eg Nanjing Nion Pe Ye (flat petiole), Chingchow San Yu Pe (half round petiole) etc.

CC. Green petioled group - eg Shanghai San Yu Man (flat petiole) Yangchow Er Ching (half round petiole) etc.

BB. Late spring form: mature in late spring, bolting and harvest in early to middle April.

C. White petioled group - eg Nanjing Si Yu Pe (flat petiole) Wu Xi Si Yu Pe (half round petiole) etc.

CC. Green petioled group - eg Shanghai Si Yu Man (flat petiole) Zheng Tiang Lo Tsai (half round petiole) etc.

AAA. Summer type: cultivated and supplied from June to September, grows rapidly, resistant to high temperature, thunderstorm, diseases and insects. Seedlings or mature plants are used as fresh vegetables eg Shanghai Huo Pe Tsai, Guangchow Ma Er Pe Tsai. Nanjing Dwarf Hybrid No. 1 (a new cultivar bred by Professor Cao Shouchun) etc. On the other hand, some long-petioled cultivars of autumn-winter type can also be cultivated in summer eg Zhan Jiang Po Touipe Tsai, Hangchow He Ye Pe Tsai etc.

II. Tai Tsai Variety (var. rosularis Tsen et Lee, var. atrovirens Mao) Plant growth habit prostrate or semi-prostrate, leaves small to

medium in size, obovate or round in shape, deep green or dark green in colour, leaf margin entire, its surface glabrous or wrinkled and glossy, fairly cold resistant. Sown in late autumn, harvested from January to February, with good quality, divided into two types based upon plant growth habit:

A. Prostrate type: plant height 5-10 cm eg Changchow Wu Ta Tsai, Shanghai Xiao Pe Ye etc.

AA. Semi-prostrate type: plant height 15-25 cm eg Nanjing Piao Er Tsai, Hefe Hei Hsin Wu etc.

III. Tsai Tai Variety (var. Tsai Tai Hort.)
Growth habit spreading or semi-erect. Leaves ovate or semi-round, margins wavy or irregularly serrated, pinnately-lobed at base. Petiole long and narrow-winged. Leaves on flowering stems short-petioled or clasping. Sown in autumn, bolts and harvested in winter or early spring. The plump and delicate flowering stem is the main edible part of this type. Appropriate combinations of various cultivars could be cultivated and supplied all the year round in southern China. It may be divided into two forms, according to the colour of leaves and flowering stem.

A. Green form (var. parachinensis Bailey): flowering stems and leaves green or yellowish green. Early cultivars eg Guangchow Sij Chiu Tsai Hsin etc, intermediate cultivars eg Kweilin Zhong Tsai Hsin etc, late cultivars eg Guangxi Lui Ye Tsai Hsin etc belong to this form.

AA. Purple form (var. purpurea Mao): flowering stem, petiole and veins of leaves are all purple-red in colour, leaves dark green or purple-green. Early cultivars eg Wuchang Tau Gutze Tsai Tai, Nanjing Tze Tsai Tai etc. Intermediate cultivars eg Chengdu Er Zao Zi Tsai Tai etc, and late cultivars eg Wuchung Yanzhi Hong etc, belong to this form.

IV. Tai-Tsai variety (var. Tai Tsai Hort.)
Plant growth habit spreading. Leaves long-ovate or long-obovate, upper margins entire or serrate, lower margins deeply pinnately-lobed, yellowish green, green or dark green. Stem leaves clasping, cultivars are sown in autumn and harvested from winter to spring. The leaves and plump roots are the edible parts of Tai Tsai. Cultivars cultivated popularly in the region of Hwang-Huai river, cold and alkali resistant, divided into two types, according to the period of bolting.

A. Early to intermediate type: cultivars sown in early autumn and harvested in the same year, but those sown in late autumn and harvested in March of the next year before bolting, eg Xu Chow Zao Tai Tsai, Jinan Hwa Ye Tai Tsai etc.

AA. Late type: cultivars sown in late autumn, bolt and harvested from March to April in the next year, eg Xu-chow Ben Tai Tsai Ta An Yuan Ye Tai Tsai etc.

V. Fen Nei Tsai variety (var. multiceps Cao)
Plant height 30-40 cm, 10-15 radical leaves emerge during the first growth stage, later many vigorous axillary buds appear at the end of the short stem, and each 'tiller' develops into 10 or more leaves before bolting. Leaves green or dark green, margins entire or divided into pinnate lobes, petioles greenish white or light green, narrow, flesh half round in shape. Strongly cold resistant. Cultivated popularly in Nanton region, divided into two types according to the period of bolting.

A. Early spring (intermediate maturity) type: cultivars sown in late autumn, bolt and harvested in following March eg Nan Ton Ma Er Tou etc.

AA. Late spring (late-maturity) type: cultivars sown in late autumn, bolt and harvested in the following April eg Nan Ton Shi Yue Pu Nao etc.

VI. Yu Tsai variety (var. utilis Tsen et Lee, var. oleifera Makino)
It is commonly well known as Yu Tsai or rape, cultivars are grown for seeds or both seeds for oil and leaves for vegetables eg Kiangsu Shin Hwa Piao Er Pe Yu Tsai etc.

3. The theoretical significance and practical application of horticultural classification of non-heading chinese cabbage

A. The taxonomic status of non-heading Chinese cabbage has been further clarified by this study and mistakes committed by former authors corrected such as: Tai Tsai was erroneously confused with Tsai-Tai, likewise Yu-tsai and Tsai-Hsin were listed as the same variety, and so on.

B. In viewing that the different species of Chinese cabbage, cabbage and mustard possess identical parallel variations, two representative varieties Tai Tsai and multiceps were supplemented in the subspecies of non-heading Chinese cabbage, thus more accurately reflecting the actual situation of Chinese non-heading cabbage cultivars.

C. More importantly the former classification based only on morphological characteristics, has been substituted by a horticultural classification which will convey theoretical and practical impact on research and utilisation of the rich germplasm resources in non-heading Chinese cabbage.

In addition, the horticultural classification will provide a theoretical basis for the improvement and balanced supply of the vegetable production all the year round, and the formation of co-ordinated combinations of Chinese cabbage cultivars in different areas of production, and for understanding the evolutionary relationship or genetic correlation among non-heading Chinese cabbage.

Application of a pattern analysis (quantification method III) for the isozyme relations in crop Brassicas

Y. Takahata and K. Hinata

The esterase and peroxidase isozymes of anther were investigated by polyacrylamid gel isoelectric forcusing for 26 strains of crop Brassicas that composed of A, B, C, AB, AC and BC genomes. Isozyme zymograms showed wide variations in number and intensity of bands in the same genome as well as in different genomes.

As for the monogenomic species;
1)Two common bands were observed in all the strains in peroxidase isozyme as well as esterase.
2)Two genome specific bands were detected in the A genome species in peroxidase.
3)There were four common bands between A and C genomes in peroxidase, while two common bands were found between A and B.
4)It was difficult to discriminate between A and C genomes by esterase isozyme, owing to no genome specific bands in them and many overlapping bands between strains of A and C genomes.
5)The B genome could be easily distinguished from the other genomes by five bands in esterase and four in peroxidase.
These results indicated the close relationships between A and C genomes. Such situation was supported by other evidences such as cytogenetics(Mizushima 1968), seed protein(Vaughan et al. 1966) and numerical taxonomy(Takahata and Hinata 1986).

Many bands of the digenomic species were composed of those of parental species. However, some bands in the monogenomic species were not found in the digenomic ones, and some digenomic unique bands were detected in B.juncea(AB) and B.carinata(BC). Further studies may be needed if these unique bands could be attributed to the occurence of mutations in the amphidiploids and/or their parental species or insufficiency of the material strains examined.

Since the zymograms under investigation were overlapping each other within the same genome as well as between different genomes, we have carried out one of pattern analysis so called quantification method III proposed by Hayashi (1956). By this method, the species relationships are presented based on the 0 - 1 data. Fig. 1 and 2 shows the results of this method for peroxidase and esterase data, respectively. In the case of peroxidase, strains having the same genome were closely located each other in spite of intra-genomic variations (Fig. 1). Wide variations of A and C genome species may be due to their wide variation of cultivated varieties. Digenomic species located between their parental genomes, and this agrees well with the cytogenetical relations (U 1935) and the numerical taxonomic ones (Takahata and Hinata 1980). On the other hand, in the case of esterase, the material strains were divided into two groups on the scatter diagram (Fig. 2). The species having B genome were placed in the left side and the other species were at the right. The A and C genomes were not distinguished. This is considered

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to be due to absence of genome specific bands between A and C genomes. Wide variation of esterase isozyme within a genome was also reported in the Brassica seedlings by Nakai (1970).

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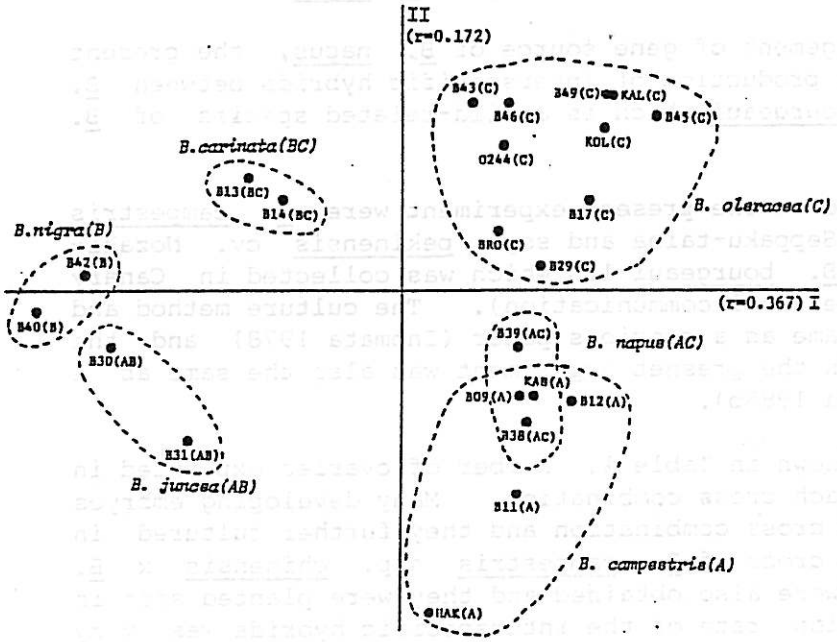

Fig. 1 Scatter diagram obtained by pattern analysis of peroxidase in Brassica.
B09=B.campestris var. toria,
B11=B.c. var. chinensis,
B12=B.c. var. campestris,
HAK=B.c. var. pekinensis,
KAB=B.c. var. rapa
B46=B.oleracea var. capitata,
B43,B45 and KAL=B.o.var.acephala
BRO=B.o. var. italica,
KOL=B.o. var. gongyloides,
O244=B.o. var. gemmifera,
B17=B.drepanensis,
B29=B.incana,
B49=B.rupestris,
B40 and B42=B.nigra,
B30 and B31=B.juncea,
B13 and B14=B.carinata,
B38 and B39=B.napus.
( ): genome symbol

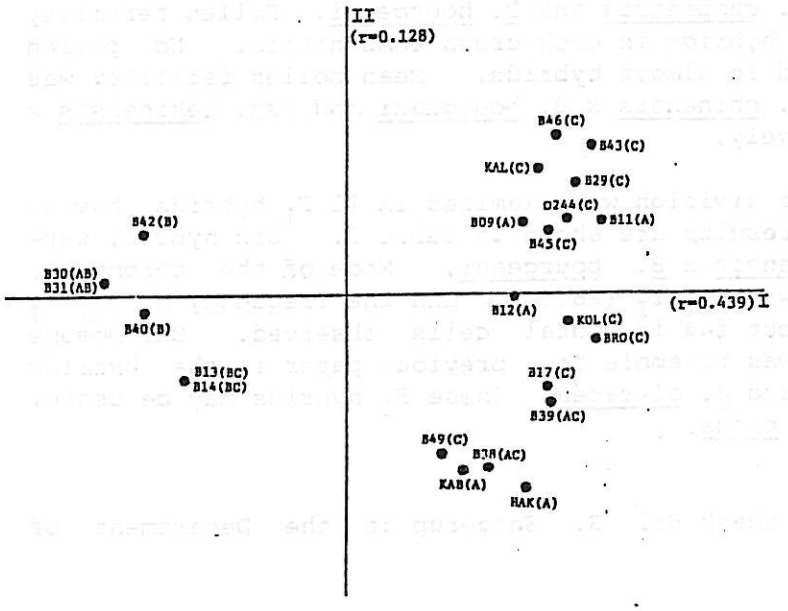

Fig. 2 Scatter diagram obtained by pattern analysis of esterase in Brassica.
Other details as Fig. 1.

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

Nobumichi INOMATA

It was already reported that Brassica campestris and B. oleracea were important gene source of the breeding of B. napus (Inomata 1983, 1985a). For the enlargement of gene source of B. napus, the production of interspecific hybrids between B. campestris and B. cretica which is wild-related species of B. oleracea, was intended in a previous paper (Inomata 1985b). Many F_1 hybrids were obtained and the cytological studies and pollen fertility on the F_1 hybrids showed like these of the F_1 hybrids which were obtained from the cross between B. campestris and B. oleracea. It is suggested that B. cretica become a gene source of the breeding of B. napus.

For further enlargement of gene source of B. napus, the present paper deals with the production of interspecific hybrids between B. campestris and B. bourgeaui which is a wild-related species of B. oleracea.

The materials used in the present experiment were B. campestris ssp. chinensis cv. Seppaku-taina and ssp. pekinensis cv. Nozaki-hakusai No. 2, and B. bourgeaui 120 which was collected in Canary Islands (Snogerup, personal communication). The culture method and condition were the same as a previous paper (Inomata 1978) and the culture medium used in the presnet experiment was also the same as a previous paper (Inomata 1985b).

The results are shown in Table 1. Number of ovaries explanted in the medium was 50 in each cross combination. Many developing embryos were obtained in each cross combination and they further cultured in the medium. In the cross of B. campestris ssp. chinensis x B. bourgeaui, many seeds were also obtained and they were planted soon in the medium. Production rate of the interspecific hybrids was very high and it was about same in the previous paper on B. campestris x B. cretica (Inomata 1985c). Morphological characteristics of leaf was intermediate between B. campestris and B. bourgeaui. Pollen fertility was examined in 24 F_1 hybrids in each cross combination. No pollen fertility was observed in almost hybrids. Mean pollen fertility was 0.06% and 0.13% in ssp. chinensis x B. bourgeaui and ssp. pekinensis x B. bourgeaui, respectively.

The first meiotic division was examined in 11 F_1 hybrids having 19 chromosomes. The results are shown in Table 2. Six hybrids were examined in ssp. chinensis x B. bourgeaui. Mode of the chromosome configuration at PMCs was $9_{II}+1_I$ (28.5%), and the frequency of $9_{II}+1_I$ and $1_{III}+8_{II}$ was about 50% in total cells observed. Chromosome pairing of the PMCs was resemble to a previous paper in the hybrids between B. campestris and B. oleracea. These F_1 hybrids may be useful for the breeding of B. napus.

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

Cross combination (B. campestris x B. bourgeaui)	No. of capsules examined (A)	No. of embryos further cultured			No. of seeds obtained	No. of hybrids obtained (B)	B/A x 100
		Late torpedo	Walking-stick	Full-grown embryo			
ssp. chinensis[1] x B. bourgeaui	44	8	17	17	110	102	231.8
ssp. pekinensis[2] x B. bourgeaui	43	37	37	21	0	39	90.7
Total or mean	87	45	54	38	110	141	162.1

1: cv. Seppaku-taina x B. bourgeaui 120. 2: cv. Nozaki-hakusai No. 2 x B. bourgeaui 120.

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

Cross combination (B. campestris x B. bourgeaui)	No. of PMCs observed (%)	First meiotic division					
		$_{I}I^{+8}_{II}$	$_{I}I^{+7}_{II}{}^{+2}_{I}$	$_{I}I^{+6}_{II}{}^{+1}_{I}$	$_{I}I^{+5}_{II}{}^{+3}_{I}$	Other types	
ssp. chinensis[1] x B. bourgeaui	181	42 (23.2)	13 (7.2)	46 (25.4)	12 (6.6)	68 (37.6)	
ssp. pekinensis[2] x B. bourgeaui	156	32 (20.5)	12 (7.7)	50 (32.1)	15 (9.6)	47 (30.1)	
Total or mean	337	74 (22.0)	25 (7.4)	96 (28.5)	27 (8.0)	115 (34.1)	

1: cv. Seppaku-taina x B. bourgeaui 120. 2: cv. Nozaki-hakusai No. 2 x B. bourgeaui 120.

HYBRIDIZATION OF TWO CULTIVATED BRASSICA SPECIES WITH WILD RELATIVES OF X=7 CHROMOSOMES

C. F. Quiros, O. Ochoa and S. F. Kianian

The basic genome number of Brassica is assumed to be x=6 (Robbelen, 1960). There are no record of any x=6 Brassica species, but there are several species with x=7 chromosomes such as B. adpressa, B. deflexa and Diplotaxis erucoides. Only a few reports dealing with the study of these species are found in the literature. The following hybridizations have been reported: D. erucoides x B. oleracea by Mizushima (1980); D. erucoides x B. napus, B. adpressa x B. campestris and B. adpressa x B. napus by Harberd and McCarthur, 1980 (Brassica Crop and Wild Allies ed. Tsunoda et al., 1980). In order to gain further understanding of the relationships between some of these species and the cultivated diploids, select hybridizations were undertaken to obtain the new hybrids reported in this note. These were confirmed by DNA restriction fragment patterns using rDNA probes, isozymes and chromosome number.

1) Diplotaxis erucoides x B. adpressa. Successful hybridization was possible using Diplotaxis as the pistillate parent, however, embryo rescue was necessary. Pollen fertility was very low in the hybrids. This was due to lack of complete chromosome pairing of the Diplotaxis and Brassica genomes. The hybrids had the expected chromosome number of 2n=14. The exception was a single hybrid plant which had a combination of haploid and diploid pollen mother cells before the first meiotic reductional division. This spontaneous chromosome doubling resulted in high pollen fertility. Their plants had the white flower characteristic of the Diplotaxis parent.

2) Diplotaxis erucoides x B. nigra. Successful hybridization was possible using Diplotaxis as the pistillate parent, however, embryo rescue was required. Pollen fertility was low due to poor chromosome pairing. The hybrids had the expected chromosome number, 2n=15 and the white flowers of Diplotaxis. One of the plants displayed an unstable anthocyanin color character not present in any of the parental species. Some of its sepals, petals, anthers, styles and leaves displayed red, anthocyanin stripes. Furthermore, a few flowers in the plant were found to have yellow petals typical of the nigra parent. The possibility of a transposable element is under investigation.

3) B. oleracea x B. adpressa. Successful hybridization was achieved using B. oleracea as the pistillate parent. Embryo rescue was required. The hybrids were very vigorous having the gray-green color and white flower of the B. oleracea parent (form alboglabra). Sterility in the hybrid was very high, and they had the expected chromosome number of 2n=16.

Chromosome numbers in the hybrids are being doubled with colchicine. Addition lines will be generated to determine the genetic composition of the chromosomes in the x=7 genomes.

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INTERGENERIC HYBRIDIZATION OF *ERUCA*, *BRASSICA* AND *RAPHANUS*

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Genus *Eruca* consists of 10 species, some of which are cultivated for green vegetable and oil seed in the restricted areas. It is rarely grown in Japan, alyhough it may provide the new genetic diversity for the breeding of cruciferous crops.

In this study, the intergeneric cross-compatibility was examined and young *Eruca* ovaries pollinated by allied species were cultured in vitro on White basal medium supplemented with 0.5 g/l casein hydrolysate and 30 g/l sucrose. The observation of pollinated pistils by means of fluorescence revealed that the pre-zygotic barrier of pollen germination and pollen-tube growth was normal for the most part. After culturing for ca. 60 days at 25°C, juvenile seedlings which were reared in cultured ovaries were removed to root forming medium. Their hybridity was ascertained cytologically(Table 1).

In the intergeneric hybrids of *E. sativa* x *R. sativus*, following generation was bred in backcrossing to *R. sativus*. These hybrid progenies may be valuable materials to incorporate agronomical characteristics of *E. sativa* to other cruciferous crops through generic recombination and cytolpasmic substitution.

Table 1. Intergeneric cross-compatibility and hybridization by ovary culture in *Eruca* x *Brassica* and *Eruca* x *Raphanus*(1985,1986)

Cross combination	P.G.I.**	No.ovaries cultured	No.seedlings obtained
E. sativa x *B. campestris*(5)*	3.1-3.3	218	2
E. sativa x *B. nigra* (5)	1.8-3.0	243	0
E. sativa x *B. oleracea* (5)	1.8-3.2	233	11
E. sativa x *R. sativus* (12)	2.0-3.5	552	8

* : Numbers in parentheses indicate lines of pollen donors examined.
**: Range of pollen germination index ($0 \leqq P.G.I \leqq 4$) after Matsuzawa 1983.

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INTERSPECIFIC HYBRIDIZATION BETWEEN BRASSICA FRUTICULOSA AND B. CAMPESTRIS

P. B. A. Nanda Kumar and K. R. Shivanna

Interspecific hybridization, particularly using wild species, offers considerable prospects in the improvement of Cruciferous crops. Interspecific crosses between B. campestris X B. fruticulosa (wild sp.) are successful while the reciprocal cross is rather difficult. During 1985, only two seeds were obtained out of 144 pollinations; they failed to germinate when grown in soil. Hybrids using B. fruticulosa as female parent are very useful in obtaining cytoplasmic substitution of the cultivars. In recent years, interspecific hybrids of many crucifers have been obtained through ovary and ovule culture. We have attempted to obtain interspecific hybrids of B. fruticulosa X B. campestris through ovary culture.

Pollinated ovaries were excised 4-5 days after pollination and cultured on Murashige and Skoog's (1962) medium containing 5% sucrose, 0.8% agar and 500mg/l casein hydrolysate. Seeds were harvested after the fruits turned brown. Some of the seeds were cultured on fresh medium to raise seedlings.

Over 88% of cultured ovaries developed into fruits (Table 1) and yielded considerable number of healthy seeds (1.15 healthy seeds/ovary cultured). The number of seeds realized in self-pollinated cultured ovaries was much less (3.4/ovary cultured) compared to field pollinations (7.4/pollination).

Table 1. Details of fruit and seed set in cultured ovaries

	No. ovaries cultured	No. of fruits	No. healthy seeds	No. seeds/ ovary cultured
B. fruticulosa X B. campestris	115	102	133	1.15
B. fruticulosa self	74	73	254	3.43

Healthy seeds collected from in vitro cultured fruits germinated and gave rise to seedlings (Table 2). Hybrid nature of the seedlings was confirmed by morphological characters of the leaves as well as by electrophoretic analysis of leaf esterases and acid phosphatases of the seedlings of parents and hybrids.

Table 2. Germinability of seeds

	No. seeds cultured	No. seeds germinated
B. fruticulosa X B. campestris	100	64
B. fruticulosa self	47	7

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AN UNUSUAL FOLIAGE TYPE IN BRASSICA

M.L.Gupta, S.S.Banga and K.S.Labana

The shape of adult leaf is an important morphological key for taxonomic and phylogenetic identification. In the tribe Brassicae, four basic leaf types namely, simple, partite, divided and pinnatisect are recognised. The B.juncea group generally have pinnati-divided leaves with lobbed margins. During 1984-85 few plants with deeply divided or pinnatisect leaves were observed in a population of an intervarietal cross between RLM 198 and RLM 240 of B.juncea. The plants had reduced vigour and pale yellow, glabrous leaves. However, the leaf shape was not comparable with any of available leaf mutant in germplasm collection of B.juncea. Out of many wild species, the leaf shape of the variant resembled most with Sinapis allioni. Plants had very low pollen fertility (25%). Sowing of forced self seeds led to about 30 adult plants. However, the leaf shape of the plants varied from normal B.juncea type to deeply divided leaves. Meiotic analysis of these plants indicated them to be aneuploids with chromosome number ranging from 36 to 45. Out of the various chromosome configurations observed in different plants, 19 II + 2 I and 16 II + 1 III + 1 IV + 1 I were more frequent. Anaphase distribution was irregular with large number of laggards. An interesting observation was that the pinnatisect leaf trait was more apparent in plant with higher chromosome number, while plants with 2n = 38 resembled B.juncea more closely. It appears that the leaf variation has resulted from a natural interspecific cross between B.juncea and an unknown species/genera. We are plan to identify the pollen parent through chromosome pairing behaviour and isozyme analysis. The potential candidates include Diplotaxis muralis and Sinapis allioni.

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A CYTOGENETIC STUDY OF HYBRIDS BETWEEN *BRASSICA NAPUS* L. and *B. CAMPESTRIS* L.

R.Y. Chang and W. Tai

Crosses were made between two lines of *Brassica napus* "Regent" and "83-1470", with two lines of *B. campestris*, "S75-7460-R" and "Hi Palm". Meiotic behaviour of 18 F_1 and six F_2 plants was analyzed. The expected genome formula of the hybrid is "AAC" with one "A" derived from *B. campestris* and "AC" derived from *B. napus*. At first meiosis, the chromosomes of the two A genomes are expected to be present as univalents.

Chromosomes paired as expected at diakinesis, the most prevailing chromosome association was ten bivalents and nine univalents. Among a total of 157 pollen mother cells (PMC) of the F_1 studied, 150 cells or 95.5% had $10^{II} + 9^{I}$. Of the remaining seven PMC's, three cells had $9^{II} + 11^{I}$, two cells had $11^{II} + 7^{I}$, and one each had $1^{III} + 9^{II} + 8^{I}$ and $1^{III} + 10^{II} + 6^{I}$. Occasionally, a bivalent was loosely connected with a univalent but the association was not tight enough to be considered as a trivalent.

Similar results were observed by U (1935), Mizushima (1950a, b), and Li (1978), Ogura (1970) reported that a substantial number of PMC's had zero to three bivalents in addition to the 10 A-genome bivalents. There seems to be considerable homoeology between chromosomes of the A and C genomes which should account for the formation of trivalents and extra bivalents in the hybrids.

Chromosomes were usually packed to form an equatorial plate at metaphase I with a few exceptions, in which several chromosomes were scattered randomly in the cytoplasm.

Precocious division, separation of chromatids at first meiosis, was common at anaphase I. With the high frequency of univalent formation, it was expected that the univalent chromosomes would line up on the equator at metaphase I and thus the separation of chromatids at anaphase I. Owing to this, a wide range of chromosome segregation was recorded. A minimum of 12 and a maximum of 20 chromosomes were found at a pole. The most common types were (15-14), (16-13), and (17-12). Most cells had single-stranded chromosomes at each pole. If two single-stranded chromosomes were counted as one, the chromosome number of the two poles would add up to 29, the expected number. For instance, if a cell had a (16-15) segregation, it would have 14 double-stranded and 2 single-stranded chromosomes at one pole and 13 double-stranded and 2 single-stranded at the other.

Laggards and chromatin bridges were common at anaphase I. Snogerup and Persson (1983) reported similar irregularities.

Only two anaphase II cells were scored and they had (16-16-13-13) and (16-15-14-13) segregation.

Among a total of 176 quartets recorded, 96 or 54.5% of them appeared

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normal. The remainder had one to three micronuclei with an average of 0.76 micronucleus per quartet.

Among the six F_2 plants studied cytogenetically, two of them had similar meiotic pairing of $17^{II} + 2^{I}$ with total chromosome number of 2n = 36. We believe that 10 of the 17 bivalents were the result of pairing between chromosomes of the A genome and the other seven bivalents were derived from pairing of C genome chromosomes with the two univalents also belonging to the C genome. Therefore, we are very close to recovering a synthetic hybrid with a genome formula similar to B. napus.

Two of the remaining four plants had $16^{II} + 3^{I}$. The other two had $10^{II} + 9^{I}$, and $10^{II} + 7^{I}$, respectively.

Further cytological studies are currently undertaken to determine the transmission of individual chromosomes and the possible use of aneuploids for genetic analysis and in the rapeseed breeding programs.

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EVOLUTIONARY TRENDS IN BRASSICA: GATHERING EVIDENCE FROM CHROMOSOME ADDITION LINES

C. F. Quiros, O. Ochoa, S. F. Kianian and D. Douches

During the past year we have focused our efforts on the creation of B. campestris-oleracea, B. nigra-oleracea, B. oleracea-nigra and B. oleracea-campestris chromosome addition lines. Most of our progress has been in the generation of B. campestris-oleracea addition lines by crossing and backcrossing the amphidiploid B. napus to B. campestris. The pollen fertility of the sesquidiploid hybrid resulting from the initial cross was approximately 66%, while those of the derived hyperploids from subsequent crosses ranged from 83 to 94%. Monosomic and disomic addition lines, had a pollen fertility of 94 and 91%, respectively. The frequency of chromosome numbers and average pollen fertility in plants derived from crossing various hyperploids to B. campestris is shown in Table 1. Selfing of monosomic addition lines resulted in disomic addition lines (19% of the progeny). The addition lines were genetically characterized by genome specific markers (Quiros et al. Cruciferae Newsletter 10:21-23). The isozymes 6PGD, LAP, PGI and PGM, and the DNA restriction fragments generated by EcoRI digestion and probed with rDNA were found to be useful for this purpose. A total of eight monosomic and eight disomic plants were identified and characterized on the basis of these markers. Another 51 plants remained uncharacterized due to the lack of additional markers. A few derivatives were self-compatible, but this trait could not be associated with a specific oleracea marker. The possibility exists that having the S locus in a polysomic condition might weaken its expression resulting in self-compatible plants; this is what occurs in the amphidiploid species which are self-compatible. rRNA genes were found to be distributed in more than one chromosome, most likely two according to the cytological evidence. Each of these was found to differ in its restriction sites, indicating initial duplication and subsequent divergence of these sequences. Intergenomic recombination for some of the markers was detected at a frequency below 20%. Duplicated loci for several of these markers were observed in Brassica campestris, B. oleracea and B. nigra, supporting the hypothesis that these diploid species are actually secondary polyploids. The isozymes of 6PGD were found to be encoded by four loci in the three cultivated diploids. Locus 6pgd-1 has a duplicated counterpart 6pgd-1', while locus 6pgd-2 has a duplicated counterpart 6-pgd-2', at least in B. oleracea and B. nigra. The B. campestris-oleracea addition lines disclosed that the 6PGD-1 and 6PGD-2 loci are located on different chromosomes of the oleracea genome (c, x=9). On the other hand, hyperploid plants of B. oleracea-nigra, derived from crossing and backcrossing B. carinata to B. oleracea, disclosed that 6PGD-1 and 6PGD-2 are on the same chromosome in the nigra genome (b, x=8). Assuming that these sets of loci have derived by tandem

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duplications, their location on different chromosomes in oleracea suggests that a secondary event, namely a translocation has taken place during the origin of the oleracea genome. Thus, it is likely that the evolution in genomic number has been in ascending order, from x=8 to x=9.

Radish (Raphanus sativus, x=9), a close Brassica relative, was found to have an rRNA restriction fragment pattern similar to B. oleracea and B. nigra, except for a 6.0Kb fragment which is replaced by a 5.3 and a 4.1Kb fragment of B. campestris. The combinations of the fragments of these species suggests that radish might have arisen by hybridization of a turnip (B. campestris, x=10) x B. nigra (x=8). Further investigation is needed to test this hypothesis.

TABLE 1. Frequency of chromosome numbers and average pollen fertility in plants derived from crossing various hyperploid plants to B. campestris.

2n	29x20	26x20	24x20	23x20	22x20	21x20	% pollen fertility
20	0	2	1	3	3	8	96.3
21	1	6	3	7	10	6	94.2
22	3	3	6	2	3	5	91.2
23	2	0	5	2	0	0	87.6
24	2	2	7	0	0	0	85.0
25	1	1	0	0	0	0	83.1
26	3	0	1	0	0	0	83.3
Other[1]	2(30)	1(40)	1(34)	1(19)	0	1(30)	

[1]Chromosome markers in parentheses.

CHIASMA FREQUENCY VARIATION IN BROWN SARSON

T S Sareen, S Rani and S C Verma

Brown sarson (Brassica campestris var. brown sarson, syn. B. campestris var. dichotoma - brown sarson) is an important oilseed crop, and all its cultivars screened by us are self-incompatible (Verma et al. 1984). Despite several studies on the chromosomes of Brassicas there is very little information available on the form of bivalents and on the effects of deliberate selfing or close breeding on mean cell chiasma frequency (Prakash and Tsunoda 1983). Since F-1 breeding programmes require the production of S-allele homozygous lines through bud-selfing, it is worthwhile to discover the effect of such breeding practices on the mean cell chiasma frequency. Verma and Chauhan (1985) reported on the form of bivalents in several crucifers from cultivated material, and we have extended similar type of observations to the several lines of crop Brassicas being maintained (and accessioned) at the Haryana Agricultural University (HAU), Hissar. This communication summarises information in brown sarson.

Seeds of 12 experimental lines (cultivars) of brown sarson, from HAU Hissar (Verma et al. 1984), were sown in the University Botanical Garden at Chandigarh, and all these were found to be fully SI. Meiotic studies were made from aceto-carmine squashes of pmc's from young anthers fixed in 1:3 acetic alcohol. Chiasma frequency per cell was determined at metaphase-I.

The bivalents were small, and largely of the rod type. Bivalents with two chiasmata, one in each arm (ring type) occurred in some of the pmc's but with low frequency (Table I). The pooled data indicate that chiasma frequency per cell was 10, 11 and 12 in approximately 80.5 %, 17 % and 2.5 % of the pmc's analysed, respectively. There are two important observations. Firstly, the mean cell chiasma frequency of the 12 cultivars is low as compared to the report of Verma and Chauhan (1985); they showed nearly 18.5 chiasmata per cell while the overall mean of the 12 samples of brown sarson analysed here is 10.24. Secondly, the analysis of variance for the data set out in Table I shows significant difference between the means at 5 % level (also at nearly 1 % level). However, it is not that every cultivar is different from every other. Rather, Table I reveals some six groups when cultivars with similar means are clustered.

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Table I
Distribution of xta per cell in 12 cvs of brown sarson

No.	Cultivars*	Frequency of pmc's with xta per cell			xta per cell	
		10	11	12	Mean	Std.error
1.	BSC-9	20			10.00	
2.	BSC-57	19	1		10.05	0.050
3.	BSC-66	18	2		10.10	0.069
4.	BSC-5	9	1		10.10	0.100
5.	BSC-1	17	3		10.15	0.081
6.	BSC-56	8	2		10.20	0.133
7.	BSC-8	9		1	10.20	0.200
8.	BSC-55	16	3	1	10.25	0.123
9.	BSC-6	12	8		10.40	0.112
10.	BSC-50	14	4	2	10.40	0.152
11.	BSC-67	6	4		10.40	0.163
12.	BSC-13	5	4	1	10.60	0.221

*Accession numbers of experimental lines of HAU, Hissar

In two SI and habitually outbred species, namely Secale cereale and Raphanus sativus, the inbred lines revealed significant reduction in mean cell chiasma frequency when tested against the parental material (Rees 1961, Dayal 1977). Comparing our data with those of Verma and Chauhan (1985) on population material, the markedly low mean cell chiasma frequency of the 12 "cultivars" (Table I) may, therefore, be due to the closely-bred nature of these experimental lines.

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EFFECT OF GAMMA RAYS ON CHROMOCENTRES IN RADISH

U.C. Mehta and N. Dayal

In radish, chromocentres have been studied quite extensively and the genetic system responsible for the maintenance of an optimum level of chromocentre frequency (Cfr) and its distribution is understood to certain extent (Dayal, 1975; Dayal et al., 1982, 1983)

There are only few studies on the effect of radiation on radish. It is known that crucifers in general and radish in particular are highly radio-resistant (Gomez-Campo & Delgado (1964). Here we report for the first time the study on the effect of different doses of radiation (100 Ke, 150 Kr & 200 Kr Gamma rays) on the number & distribution of chromocentres representing pericentric constitutive heterochromatin in radish.

Four varietal populations of radish (Raphanus sativus L.), namely Japanese White (JW), Pusha Himani (PH), Doppel Bock (DB) and Violet de Gourney (VG) have been used in the present study. JW and PH are Indian varieties and DB and VG are European. These varieties differ in several morpho-physiological characteristics. About 100-200 air dried seeds of each variety were irradiated at 100 Kr, 150 Kr & 200 Kr of Gamma rays at a dose of 6 KR/minute with Co60 source at the Gamma Cell of the Projects & Developmental Co.Ltd., Sindri, Bihar. After irradiation seeds were brought back, sowh simultaneously in the field & plants raised. Cytological studies were made as reported earlier (Dayal, 1975). Scoring was made in 20 cells per plant. A total of 160 plants, 10 from each item, were cytologically examined.

The varietal populations of radish varied significantly in the number and the distribution of chromocentres per nucleus. Interestingly, the Indian varieties had significantly higher mean Cfr than their European counterparts. The distribution pattern of chromocentres also varied noticeably; the European varieties had more uniform distribution pattern of chromocentres than the Indian varieties (Table I). Individual plants within a population however, showed negligible variation. Irradiation by Gamma rays had a marked effect on the Cfr and its distribution pattern, significantly reducing the mean Cfr in all the varieties. However, the effect of irradiation was more drastic in the Indian varieties than in the European ones. DB was practically unaffected. The distribution pattern of Cfr was also significantly affected in them, more particularly in the Indian varieties.

The effect of irradiation on various cytological parameters have been an object of cytological studies for quite some time. Radish is specially suited for studying the amount & distribution of constitutive heterochromatin represented by chromocentres in interphase nuclei. Our study shows that the Indian & the European varieties of radish respond differently to different doses of Gamma rays and that they are highly radio-resistant. The different doses of radiation also alter noticeably the distribution pattern of chromocentres in the nuclei. The Indian & European varietal populations vary in this respect too, which indicates the genotypic peculiarities of these populations. In general radiation has a reducing effect on the mean Cfr.

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At this stage, we do not exactly know as to what factors are actually responsible for the differential behaviour of different populations of radish to irradiation by Gamma rays. Chromocentres have been considered as an adaptive characters. Besides the role of genome size, genetic factors & DNA content may not also be over ruled. Ionizing radiation probably breaks up the heterozygosity and consequently the genetic balance and the buffering properties of the varietal populations of radish in different ways. Here it is shown that irradiation by Gamma rays reduces the mean Cfr and its distribution pattern in radish.

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Table 1. The number & distribution of chromocentres/nucleus in Indian & European radishes at different doses of radiation

Materials	No.of chromocentres in nuclei										Chromocentres/ Nucleus Mean ± S.E.	CV (%)
	7	8	9	10	11	12	13	14	15	16		
Indian Radishes												
JW (K)				-	6	16	18	54	80	26	14.3±0.10	10.4
JW (100 KR)				43	30	47	36	34	5	5	12.1±0.01	0.32
JW (150 KR)				28	37	48	38	33	16	-	12.3±0.01	0.31
JW (200 KR)				29	46	45	34	32	13	1	12.2±0.01	0.32
PH (K)				04	06	16	18	54	76	26	14.2±0.09	9.8
PH (100 KR)				40	33	45	38	34	5	4	12.1±0.11	12.8
PH (150 KR)				30	35	50	36	30	19	-	12.3±0.10	12.4
PH (200 KR)				30	45	40	39	30	15	1	12.2±0.10	12.4
European Radishes												
DB (K)	21	48		50	55	46					10.3±0.12	15.8
DB (100 KR)	25	44		57	46	48					10.2±0.11	16.1
DB (150 KR)	30	39		58	48	45					10.1±0.10	16.5
DB (200 KR)	35	34		60	46	45					10.1±0.11	16.8
VG (K)	10	17		65	44	64					11.2±0.16	14.8
VG (100 KR)	21	36		50	45	48					9.3±0.09	13.8
VG (150 KR)	30	27		55	44	44					9.2±0.12	19.3
VG (200 KR)	40	17		65	34	44					9.1±0.13	20.9

CHROMOCENTRES IN THE AMERICAN RADISHES

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In continuation of our previous studies on hetero-chromati n phenotype represented by chromocentre frequency (Cfr) in the Indian and European populations of radish, we studied the number and the distribution of chromocentres in the American varietal populations of radish in order to understand the degree of heterochromatinization in them vis a vis their Indian and European counterparts.

Materials for the present investigation are listed in Table 1. Methods for cytological analysis are the same as used earlier (Dayal 1975).

The American radishes on the whole had almost the same mean Cfr-13.1 as the Indian-13.6 but higher than that of European radishes-11.3. However, the lowest and the highest Cfr was noted in CB and CG respectively. FB occupied an intermediate position-13.4 in this parameter.

The American radishes like their European and Indian counterparts have both high and low number of chromocentres per nucleus. Taking mean Cfr as an index of visible heterochromati n it appears that American radishes in general are less heterochromatinized than the Indian but more heterochromatinized than the European radishes, if chromocenters are any indication of visible heterochromatin. This means that the American radishes are less heterozygous than the European but more heterozygous than the Indian radishes. Thus they occupy an intermediate position in this regard. Our study also shows that they have a narrower distribution pattern of Cfr than the European and the Indian radishes.

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Table 1 Chromocentres per nucleus in American, European and Indian radishes

Materials	Chromocentres/nucleus Mean ± S.E.	CV (%)	Source
Indian radishes			
Long White Green Top (GT)	13.1±0.12	8.9	Dayal & Prasad, 1983
Japanese White (JW)	14.2±0.12	8.5	- do -
Kalamikati Red (KR)	13.4±0.15	11.5	- do -
Scarlet Globe (SG)	12.6±0.14	10.8	- do -
Rainy Season Red (RR)	12.9±0.17	13.0	- do -
Pusa Himani (PH)	14.6±0.14	9.3	- do -
Chinese White (CW)	15.2±0.16	10.6	- do -
Jaunpur Giant (JG)	12.4±0.10	8.3	- do -
Contai Long (CL)	14.1±0.17	7.6	- do -
Pusa Desi (PD)	13.6±0.13	2.9	- do -
European radishes			
Rabano Ravanello (RRN)	10.0±0.18	18.7	Panicker, et al, 1985
Demilong Ecarlet (DE)	11.5±0.14	12.2	
Riesenbulter Wez (RW)	11.4±0.18	10.8	- do -
Saxa Trieb (ST)	13.0±0.17	13.3	- do -
Doppelbock (DB)	10.6±0.14	13.2	- do -
Violet de Gournay (VG)	11.2±0.16	14.8	- do -
Round Rose (RRO)	11.1±0.12	10.8	- do -
American Radishes			
Red Devil (RD)	13.2±0.04	1.38	Present study
Early Scarlet Globe (ESG)	11.5±0.03	9.9	- do -
Crimson Giant Large solid Flesh(CG)	13.1±0.08	6.1	- do -
French Breakfast (FB)	12.1±0.01	13.6	- do -
Cherry Belle (CB)	12.5±0.01	1.4	- do -
Icicle Short Top (IST)	14.5±0.09	3.1	- do -
Champion (CP)	13.7±0.01	4.8	- do -
Scarlet Turnip White Tipped (STW)	13.8±0.04	13.4	- do -
Crimson Giant (CG)	12.3±0.16	6.2	- do -
Sparkler (SP)	13.0±0.04	15.6	- do -

DEVELOPMENT OF PRIMARY TRISOMICS OF RAPID-CYCLING *BRASSICA CAMPESTRIS*

Curtis B. Hill, Keliang Tang and Paul H. Williams

Development of trisomic stocks of rapid-cycling *B. campestris* was begun by crossing tetraploids with diploids to produce triploid plants. Populations of 96 plants of tetraploid CrGC 91 were grown under standard conditions (CrGC Resource Book, 1985). Individuals of tetraploid populations were pollinated with mixed bulk pollen from 96 diploid plants (CrGC 1). Preliminary work indicated that the tetraploids were self-incompatible, permitting the pollination of open flowers. Seed sets were higher when tetraploids were pollinated with diploid pollen than the reverse.

Of the 480 tetraploid plants that were pollinated, 177 set from 1 to 10 seeds. Twenty two tetraploid x diploid families have been analyzed and 49 progeny have been examined cytologically using the following procedure.

Young, rapidly growing leaves, collected between 9-11 A.M., were pretreated with 0.002M 8-Hydroxyquinoline at 22-24C for 2-3 hrs., fixed in Carnoy's fixative for 1 hr.-3 days, hydrolyzed in 1N HCl for 6- 10 min. and stained with modified phenol-basic fuchsin staining solution (see below) for 0.3 hr.-overnight before using standard squashing techniques. Chromosomes are clearly resolved under 1000X.

Of the 49 progeny examined, 35 were tetraploid, 12 were triploid, one was diploid and the ploidy of one could not be determined. Many of the cells examined had chromosome numbers ranging between tetraploid, triploid or diploid numbers. At least 10 cells/plant were examined and the median chromosome number/cell was used to estimate the ploidy.

Some of the triploid plants appeared to be sterile but most were fertile. They wer intercrossed and crossed with diploid CrGC 1. Progeny from the triploids will be examined for morphological and cytological evidence of trisomy. Tetraploid plants with a propensity for producing triploids are being intercrossed.

Similar methods are being used to develop primary trisomics of rapid-cycling *B. oleracea* and *B. nigra*. Primary trisomics will facilitate mapping studies in *Brassica*.

Modified Phenol-fuchsin Staining Solution

Mix 3 g basic fuchsin in 100 ml 70 % ethanol (solution A) (this solution can be stored for long periods). Put 10 ml of solution A into 70 ml of 5 % phenol (solution B). Add 45 ml of solution B to 6 ml glacial acetic acid and 6 ml of 37 % formaldehyde (solution C). Combine 10 ml of solution C with 30 ml 45 % acetic acid and 1 g sorbitol to make modified phenol-fuchsin staining solution.

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GENETICS OF SEED YIELD AND SOME IMPORTANT ATTRIBUTES IN INDIAN MUSTARD

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The present investigation was conducted to know the nature and magnitude of genetic components involved in the inheritance of seed yield and its contributing traits in 3 crosses of Indian Mustard using the NC III design of Comstock and Robinson(1952).

The F_2 families included in the present study were obtained from three crosses namely; RH-785xRH-30, RH-30xRC-1425 and RH-30xRC-1426. The plants were randomly selected from these F_2's and crosses were attempted as per NC III design of Comstock and Robinson(1952). The progenies obtained there of were grown alongwith their parents in a randomized block design consisting of 3 replications. Each progeny was represented by a single row of 6 m length with a spacing of 30 x 15cm between and within lines, respectively. Five competitive plants from each progeny family per replication were taken and data were recorded with respect to plant height, primary branches, secondary branches, length of main raceme, siliquae on main raceme, siliqua length, seeds per siliqua and seed yield. The data recorded on these traits were subjected to statistical analysis as per method suggested by Comstock and Robinson (1952) for the estimation of genetic components of variance.

The data presented in table-1 indicated that mean squares due to males and females in sets were significant in all the three crosses for all the characters except for primary branches and seeds per siliqua in cross RH-30xRC-1425 and for length of main raceme in cross RH-30xRC-1426. The differences among both males and females in sets were also significant in all the three crosses for all the traits except for primary branches and seeds per siliqua in cross RH-30xRC-1425 and for length of main raceme in cross RH-30xRC-1425 for only females. The mean squares due to malesxfemales interaction showed non-significant differences for plant height, seeds per siliqua in two crosses RH-785xRH-30 and RH-30xRC-1425 whereas this interaction showed significant differences for all the traits in cross RH-30xRC-1426. The genetic variances such as additive, dominance alongwith their standard errors and degree of dominance estimated for different characters in all the crosses have been presented in table-2. In cross RH-785xRH-30, the additive genetic component was observed to be significant for plant height, secondary branches, siliquae on main raceme, siliqua length, seeds per siliqua and seed yield.

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The dominance component was significant only for secondary branches, length of main raceme and seed yield. Further, the estimates of degree of dominance indicated the presence of partial dominance for plant height, primary branches, siliquae on main raceme, siliqua length and seeds per siliqua; and over dominance for seed yield, secondary branches and length of main raceme. In cross RH-30xRC-1425, the additive and dominance components were significant for primary branches, secondary branches, length of main raceme, siliqua length and seed yield. For plant height and siliquae on main raceme, only the additive genetic component was observed to be significant. Further, the degree of dominance estimated for different traits indicated the presence of partial dominance for all the traits except for seed yield for which the presence of over dominance was observed. In cross RH-30xRC-1426, both additive and dominance component was significant for plant height, primary branches, secondary branches, length of main raceme, siliqua length and seed yield. Like other crosses, only dominance component was significant for siliquae on main raceme. The less than one value recorded for degree of dominance with respect to all the traits except for seed yield indicated the presence of partial dominance. For seed yield over dominance was observed.

The results obtained from present study have indicated the presence of either additive or dominance in some cross/crosses, where as in some other crosses both the genetic components were operative for the inheritance of different traits under study. Under such situations, the maximum improvement in characters where there is preponderance of additive genetic component, the use of conventional breeding procedures would be most appropriate. Further, to utilize both additive and dominance type of components, as observed for seed yield and secondary branches, it is suggested to make use of breeding procedures which may mop up the fixable gene effects and at the same time maintain considerable heterozygosity for exploiting the dominance gene effects, the use of non-traditional breeding methodologies such as reciprocal recurrent selection, S_2 selection and biparental cross are likely to bring the desired results. Since Indian Mustard is a self fertilized crop, intermating would be difficult. But the conditions like self incompatibility or protogyny may facilitate recombinants.

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Table 1: Analysis of variance for different quantitative traits in 3 crosses of Indian mustard using NC III design.

S.V.	d.f.	Plant height	Primary branches	Secondary branches	Lenth of main raceme	Siliquae on raceme	Siliqua length	Seeds per siliqua	Seed yield
				Cross I RH-785xRH-30					
Sets	6	406.92	1.49	21.40	198.76	32.95	0.04	1.12	14.13
Replication in sets	14	273.79	0.89	6.17	29.19	14.45	0.10	0.42	0.73
Females(F) in sets	7	835.24*	0.72*	59.08*	103.58*	203.66*	0.78*	1.20*	21.43*
Males(M)in sets	14	337.73*	0.83*	12.59*	62.41*	54.29*	0.15*	1.74*	14.18*
MxF interaction in sets	14	98.43	0.37	15.01*	107.74*	42.15*	0.08	0.75	14.62*
Error	70	149.78	0.28	2.92	26.99	12.23	0.05	0.48	0.79
				Cross II RH-30x RC-1425					
Sets	6	290.45	1.50	15.06	330.21	114.28	0.13	1.18	7.31
Replication in sets	14	685.80	2.37	5.61	53.16	13.84	0.04	0.44	0.35
Females(F) in sets	7	543.56*	0.37	20.36*	127.09*	179.15*	0.31*	1.02	19.49*
Males(M)in sets	14	489.36*	1.20*	11.82*	49.92*	46.46*	0.13*	1.68*	6.41*
MxF interaction in sets	14	27.15	0.71*	5.76*	141.92*	63.01*	0.09*	1.11	8.95*
Error	70	129.86	0.21	1.49	10.67	2.89	0.04	0.75	0.58
				Cross III RH-30xRC-1426					
Sets	7	328.00	2.22	46.19	198.31	77.17	0.30	3.49	31.47
Replication in sets	16	52.92	0.40	6.39	40.45	13.42	0.04	1.66	2.167
Females(F) in sets	8	3298.9*	3.36	33.22*	46.35	510.78*	2.48*	4.69*	50.25*
Males(M) in sets	16	249.23*	2.33*	12.64*	127.80*	42.36*	0.20*	2.05*	27.39*
MxF interaction in sets	16	185.88*	1.54*	11.91*	94.05*	21.01	0.15*	2.13*	21.03*
Error	80	56.73	0.24	1.77	29.83	13.84	0.04	0.94	0.71

* denotes significance at P=0.05.

Table 2: Estimates of components of genetic variance and degree of dominance in some Indian mustard crosses.

Characters	RH-785xRH-30			RH-30x RC-1425			RH-30xRC-1426		
	σ^2_A	σ^2_D	$\bar{a}$	σ^2_A	σ^2_D	$\bar{a}$	σ^2_A	σ^2_D	$\bar{a}$
Plant height	125.32* ±61.33	34.20 ±28.55	0.52	239.68* ±116.24	98.86 ±66.44	0.64	128.33* ± 55.70	86.10* 41.73	0.82
Primary branches	0.36 ± 0.19	0.06 ± 0.09	0.41	0.68* ± 0.28	0.34* ± 0.17	0.71	1.39* ± 0.52	0.86* 0.34	0.78
Secondary branches	6.44* ± 2.99	8.06* ± 3.55	1.12	6.88* ± 2.79	2.84* ± 1.37	0.64	7.25* ± 2.82	6.76 ± 2.65	0.97
Length of main raceme	23.60 ±15.01	53.84* ±25.57	1.51	157.0* ± 11.83	87.50* ±33.47	0.75	65.31* ± 28.57	42.81* ±21.13	0.81
Siliquae on main raceme	27.36* ± 12.88	19.28 ±10.04	0.84	80.16* ± 10.96	14.52 ±14.86	0.43	19.01* ± 9.52	4.77 ± 4.89	0.50
Siliqua length	0.080* ±0.036	0.02 ± 0.02	0.50	0.08* ± 0.03	0.04* ± 0.02	0.71	0.71* ± 0.04	0.07* ± 0.03	0.80
Seeds/Siliqua	0.84* ± 0.41	0.18 ± 0.19	0.46	0.64 ± 0.41	0.24 ± 0.28	0.61	0.74 ± 0.47	0.60 ± 0.48	0.92
Seed yield	8.92* ± 3.34	9.22* ± 3.45	1.20	3.88* ± 1.51	5.58* ± 2.11	1.20	17.79* ± 6.09	18.54* ± 5.67	1.20

GENETIC ARCHITECTURE OF OIL CONTENT IN INDIAN MUSTARD (BRASSICA JUNCEA L. CZERN & COSS) GROWN IN TWO ENVIRONMENTS

Yash Pal and Hari Singh

Increase in oil yield per unit area and in seed oil content are important objectives for Indian mustard. As new exotic sources possessing significantly higher oil content have become available, the inheritance of oil content was analysed in two crosses between the high yielding line EC 126743 of Russian origin and two Indian cultivars, Varuna and Prakash.

Six generations (P_1, P_2, F_1, BC_1, BC_2 and F_2) from the crosses Varuna x EC 126743 and Prakash x EC 126743 were grown in a randomised block design with three replications in two environments (normal and late sown). The oil content of the seed produced was estimated by NMR. The results were analysed by the generation mean analysis of Hayman (1958) and the scaling test of Cavalli (1952).

Line EC 126743 possessed significantly higher values of oil content than the cultivars Prakash and Varuna (Table 1). The oil content of all samples was higher from the normal than the late sowing and there were few differences in relative rankings of the generations in the two environments. Lower values of oil content in late sowing could be attributed to more damage to the crop by insect pests and diseases. Over dominance was observed in F_1 except for Prakash x Ec 126743 in E_1. BC_1 and BC_2 generations tended towards their respective parental values.

Joint scaling test (Table 2) indicated the adequacy of a simple additive dominance model for oil content in both the environments. Absence of epistasis in the inheritance of economically important characters has been reported in Indian mustard by Singh et al. (1970 and 1981). The appreciable additive genetic effect in these crosses, particularly in Varuna x EC 126743, could be exploited by simple selection for improvement of the oil content both in normal and late sown conditions.

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Table 1. Mean performance of six generations of two crosses for oil content (%) in two environments

Envt	Cross	P_1	P_2	F_1	F_2	BC_1	BC_2
E_1	Varuna x EC 126743	41.47 ±0.070	44.11 ±0.116	45.91 ±0.110	45.71 ±0.314	42.09 ±0.229	44.19 ±0.341
E_2	Varuna x EC 126743	34.46 ±0.106	37.29 ±0.112	40.36 ±0.119	36.61 ±0.211	36.32 ±0.228	37.73 ±0.183
E_1	Prakash x EC 126743	40.76 ±0.226	43.99 ±0.096	41.56 ±0.711	38.78 ±0.356	40.79 ±0.298	40.41 ±0.410
E_2	Prakash x EC 126743	35.62 ±0.133	37.31 ±0.114	40.06 ±0.067	37.45 ±0.292	38.01 ±0.276	37.77 ±0.271

Table 2. Estimates of components of generation means on three parameters model in two environments for oil content

Envt	Cross	Gene effects			Joint scaling test (3 d.f.)
		m	(d)	(h)	
E_1	Varuna x EC 126743	42.17 ±0.295	-0.507** ±0.196	3.500** ±0.571	5.682
E_2	Varuna x EC 126743	35.77 ±0.336	-1.413** ±0.337	4.325** ±0.627	2.341
E_1	Prakash x EC 126743	43.04 ±0.528	-1.121* ±0.529	-3.175** ±0.935	5.807
E_2	Prakash x EC 126743	36.30 ±0.305	-0.544* ±0.266	3.736** ±0.432	1.062

*Significant at 5% level: **Significant at 1% level.

GENETICS AND COMBINING ABILITY STUDIES IN CAULIFLOWER

S.C.Pandey and G.Naik

Genetical investigations by Watts (1963, 1964 and 1965) revealed some information regarding inheritance of polygenic characters of temperate type cauliflower. Indian cauliflowers are distinct from temperate or Erfurt type cauliflower groups (Swarup and Chatterjee, 1972). The present investigation was undertaken to study the genetics and combining ability of earliness, maturity and curd characters in Indian cauliflower.

Materials and Methods

Experimental materials were fourteen hybrids derived by mating seven female parents with two male parents. These hybrids along with parents were transplanted with a spacing of 60 x 45 cm. Data were recorded for days to initiation of curd, days to maturity of curd, number of leaves per plant, weight of leaves (g), plant height (cm), curd weight, (g) and curd size index. Analysis of variance for combining ability, general and specific combining ability effects and $\hat{\sigma}^2_A/\hat{\sigma}^2_D$ were calculated according to Kempthrone (1957).

Results and Discussion

Mean square due to parents, parents -vs- crosses, crosses, females, males and female x male for all characters were found significant. Significance of female x male mean sum of squares indicates the presence of both additive and non-additive genetic variances in expression of these characters. In cabbage (More and Wallace, 1984) and in cauliflower (Lal *et al*., 1976; Neog, 1983) reported similar type of genetic variances for these characters. This may be substantiated the ratio of $\hat{\sigma}^2_A/\hat{\sigma}^2_D$. In case of characters such as number of leaves, leaf weight, curd weight and plant height the additive genetic variances ($\hat{A}$) were predominant whereas in case of days to initiation of curd, days to curd maturity and curd size index. The prepoderance of non-additive ($\hat{D}$) genetic variances were found. The best *per se* performance among female and male parents were also selected. Among females IHR-3, IHR-8 and IHR-36 and in case of males IHR-9 was found good for all the characters.

GCA effects for each female/male were estimated separately for each character. Among females IHR-36 was found good general combiner for all the characters, IHR-3 for earliness, IHR-4 for number of leaves, leaf weight and plant height whereas IHR-8 for curd weight and curd size index. Among males, IHR-9 was found best general combiner for all the characters. S.C.A. effects for each cross were

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estimated for all the characters. IHR-8, IHR-9 for earliness, IHR-4 x IHR-6 for number of leaves and weight of leaves, IHR-4 x IHR-9 for plant height, IHR-3, IHR-6 and IHR-36, IHR-9 for curd weight were found good specific combination. In general, majority of the best specific crosses at one or both of the good general combiners involved. Hoser Krauze (1972), More and Wallace (1984) Selected heterotic crosses on the basis of general combining ability and good per se performance and ability to transmit desirable characters into the hybrid would lead to maximum performance of the hybrid cultivars. Selection of parents on the basis of per se performance and general combining ability effects, lines namely IHR-3, IHR-4, IHR-8, IHR-9 and IHR-36 having majority of the good characters to produce hybrids and synthetics in cauliflower.

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HETEROSIS IN INTERSPECIFIC HYBRIDS OF BRASSICA

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Appreciable amount of heterosis have been reported in Indian Cauliflower by Swarup and Pal (1966), Swarup and Chatterjee (1972). Heterosis is almost negligible in case of Snowball or Erfurts except in curd weight (Watts, 1965). The present investigations were designed to provide information on heterosis in interspecific and intervarietal crosses on broccoli and cauliflower.

Materials and Methods

Twenty two hybrids were made from one Broccoli line IHR-137, reported to be black rot (Xanthomonas Campestris) tolerant and nine tropical cauliflower lines (Pandey and Naik-1985). The resulting hybrids and parents were evaluated in randomised block design with three replications. Seedlings were transplanted with a spacing of 60 x 45 cm accommodating ten plants in each treatment. The observations were recorded on days to curd initiation, days to curd maturity, number of leaves per plant, total plant weight (Kg), leaf weight per plant (Kg) and net curd weight (Kg). Heterosis were calculated as percentage increase or decrease over better parents.

Results and discussion

Hybrid vigour for earliness (days to initiation of curd) were recorded in twenty out of twenty-two hybrids observed. The percentage of heterosis ranges from -4.54 to -63.93. The highly heterotic combinations were 137 x 111 (-63.13**) followed by 112 x 138 (-62.93**) and 111 x 137 (-48.21**). Reciprocal differences for heterosis in earliness were evident in broccoli and cauliflower. Heterosis for maturity of curd were observed in sixteen hybrids having the range from -6.25 to 37.50. Maximum heterosis for curd maturity were recorded in 137 x 116 (-37.50**), 138 x 112 (-33.33**) and 138 x 114 (-20.00**). Heterosis is a function of the square of the difference in the gene frequencies controlling a quantitative character and also the amount of dominance in the parent (Faleoner, 1964). Heterosis for earliness, curd maturity was also reported by Swarup and Pal (1966), Swarup and Chatterjee (1972) and Watts (1965). Fourteen hybrids, exhibited heterosis ranging from -25.00 to 33.33. Highly heterotic hybrids were 111 x 137 (25.oo**). Thirteen hybrids expressed heterosis for plant weight. The range of heterosis for plant weight varies from -99.72 to 96.82. The maximum heterosis were recorded in hybrids 137 x 116 (96.82**), 138 x 114 (96.52**) and 138 x 111 (51.66**). Heterosis for leaf weight per plant were recorded in eleven combinations. The range of percentage heterosis varies from -99.80 to 75.00. The highly heterotic combinations were 137 x 113 (75.00**) followed by 137 x 118

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(73.00**) and 137 x 117 (72.80**). Heterosis for net curd weight was recorded in only two combinations namely 111 x 137 (52.17**) and 112 x 137 (41.30**). Number of leaves were directly correlated with net curd weight (r = 993**) as evident by the heterotic combinations of these two characters. Heterosis observed at seedling stage also expressed at reproductive stage in interspecific hybrids of broccoli and cauliflower lines namely 111 x 137, 137 x 113, 137 x 116, 112 x 137, 112,x 138, 138 x 116 and 138 x 114 can be exploited for developing hybrids and synthetics.

Acknowledgement

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EFFECTS OF INBREEDING ON GROWTH VIGOUR OF RADISH (RAPHANUS SATIVUS L.)

M. Nieuwhof

Radish is a cross pollinating crop. However, the incompatibility mechanism present in Early Round Red and related early radish cultivars is rather weak (Nieuwhof, Cruciferae Newsletter 10, 1985, 72-73). This situation also occurs in early cauliflower. In this crop no inbreeding depression is observed after propagation by line selection. If the activity of the incompatibility system and sensitivity to inbreeding depression should be correlated, it might be possible that in early radish also no loss of vigour occurs after inbreeding. As practically no data on this subject are available, plants selected from early radish families were propagated for three or four generations by selfing to test this hypothesis.

Some results of crops grown in winter in glasshouses are summarized in Table 1. Already after one generation of selfing, 43% of the lines showed, compared with the I-0 generation, a slower root and leaf growth, 24% only a decreased root growth and 13% only a decreased leaf growth. Twenty % of the lines showed the same or an increased root and leaf growth. The same situation was observed for the I-2 lines. When selfing was applied for three or four generations the I-3 and I-4 lines obtained mostly showed a further decrease in growth vigour. The slower growth of succeeding inbreeding generations also becomes evident when the lines are compared with that of cv Robino, which was included in the trials as standard. Twenty % of the I-1 lines were later than Robino, and respectively 58%, 80% and 83% of the I-2, I-3 and I-4 lines. Among the I-3 and I-4 lines still a number with the same earlines as Robino was found, sometimes also with the same amount of leaves as Robino.
In successive generations plants were selected from lines showing a very slight or no decrease in leaf growth and earliness. Table 2 mentions growth data of some of the I-3 and I-4 lines thus produced together with growth data of their parents. The material was sown out on November 6, 1980, in a cold glasshouse. From this table it appears that all the I-3 and I-4 lines showed a decreased leaf growth and a delay of the date of maturity, varying from 2 to 9 days.

Summarizing it can be said that propagation by selfing of early radish induces inbreeding depression, resulting in decreased leaf and root growth. The rate of inbreeding depression, however, was sometimes limited. After three or four generations of inbreeding a small number of lines still showed rather good growth vigour with a growth retardation of the roots of only a few days compared with the parent population. Leaf growth of such lines was more strongly inhibited than root growth.

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Table 1. Growth vigour of radish lines compared with the preceding generation. (- : decrease of vigour; = : same vigour; + : increase of vigour).

99 I-1's compared with their I-0's		96 I-2's compared with their I-1's		55 I-3's and I-4's compared with their I-2's and I-3's respectively	
root	leaf	root	leaf	root	leaf
- 67[1]	56	51	43	70	34
= 16	26	21	43	19	47
+ 16	17	27	13	9	7

1) % of 99 I-1's with decreased growth vigour of the roots compared with the I-0's, etc.

Table 2. Growth vigour of selected I-4 lines of Robijn and I-3 lines of Triplo compared with their parent populations (early families).

		I-0			I-4		
Cultivar ↓	Family ↓	L[1]	R[2]	E[3]	L	R	E
Robijn	1	3.5	5.3	29	3.0	5.2	32
					2.0	4.7	34
					2.7	4.3	35
	2	4.0	4.5	32	2.0	4.7	34
					2.5	3.7	35
					1.7	3.9	37
	3	2.8	5.3	31	3.5	4.4	37
					3.3	3.8	37

		I-0			I-3		
Cultivar ↓	Family ↓	L	R	E	L	R	E
Triplo	1	3.5	5.7	28	2.5	3.9	37
					3.0	3.4	36
	2	3.5	5.4	28	3.0	4.5	35
					3.0	5.0	33
	3	4.3	4.9	29	2.0	4.4	34
	4	4.0	5.6	28	1.5	3.7	37

1) L = leaf size on January 6; 1 = small leaves, ..., 5 = large leaves; 2) R = root size on January 14 and 26 (average); 2: 0 < 4 mm, 3: Ø 4 - 8 mm, 4: Ø 8 - 12 mm, ..., 9: Ø > 28 mm; 3) E = date of maturity (days in January), Ø roots 25 mm.

HERITABLE DEVIATIONS IN LINES OF EARLY RADISH (RAPHANUS SATIVUS L.)

M. Nieuwhof

In a programme to produce radish lines of the type Early Round Red observations were made on the incidence of heritable deviations. In a fairly large number of lines white seedlings were observed and in a smaller number heartless plants.

1. White seedlings

Seeds producing chlorophyl deficient seedlings germinated normally, bud such seedlings died off some weeks after emergency. White seedlings were found regularly in lines of various origin. In 1977 white seedlings were noted in 2 out of 40 I-1's of Robijn and in 1978 in 1 out of 7 I-1's of Rota and in 3 out of 18 I-1's of Triplo. In 14 I-1's originating from Katra, Minitas, Novired, Radar and Verano, however, no deviating seedlings were observed. The %'s of white seedlings varied from 0 to 31. Environmental conditions do not seem to strongly influence the incidence of white seedlings. In 9 lines with white seedlings sown out in 1979 as well as in 1980 the average %'s of white seedlings in these years were 11 and 9 respectively, and in 13 lines which were sown in 1980 and 1981 8 and 10% respectively. Between years no significant differences were assessed. To study the effect of temperature on the incidence of white seedlings, seeds of 3 lines were sown out in petri dishes, which were allowed to germinate in the dark at constant temperatures varying from 10°C to 26°C. The average % of white seedlings at 10°C was 17%, and at 17°C and 26°C 21 and 17% respectively, so no effect of temperature could be demonstrated.

When white seedlings were found in I-1's, they were generally also found in later inbreeding generations, though mostly in decreasing %'s. In 2 out of 15 I-5's originating from I-1's of Robijn with white seedlings such defects were still present.

The incidence of white seedlings must be governed by recessive genes. Mostly the % of white seedlings was significantly lower than 25%. This indicates that not one but several recessive genes may be involved. Sometimes a few white seedlings were observed in progenies which were completely free from white seedlings in preceding generations, which may point to mutation.

2. Heartless plants

In a small number of lines seedlings occurred with normal cotyledons, but with growing points dying off in an early growth stage, eventually causing collapse of the young plants. Heartless plants were observed in I's of very different origin (Robijn,Triplo, Rota, Saxa). When heartless plants were found in a I-1 line, they were mostly also found in later generations.

The %'s of heartless plants were counted in a trial in 1984 and varied from 2 to 22%, in most lines this % varied from 12 to 19%. A simple heritability seems not very likely. The %'s of heartless plants could be reduced by selection, though this phenomenon also could be observed in later generations. In one I-6 line no less than 15% of heartless plants were produced.

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INHERITANCE OF LATERAL SUPPRESSION AND LEAF NUMBER IN BROCCOLI (*BRASSICA OLERACEA* L. ITALICA GROUP)

Katherine A. Keyes and S. Honma

Reciprocal crosses between a sister line of 'Solohead' and 'Spartan Early' suggest that lateral suppression was dominant over non-suppression. The observed F_2 ratio suggests a 2-gene 9:7 epistatic (recessive-suppression) model. $\underline{Ns}_1\ \underline{Ns}_2$ conditions lateral suppression.

F_2 distribution from crosses involving high (22) and low (16) leaf number parents showed skewness toward low leaf number parent suggesting dominance for low leaf number. Partitioning of classes using the arithmetic mean of the parents as the dividing point a 13:3 dominant - recessive epistatic model was obtained. It appears 2 major genes and modifiers determine leaf number in broccoli.

RELEASE OF 'SOLOHEAD' BROCCOLI

S. Honma

'Solohead' as the name suggests is a broccoli (*Brassica oleracea* L., Italica Group) that bears only the primary head with no lateral shoot development prior to head maturity. Lateral shoots may appear at a later date after harvesting the central head. This cultivar shows resistance to black rot (*Xanthomonas campestris*). The pedigree of this cultivar is 'Self-Blanche' cauliflower x 'Early Fiji' cabbage which was crossed to 'Spartan Early' broccoli.

A DIALLEL ANALYSIS OF BOLT RESISTANT GERMPLASM IN SEVERAL *BRASSICA* SPECIES

Katherine A. Keyes and S. Honma

Combining ability analysis for bolting response was performed on data from 12 parent incomplete diallel representing 7 species. Some difficulty was encountered in obtaining seeds from certain crosses and reciprocal crosses. Results suggest diversity among parents and that increase bolting resistance could be selected. The Hakuran group was found to contribute the greatest degree of bolt resistance to its progeny and with ease than the others.

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CHROMOSOMAL MONOGENIC DOMINANT MALE STERILITY IN CHINESE CABBAGE (BRASSICA RAPA SUBSP. PEKINENSIS (LOUR.) HANELT).

Q.P. van der Meer

Male sterile B_1 descendents were found after back crossing a Chinese cabbage x pak choi (B. rapa subsp. chinensis) - hybrid to Chinese cabbage. After crossing one of the male sterile B_1 plants with a Chinese cabbage plant (cultivar Monument) its offspring showed about 50 % of male sterile descendants wheras the offspring of the Monument plant were completely male fertile.
Pair crosses of male sterile descendents from following generations of male sterile plants with male fertiles (inbreds) from following generations of the Monument plant again resulted in about 50 % male sterility in the offspring of male sterile plants and 100 % male sterility in the offspring of the Monument inbreds. These results fit in completely with the genetical background as defined in the above title. Details were described in an article that was sent to Euphytica in July 1986.

Recently results were obtained from crosses between individual male sterile and male fertile descendents (full sibs) of the same male sterile plants. These results are given in table 1.

Table 1. Sex expression in offspring of pair crosses between male sterile and male fertile plant both originating from the same sterile plant.

Pair number :	Offspring of:			
	male sterile plant		male fertile plant	
	m.s. plants	m.f. plants	m.s. plants	m.f. plants
1	14	11	0	22
2	10	9	0	1
3	0	1	0	16
4	15	7	0	25
5	10	11	0	21
6	14	9	0	23
7	9	16	0	21
8	6	16	0	24
	78	69		

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These results also fit in very well with the inheritance as formulated above.
The male sterility in question shows clear-cut symptoms (strongly reduced stamens) and is very stable. No remarkable differences in seed yield between male sterile and male fertile plants were observed. Consequently this material seems to be rather suitable for the breeding of hybrids and for the production of hybrid seed. However a considerable, but not insurmountable, handicap is the obligatory removal of about 50 % of fertile plants from the seed parent population. This problem might be tackled by androgenesis or gynogenesis, yielding (after doubling the chromosome number of haploids) homozygous dominant male sterile plants giving a completely male sterile offspring. However this necessitates obligatory vegetative propagation of the homozygous dominant male sterile plants in order to produce (after pollination) sufficiently large populations for hybrid seed parents.

INHERITANCE OF THREE GLUCOSINOLATE COMPONENTS IN CABBAGE (Brassica oleracea L. ssp. capitata L.)

M.S. Chiang, C. Chong, G. Chevrier and R. Crête

The inheritance of the three glucosinolate components, goitrin, volatile isothiocyanates and the thiocyanate ion was studied in cabbage plants.

Six genetic populations were used including P_1 and P_2 (moderate inbred lines selected for low and high goitrin content, respectively), F_1 (P_1 x P_2) and reciprocals, F_2, B_1 (P_1 x F_1) and B_2 (P_2 x F_1).

Procedures for analysis of the three glucosinolate components were the same as described previously (Chong et al. 1985). Results indicated that all three glucosinolate components showed a strong heterosis towards lower concentrations, the maternal effect in inheritance was observed for goitrin only. Lower concentrations of goitrin and volatile isothiocyanates were controlled by four to six genes but the inheritance of thiocyanate ion was governed by only two to three loci.

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THE INFLUENCE OF TEMPERATURE ON THE EXPRESSION OF CYTOPLASMIC MALE STERILITY IN *BRASSICA NAPUS* L.

P.L. Polowick and V.K. Sawhney

In most systems, the phenotypic expression of cytoplasmic male sterility is highly variable. Temperature is known to be one of the factors responsible for this variability in several crops e.g. onions (1, 2, 3), cotton (4, 5), petunia (6, 7, 8) and chives (9) as well as two lines of CMS *Brassica napus* (10). This study provides an account of the influence of temperature on the variable nature of a CMS line containing Ogura's *Raphanus* cytoplasm (11) in the nuclear background of *B. napus* cv. Westar.

Under the three temperature regimes used, i.e. high (HTR, 28°C day/23°C night), intermediate (ITR, 23°C day/18°C night) and low (LTR, 18°C day/15°C), the development of normal flowers was not affected. However, in the CMS line, the development of the four long stamens, but not that of short stamens, was altered in response to different temperatures.

The stamen development of CMS was variable under different temperatures and could be classified into 3 types. Type 1 stamens had a distinct separation of anther and filament, and at the scanning electron microscope (SEM) level, the surface features of anthers resembled those of the normal. The cross sections of anthers revealed one to four locules containing the sporogenous tissue, and in some cases development proceeded to the microspore stage. Such stamens were predominent under HTR (Table 1).

Type 2 stamens displayed features of both the stamens and carpels. Stamens showed the separation of anther and filament, and the surface features of anthers were comparable to those of normal stamens. Also, the stamens contained one to four locules, with stages of microsporogenesis as advanced as the tetrad stage. In addition, features resembling those of a gynoecium were evident on the stamens. External ovules were often present at the base of the anther and, less frequently, a stigmatic surface was visible on a lateral edge of the anther. Type 2 stamens were common under ITR and LTR (Table 1); those with the lateral stigmatic surface were more common under LTR.

Type 3 stamens resembled carpels more than stamens. These 'carpelloid-stamens' had a stigmatic surface at the distal end, proximal to which was a style-like region and the basal end was comparable to an ovary. At the SEM level, all surface features of these carpelloid stamens resembled those of a normal gynoecium. There were no regions resembling an anther or filament nor were there any signs of microsporogenesis observed in the cross sections of such stamens. Type 3 stamens were generally fused in pairs or, frequently, all four long stamens were fused to completely surround the gynoecium. Campylotropus ovules were present along the lateral edges of such stamens, especially along the fused edges. These stamens were most frequently observed under LTR (Table 1).

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These observations show that at high temperatures, the stamens of the CMS line of B. napus (ogura system) develop many features resembling those of normal stamens, including the development of microspores. At low temperatures, however, the stamens differentiate into carpel-like structures with no evidence of microsporogenesis. Normal pollen development was not observed in this CMS line under any of the three temperature regimes examined.

Table 1. The effect of different temperature regimes on the morphology of long stamens from a CMS line of Brassica napus.

Temperature regime	Frequency (%)[a]		
	Type 1	Type 2	Type 3
HTR	61	39	0
ITR	18	67.5	14.5
LTR	2	55	43

[a]Based on observations of a total of 200 stamens per treatment, from 2 trials.

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MALE STERILE FORMS IN SUMMER RAPESEED POLIMA

I.Bartkowiak-Broda and W.Popławska

Seeds of male sterile line /MS/ selected from summer rapeseed variety Polima we have obtained from China.

First observations were conducted under greenhouse conditions in 1982. These were summer plants with a very weak vigor. Male sterile plants were crossed three and four times with double low lines "OO" of winter rapeseed. Observations of 111 F1 hybrids /♀ MS plants x ♂ "OO" lines/ in the field in 1984/85 and 1985/86 made possible the following characteristic of MS plants:

1. Progenies of all observed hybrids were MS plants:
   - not producing pollen from the beginning to the end of flowering period,
   - producing little amount of pollen during the whole flowering period,
   - producing pollen only in the beginning of the flowering period.
2. MS plants are characterized by reduced corolla petals, strong reduction of filament and anther /Fig.1/. Histological examinations of anthers revealed a lack of differentiation of male archesporium into PMCs in totally MS plants. In a few cases there was observed a very late differentiation of male archesporium in single pollen sacks, but these sacks have not dehisced till the end of flowering period. In partially MS plants the anthers were also reduced. Differentiation of male archesporium is late compared to male fertile anthers, and most often it appears in one or two pollen sacks and the rest do not develope.
3. Occurence of totally male fertile plants was not observed in the progenies of examined hybrids. Double low lines of winter rapeseed which have been tested so far appeared to be maintainers or partial maintainers. Instead no full restorers were observed among them.

Conclusion:

Observations of MS plants of Polima type allow a statement that the discussed sterility appears to be similar /flower modifications, anther development/ to male sterility originating from Bronowski variety of summer rape /Thompson 1972, Bartkowiak-Broda et al. 1979/ and this one described by Shiga /1976/. However this type of sterility is more interesting because of appearance of a considerable amount of plants fully sterile during the whole flowering period and because of appearance of maintainers within "OO" lines of winter rapeseed.

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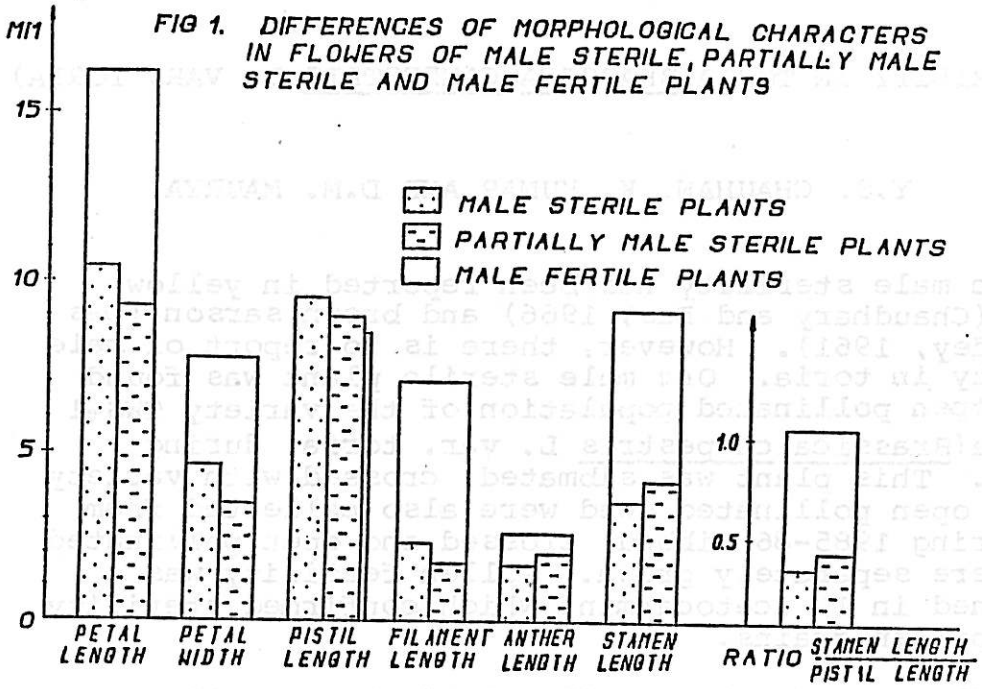

FIG 1. DIFFERENCES OF MORPHOLOGICAL CHARACTERS IN FLOWERS OF MALE STERILE, PARTIALLY MALE STERILE AND MALE FERTILE PLANTS

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MALE STERILITY IN TORIA(*BRASSICA CAMPESTRIS* L. VAR. TORIA)

Y.S. CHAUHAN, K. KUMAR AND D.M. MAURYA

Genic male sterility has been reported in yellow sarson (Chaudhary and Das, 1966) and brown sarson (Das and Pandey, 1961). However, there is no report of male sterility in toria. One male sterile plant was found in the open pollinated population of the variety TWS-1 of toria(*Brassica campestris* L. var. toria) during 1984-85. This plant was sibmated, crossed with variety T 9 and open pollinated seed were also collected from it. During 1985-86 sibbed, crossed and open pollinated seeds were separately grown. Pollen fertility was determined in 1% acetocarmine which confirmed sterility of the pollen grains.

The flowers of male sterile plants have smaller buds, narrow petals, very short filaments and anthers devoid of fertile pollen. Compared to their fertile sibs, male sterile plants are shorter, profusely branched, producing small siliquae with few seeds.

This male sterility may be used in hybrid seed production, the development of synthetics and population improvement programmes.

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MECHANISM OF MALE STERILITY IN BRASSICA JUNCEA. VI. IDENTIFICATION AND INHERITANCE OF POLLEN FERTILITY RESTORATION

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A knowledge on the inheritance of fertility restoration is useful for developing R-lines which facilitates commercial cultivation of hybrids economical. Restorer plants derived from B. nigra and B. campestris sources of restoration in the background of B. juncea (Anand et al. 1985) were selfed and crossed to ms plants for identifying the number and nature of nuclear genes involved in restoration. It was observed that the F_1s of ms x restorer plants segregated to male sterile and partial male fertile (since the restorer plants were heterozygous) for the gene(s) controlling restoration, the former being more. Monogenic segregation was a poor fit in selfed and crossed families over years. On the other hand, segregation ratio based on digenomic complementary gene action (9 fertile : 7 sterile) was a better fit for the majority of the selfed and crossed families. The results substantiated that two genes were involved for the inheritance of fertility restoration for each of the sources of restoration (B. nigra (RN) and B. campestris (RC). Shiga et al. (1977) and Sernyk (1983) reported upto four and eight genes controlling fertility restoration for cytoplasmic male sterile B. napus, respectively.

It is interesting to focus the disproportionate occurrence of sterile plants in the progeny of selfed and crossed families involving ms and restorers. The reason could be due to presence of modifying factors and or meiotic irregularities/abnormalities. The latter one appears to be more drastic in changing the segregation ratio. Even meiotic abnormalities in 30 per cent of the microspore mother cells would be enough to give rise to more number of sterile plants (i.e., 9 sterile : 7 fertile). It is natural to expect chromosomal irregularities in pairing keeping in view the difference in chromosome number between the two species B. nigra (2n=16) and B. juncea (2n=36).

Based on the ability to restore fertility, the different crucifers that were crossed with CMS B. juncea were categorised into two groups. Indigenous and exotic B. juncea were included in Group I, as they had fertile cytoplasm (F) and no dominant nuclear genes for

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fertility restoration of male sterility. This category of strains are useful as maintainer lines (B-lines) of cytoplasmic genetic male sterile line (A-line). The subspecies of B. campestris as oleifera var. trilocularis and toria, chinensis, parachinensis, pekinensis, nipposinica and B. oleracea as alboglabra, botrytis and acephala have the F cytoplasm with no genes for fertility restoration. The partially fertility restoring species as B. nigra, B. campestris var. brown sarson, B. carinata and B. napus with the fertile cytoplasm (F) and nuclear genes for fertility restoration were included in Group II. The male sterile juncea has the sterile cytoplasm (S) and is no more a result of cytoplasm alone but an interaction involving cytoplasmic factors and nuclear genes.

The genotypic symbols for group I and II species could be given as:

Male sterile	(s) rf_1rf_1 rf_2rf_2
Group I	(F) rf_1rf_1 rf_2rf_2
Group II	
B. nigra	(F) Rf_1Rf_1 Rf_2Rf_2
B. campestris	(F) $Rf_1'Rf_1'$ $Rf_2'Rf_2'$
B. napus	(F) No definite symbol could be assigned
B. carinata	(F) No definite symbol could be assigned

A further analysis to investigate whether the restorer genes of B. nigra (Rf_1Rf_1) and B. campestris ($Rf_1'Rf_1'$) are of similar or different origin have revealed that they are of independent origin and are borne on different genomes. Selection for pollen fertility restoration with improved pollen viability in each of the two sources RN and RC had resulted in the development of restorer lines with normal flower size (the male sterile and partially fertile lines have flowers with reduced petal size), well developed anther lobes and profused pollen production. However, their crosses with CMS line had invariably resulted in 60 to 75% plants with male fertility, the remaining showing male sterility in their progenies. Since B. juncea is a digenomic species involving the genomes of campestris and nigra in its amphdiploidic origin, the bringing of two sources of pollen fertility restoration (RN X RC) together had resulted in progenies with complete pollen fertility restoration. Currently, these sources of restoration are being used for development of R-lines

for B. juncea male sterility in the ongoing plant breeding programme.

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ON THE WAY TO YELLOW SEEDED BRASSICA NAPUS II. BACKCROSSES WITH BRASSICA OLERACEA OF F_1 HYBRIDS OBTAINED AS THE RESULT OF CROSSES BETWEEN B. OLERACEA AND B. CARINATA

B. Barcikowska

As was reported in Cruciferae Newsletter No. 8, backcrosses were made between F_1 hybrid B. oleracea x B. carinata (yellow seeded form) and B. oleracea. 10,027 pollinations with B. oleracea gave rise to 40 seeds, a rate of 0.31% in respect to the number of pollinated flowers. Of the 8 plants which germinated, only 3 were fertile and they had chromosome numbers oscillating from 2n=22 to 2n=28. In the next year (1985) 200 BC_1S_1 plants arose, these gave a progeny of 166 BC_1S_2 plants in 1986. Among these offspring, 8 plants were phenotypically B. oleracea. Unfortunately, all plants from this generation were characterised by having dark seed coat. In spite of this we are expecting that some yellow seeded forms of B. oleracea will appear as the result of further gene segregation in succeeding self-pollinated generations. To reach this aim, the next generation of BC_1S_2 of interesting phenotype has been chosen and sown in the greenhouse during the vegetation period 1986/87.

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SPONTANEOUS HAPLOIDY IN BRASSICA NAPUS

S.S. Banga and K.S. Labana

The last decade has seen much progress in the induction and utilisation of haploids, which are prized for producing instant pure lines. Though anther or pollen culture is the most exploited technique for haploid production, naturally occurring haploids have also been reported in a wide array of crop plants. In Brassica napus such haploids were earlier reported by Thompson (1968) and Stringam and Downey (1973). Genotypes with greater frequency of natural haploids could be very attractive for plant breeders. In this communication we present evidence for naturally occurring haploids in some varieties of Brassica napus (n=19).

Materials and Methods

Seventy eight varieties of B. napus obtained from various countries were screened under field conditions at Oilseeds Farm, Punjab Agricultural University, Ludhiana. Haploid plants were identified at flowering by their small, pollen sterile flowers, reduced plant vigour and absence of seed setting. These plants were later confirmed by chromosomal counts.

Results and Discussion

Haploids were observed in only ten varieties of B. napus. Total plant populations screened and the respective haploid frequencies in these varieties are presented in Table 1. The haploid frequency per thousand ranged from 2.58 to 19.4. Haploid frequency (8.27) in the Canadian variety Oro was much higher than that reported from Canada (Stringham and Downey, 1973). Three entries, HNS-4, HNS-5 and HNS-6 selected in India from a common exotic cultivar Norin-20 produced consistently higher frequencies of haploids over years. In general, haploids were more prevalent in harvest from late sown crop (November end) than that from normal sown crop (early October). This could be attributed to the fact that the blooming in late sown crop coincided with onset of higher temperatures during March.

Haploid plants of Sv Nikalas were more vigorous and also set some seed which invariably produced diploid seedlings.

About 73% of the cells analysed had no bivalent (Table 2), 21% had one bivalent and 6% had two bivalents. At anaphase, 7-10 distribution with two laggards was the most common. About 5% of the cells had 9-10 distribution and had no laggards.
The present study suggests that parthenogenetic haploidy is of relatively common occurrence in B. napus. Thus it will be profitable to look for such haploids both in varietal strains and the segregating generations. Diploidization of haploids observed in F_2 will be a faster approach to practical homozygosity.

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Acknowledgements

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Table 1. Haploid frequency in Brassica napus

Cultivar	Plant population	Haploids	Frequency/ thousand
HNS-4	1171	11	9.39
HNS-5	985	10	10.15
HNS-6	1410	14	9.92
Sv Nikalas	591	6	10.15
Christa	1160	3	2.58
Oro	967	8	8.27
Fido	792	9	11.36
Line	197	2	10.15
Regent	782	8	10.23
Hinchu	103	2	19.41

Table 2. Meiotic analysis of haploids in B. napus

Configuration	Frequency
13I + 3II	-
15I + 2II	7 (6.60)
17I + 1II	22 (20.75)
19I	77 (72.64)
Total PMCs	106

POSSIBILITY OF DEVELOPING TROPICAL CABBAGE

S.C.Pandey and G.Naik

In Cabbage, the character for wider adaptability to fluctuating climatic conditions has not been exploited to that extent as in cauliflower. However, reports suggest that in some varieties of cabbage head formation as well as seed set can take place in higher temperature than required for most varieties (Miller, 1955; Chatterjee and Mukherjee, 1957; Ito and Saito, 1961 and Kuroki,1970). It is believed that genes for wider adaptability is attributed to the inherent character of the parent Brassica oleraceae L. var. sylvestris L. which is found growing well in various regions from temperate to tropical (Swarup and Chatterjee, 1972). The present attempt was made to isolate and develop a line/variety which can produce successfully head as well as seed under tropical climates.

Materials and Methods

Four varieties were grown in randomised block design with three replications. These lines were obtained from U.S.A.(two), Srilanka (one) and India (one). Twenty five days old seedlings were transplanted in the field on 22nd October, 1985 with spacing 60 x 45 cm. Observations were recorded on days to head maturity, total weight of the head (Kg), net head weight (Kg) and various flowering and seed characters.

Results and Discussion

Earliest harvestable stage of head were recorded in EC 16525 (Srilanke) after 70 days of transplanting followed by EC 16100 (U.S.A.), ARU GLORY (India) and EC 158185 (USA). Total plant weight was recorded in ARU GLORY to be 1.77 Kg followed by EC 16525 (1.52 Kg) and EC 158185 (0.77 Kg). Net head weight in ARU GLORY was recorded to be 0.86 Kg followed by EC 16525 (0.79 Kg), EC 158185 (0.45 Kg) and EC 16100 (0.23 Kg). Initiation of flowering recorded in one line (EC 16252) after 88 days of transplanting. The height of the plants was recorded from 81.0 cm to 125.0 cm (average 108.5 cm), number of branches per plant from 7 to 16 (10.7), number of siliqua per plant from 66 to 360 (216), seeds per siliqua from 3 to 17 (9.5), and length of pods was 3.3cm to 7.6 cm (5.9 cm). Seed yield per plant varies from 0.54 to 10.42 g and 1000 seeds weight was recorded to be 0.45 g.

In EC 161525 line all plants produced dark green oblong medium size heads weighing around 800g. About 80% plants flowered and produced seeds under tropical climatic condition. This line does not require any chilling (5-8°C for 6 weeks) to convert vegetative phase to reproductive phase as required in almost all

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varieties of cabbage. This line flowered and produced seeds under Bangalore condition where lowest temperature was recorded 13°C.

In India cabbage seeds are produced only at hills of Uttar Pradesh, himachal Pradesh, Jammu and Kashmir, and West Bengal due to availability of chilling requirement for the cabbage varieties. Considering the immediate need to develop a variety which can produce seeds under tropical/sub-tropical regions, attempts have been made to cross cabbage line EC-16525 with cauliflower and broccoli lines. Recently Mackiewicz and Barczynska (1986) reported that there is no much reciprocal differences in the crosses between cabbager and broccoli. They observed preponderance of cabbage type leaves and seed stalks in all the three generations (F_1, F_2 and F_3) with annual habits. Yhis finding supports the present efforts to develop tropical type cabbage lines/varieties under Bangalore condition.

In the year 1986 about four hundred cabbage lines are under screening at I.I.H.R., Bangalore to isolate heat tolerant tropical and sub-tropical lines as already have been achieved in cauliflower in India, Chinese cabbage in Taiwan and Cabbage in Japan.

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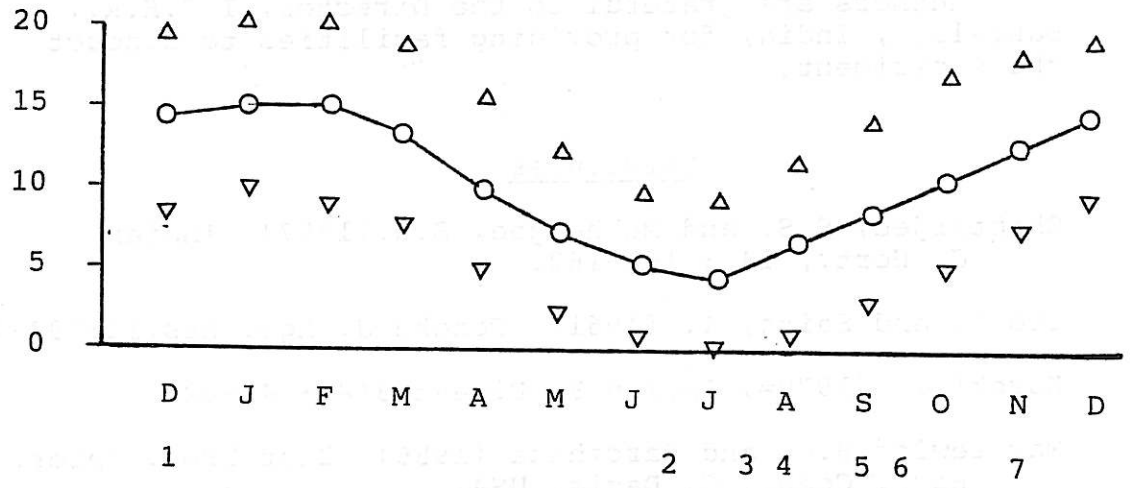
ANNUAL SWEDE BREEDING CYCLE IN NEW ZEALAND

S Gowers and S D Armstrong

Swedes (Brassica napus ssp. rapifera) are basically biennials, requiring a period of vernalisation before they will flower. They can be grown on an annual cycle by sowing in the field like winter oil-seed rape, or by growing overwinter in a cold greenhouse; in neither case is yield evaluation or morphological selection possible. When grown as a normal crop in the field and then left to flower, about 14 months are needed to set seed naturally, which leads to a biennial breeding cycle. To grow a normal crop and produce seed within 12 months in Scotland, for example, a large area of heated greenhouse would be needed. In New Zealand, however, sowing times and climatic conditions are such that an annual cycle can be obtained with a minimum of supplementary heating.

The procedure now being used at Gore is shown below in relation to a graph of mean daily temperatures.



O - daily mean temp. △ - mean maximum. ▽ - mean minimum.

1. Sow trials, early December.
2. Harvest trials, June/July.
3. Select plants, end July.
4. Into heated glasshouse, August.
5. Into unheated glasshouse, September.
6. Bag plants, mid/end September.
7. Harvest and thresh seed.

DSIR, Crop Res. Division, Private Bag, Gore, New Zealand.

This procedure allows six months growth for trials, and a further two months at mean daily temperatures of 5°C for vernalisation to occur, during which time harvesting and selection can be carried out. One of the two main glasshouses at DSIR, Gore is heated overwinter for other crops, which are mainly grown on 750 mm high benches. Over 300 swedes, potted into 10 l buckets, can be fitted under these benches and thereby utilise 'free' heating at a time when the climate is still cold. By September, when the flowering stems have grown and the plants have to be moved, the temperatures in the unheated glasshouse are rising, and further heating is not required. Flowering starts by mid-September, and the plants are bagged after removing all side inflorescences except the top five or six.

Ten litre buckets (with holes drilled in bottom) are used because they were obtained on 'Special Offer' at a third the price of similar sized plant pots, and came complete with carrying handles. Soil from the field is used for potting, except for a covering of peat compost to act as a mulch. Allowing access room for watering and bagging, 400 plants will fit into 50 m^2.

Tissue paper sheets (750 x 500 mm) glued onto newsprint backing produces very cheap bags, excluding labour costs. The newsprint becomes brittle, but two years use is obtained in most cases. Bags made out of only newsprint were tried, but growth and seed set was very poor in comparison to tissue paper fronts. The bags are quickly stapled round the plants, and onto a supporting string initially, and are shaken daily to effect self-pollination.

Management of this system is far more intensive than bagging plants grown outside. Daily watering and shaking, feeding and spraying for pests and diseases, involves much more effort during the flowering stage. Glasshouse space is also a limiting factor in the number of selections that can be taken. However, plant losses are only in the region of 5 per cent, whereas 50 per cent losses are not uncommon outside. Harvesting and hand threshing in the glasshouse, straight into a seed cleaner and then the seed packet, is quick and easy, and a computer printed label can be transferred direct from the plant pail to the seed packet.

Under different climatic conditions such an annual breeding cycle could be expensive or difficult to manage. With the climatic conditions at Gore, however, the system has been found to be easy and economical to run.

VARIATION WITHIN SWEDE CULTIVARS
THE NEED FOR INBRED LINES

J E Bradshaw

The swede (Brassica napus ssp rapifera L.) is self-fertile, tolerant of inbreeding (Davey, 1938) and hence often classified as an inbreeding crop species, although it reproduces by a mixture of self- and cross-pollination. Self-incompatible plants are occasionally found. Thus Gowers (1981) reported 17-19% outcrossing for three Wilhelmsburger cultivars but a higher rate of 46% for cv. Gullacker III where self-incompatible plants were shown to be present.

As a result of this cross-pollination the older cultivars and strains of swedes produced by phenotypic mass-selection were very variable. They could, however, be classified by their skin and flesh colour, shape, and maturity coupled with hardiness (Davey, 1946).

Modern cultivars are more uniform, and in the United Kingdom since 1973 this greater uniformity has been ensured by the regulations controlling the sale of seed (Bowring and Day, 1977). At the Scottish Crop Research Institute (SCRI) uniformity has been achieved through pedigree breeding programmes where hybridisation is followed by selfing individually selected plants from the F1 to F4 generations, before multiplying F5 families as potential cultivars. Nevertheless, even in cultivars strongly selected for morphological uniformity, like Angus and Melfort bred at SCRI, some genetical variation will remain, particularly for quantitative characters under polygenic control.

Variation within cultivars can be exploited. Tomlinson and Ward (1982) were able to select two lines with immunity to turnip mosaic virus from 420 plants of cv. Ruta Øtofte. Gowers (1984) was able to select a very high dry matter swede, Dryden (13.4%), from cv. Bangholm Wilby (11.4%).

However, variation within cultivars can make genetical studies and breeding work more difficult. Lammerink (1967) obtained conflicting results for clubroot resistance in crosses with cv. Wye and demonstrated that 2 out of 4 plants were heterozygous in resistance to race B. Gowers (1984) reported difficulties when backcrossing S alleles into swede cultivars to produce self-incompatible lines for hybrid swede production. Using different plants of the recurrent parent in different generations would often result in self-incompatible plants giving only self-compatible progeny. In these situations true breeding lines are desirable.

Furthermore, with quantitative characters biometrical genetical techniques such as diallel analysis are most powerful when working with inbred lines, although Brain and Whittington (1980) were able to analyse resistance to powdery mildew and Grant et al (1982) the inheritance of root size and yield in diallel crosses of commercially available cultivars.

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Therefore at SCRI we are using sexual and androgenetic techniques to continue to produce and use inbred lines of swedes in our genetical studies of disease and pest resistance (to clubroot, powdery mildew, turnip mosaic virus and turnip root fly); in our research projects on dry matter content of swedes and the genetical basis of heterosis for dry matter yield; and as parents in our breeding programmes.

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GSL-1, THE FIRST BRASSICA NAPUS CULTIVAR FOR PUNJAB, INDIA

K.S. Labana, S.S. Dhillon, T.R. Gupta and T.S. Goomber

Rapeseed and mustard are grown in a substantial area in the world, India being second only to China in area and production. Although several crops of Brassica campestris vars toria, brown sarson and yellow sarson; Eruca sativa (rocket) and Brassica juncea (Indian mustard) are cultivated in India, the latter predominates because of its high yield potential and better tolerance to stress conditions. During the last 5-6 years some exotic collections of Brassica napus have attracted farmers of the Punjab for cultivation under the popular name 'Gobhi Sarson'. Brassica napus is both photo and thermosensitive and is initially slow growing (October to December). As a result it evades frost, whereas raya is sometimes severely affected by frost. 'Gobhi Sarson' has wider adaptability, higher oil content and is suitable as a pot herb (Saag). In addition it will help in diversification of cropping pattern of winter crops. Looking into these characters many collections were made from farmers' fields and improvement work was started. The species in general is lower yielding than the prevalent varieties of Indian mustard like RLM 198, RLM 514 and RLM 619. However, by selection from the existing materials, an improved line of Gobhi Sarson (GS.D) was developed. It was found in trials to be superior to the local types of the respective areas and it has been released for general cultivation in the Punjab State as GSL-1 (Gobhi Sarson-Ludhiana-1). It is the first variety of Brassica napus released in India for general cultivation.

The salient features of the variety together with its yield performance are presented herein.

GSL-1 was tested in the trials as GS(D) against the best local lines at the university research stations from 1981-82 to 1984-85 and on farmers' fields during 1984-85. Yields are summarised in Table 1.

Table 1. Comparative yield performance of GSL-1 and local types

Trials	Yield kg/ha		Percent increase over local
	GSL-1	Local	
Research Stations (17 trials)	1951	1668	16.67
Adaptive trials on farmers' fields (34 trials)	1463	1401	4.43

The percent increase over the local type was higher in research station trials than in trials in farmers' fields. Table 2 gives comparative data for some of the ancillary characters.

GSL-1 had higher oil content, more primary, secondary branches and pods on the main shoot than the local types. Both GSL-1 and local lines showed similar reactions to diseases and insect pests. The

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cultivar GSL-1 has high erucic acid content. Work to improve *B. napus* with respect to yield, quality and resistance to diseases and insect pests is in progress.

Table 2. Ancillary characters of GSL-1 and local types

Character	GSL-1	Local
Days to maturity	168	166
Plant height (cm)	187	180
Number of primary branches	10.5	7.8
Number of secondary branches	9.5	7.1
Main shoot length (cm)	51.6	59.4
Number of pods on main shoot	74.2	68.1
1000-grain weight	3.52	35.50
Oil content (%)	44.65	41.25

VEGETATIVE PROPAGATION BY GRAFTING OF THE FLOWER STEM OF CRUCIFEROUS PLANTS

Lee, Soo-Seong and Lee, Kwang-Sik

Vegetative propagation of selected individuals is occasionally required to maintain their characteristics in breeding programs of Chinese cabbage (Brassica campestris ssp. pekinensis) and radish (Raphanus sativus L.). In the case of Chinese cabbage, an axillary bud cutting including a part of the midrib is taken[2]. In vitro culture of axillary bud[3] and flower stem[1] were reported as useful techniques for asexual multiplication. However such methods are not very successful yet and there is a risk of infection with microorganisms carried in incompletely sterilized tissues.

Even so, no system has been developed yet for vegetative multiplication of radish.

An attempt was made to graft flower stalks of several cruciferous plants. Results showed an excellent affinity even between plants of different genomes of cytoplasm and nucleus (Table). Since several tens of branches of the flower stalk develop from a single adult plant, sufficient flowers for self- and cross-pollination can be obtained by this technique.

The stem to use for the stock and scion should be young and tender and without an interior cavity. The stem diameter of the stock and scion should be nearly the same. The basal end of the scion with 2 to 3 leaves except the tip removed is cut to a V-shaped wedge with a sharp blade. After matching the opening of the stock with the prepared scion, the grafted part is fastened tightly with a thin and elastic plastic band about 1.5 cm wide. The scion including the grafted area is covered with a light plastic bag of an adequate size and shaded with a paper bag to prevent withering by excessive transpiration. Alternatively the grafted plant can be kept in shade without the paper bag cover. The grafted tissue is healed completely in a week, then the plastic cover and band is removed.

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Table Number of graftings between various genomes of cruciferous plants[1]

Genome of stock	Genome of scion						Total
	Aaa	Ccc	ABaabb	Aar	Raa	Rrr	
Aaa[2]	10	4	3	6	14	28	65
Ccc	5	4	-	-	-	7	16
ABaabb	2	-	5	2	8	6	23
Aar	2	-	-	-	-	-	2
Raa	14	-	3	-	3	8	28
Rrr	8	3	3	5	9	9	37
Total	41	11	14	13	34	58	171

[1] All the graftings were successful.

[2] Aaa: Chinese cabbage (B. campestris ssp. pekinensis)
Ccc: cabbage (B. oleracea var. capitata)
ABaabb: leaf mustard (B. juncea)
Aar: F_1 plant of Chinese cabbage x radish
Raa: cytoplasmic male sterile Chinese cabbage induced from CMS radish x Chinese cabbage
Rrr: radish (Raphanus sativus)

THE ASSESSMENT OF STEM HARDNESS IN FORAGE RAPE

W.H. Macfarlane Smith, P. Smith and Jane M. Dinsmore

Chemical analyses of stems of forage rape give high values for digestibility, protein and organic matter contents, although under normal grazing conditions often less than one third is consumed unless high grazing pressures are exerted (Julen, 1966; Toosey, 1972). It is therefore likely that some mechanical factor exists in the stem which inhibits intake, the most probable cause being stem hardness. The aim of current work is to find a repeatable method for measuring stem hardness, to relate this to the tissue structure of the stem and finally to select 'hard' and 'soft' stem breeding lines for animal evaluation.

None of the methods considered for measuring stem hardness, including a penetrometer which Gowers (1982) used to measure bulb hardness in swedes would exactly simulate the biting action of sheep and almost all would suffer from a progressive deterioration of a cutting edge or point which would result in a possible bias to results. However, a texture press [Food Technology Corporation TP-1 with CS-1 shear-compression cell] gave repeatable results over a large number of samples. Sections, 5 cm long, were taken from the upper, middle and lower parts of the stem and subjected to a transverse hydraulic pressure using the above equipment. Readings on a 0-300 arbitrary scale were obtained at the compression (R1) and shearing (R2) stages.

Adjacent lengths of stem were frozen at -20°C and transverse sections obtained using a freezing microtome. Lignified tissue was stained with a solution of phloroglucinol in 20% HCl and the sections photographed. Photographs make a useful permanent record and allow the 'harvesting' and measurement operations to be separated. A Quantimet 900 Image Analyser was used to measure the area and calculate the diameter and thickness of different tissue types directly from negatives. The value of this equipment is that the computer can be programmed to smooth the image for greater accuracy and to fill in missing tissue, then make measurements with a resolution of 0.05 mm per picture point.

Following such measurements on two replicates only of a trial of advanced breeding material, various combinations of texture press readings and tissue measurements were examined for any correlations. A number of these measurements were well correlated but in particular values of 0.91 and higher were found between R1 and both the area of lignified tissue [(Radius lignin + cortex = Radius cortex)^2] and the related random mean chord of the cylinder made up of this tissue which was calculated as x Area of lignified tissue/Perimeter of the same. There was a low correlation with the diameter of the cylinder.

'Hard' and 'soft' stem breeding lines have been identified and, once sufficient seed is available, they will be used in animal feeding trials.

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Full details of this work will be published following completion of measurements on all replicates of two years of trials of advanced breeding material.

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BREEDING FOR NEW CULTIVARS OF YELLOW-SEEDED B. NAPUS TO INCREASE THE OIL CONTENT IN RAPESEED

Liu Hou-Li and Gao Yong-tong

As we know, yellow-seeded rapeseed has more oil, more protein and less fibre. Yellow seed, as a very important character, has been rated as one of the objects of breeding in B. napus, in China. In recent years, some research on the breeding for yellow-seeded B. napus has been done in our Institute. The main results have been obtained as follows:

1. Yield tests between different yellow-seeded lines have been practised in field experiments since 1979. The commercial black-seeded cultivar Huayou No. 8 has been used as a control. No line which yielded higher than control had been found before 1983. Now, two new cultivars have been developed (as Table 1).

Table 1. Main characters of two yellow-seeded cultivars (1985)

Cultivars	Yield kg/ha	Yield %	Oil content %	Oil content %	Date of ripening
2328	2787 a	124.31	42.94	111.82	12th May
955	2722 a	121.41	46.69	121.59	13th May
Huayou No. 8 (ck)	2242 b	100	38.40	100	9th May

As the above data shows, yellow-seeded B. napus with high seed yield and high oil content could be developed.

2. Degeneration of offspring was quite prominent in some inbred lines. The proportion of yellow cotyledons of seedlings ranged from

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0.02 to 86.11% in these populations. Cotyledons were classified for colour on a 0-4 scale:

0 Normal green cotyledons
1 The size of yellowish spot $<\frac{1}{2}$ area of two cotyledons
2 The size of yellowish spot $>\frac{1}{2}$ area of two cotyledons
3 Total yellow cotyledons growing very slowly
4 Total whitish yellow cotyledons, no true green leaf, it died in very early seedling stage.

The seedlings of 1st to 3rd scale were underdeveloped, their stems and branches were very weak, finally, their yield was much lower than normal plants. The more severe the yellowing cotyledons, the less the seed yield per plant (as Table 2).

In the yellow cotyledons, the photopigment contents decreased, the chloroplasts were misshapen, and there were fewer lamellar structures and granula (as Table 3). The variation in ultrastructure reveals the essence of degeneration.

Table 2. The economic characters and seed yield of rapeseed plants (1981)

Scales of yellow cotyledons	0	1	2	3
Plant height (cm)	139.38	111.39	87.68	77.08
Number of primary branches	5.90	3.69	4.13	2.50
Length of main raceme (cm)	38.88	32.88	25.43	22.43
Siliqua number of main raceme	46.44	28.78	27.31	14.70
Siliqua number per plant	127.44	49.79	29.43	16.50
Length of Siliqua (cm)	5.02	4.42	3.93	3.39
Seeds per Siliqua	20.2	19.4	13.9	10.9
1000-seed weight (g)	5.44	5.39	4.19	3.58
Seed yield per plant (g)	6.93	2.79	1.72	0.60

It should be pointed out that such degeneration might be overcome by selecting lines with tolerance to continuous selfing or by open pollination in an isolated plot of the same population. But 2328 and 955 are new cultivars in which there is no sign of degeneration.

3. The relationship between seedcoat colour and oil content.
The oil contents of the yellow-seeded B. napus have been testing systematically since 1980. They were 2-5% higher than those of dark-seeded plants within the same population. Xiao D. (1982) indicated that the lighter the colour of seedcoat the higher was the oil content but this was not the case in the progeny of F_2. The oil content of brownish-yellow seeds (43.65 0.80) was not only higher than dark-yellow seeds (41.96 0.94), but also higher than the light yellow ones (40.91 1.32) (1982-1984). From the point of view of breeding for high oil content, the main goal, must be considered firstly and it is not necessary to select the lighter yellow-seed materials only.

4. Breeding for low contents of erucic acid and glucosinolate in yellow-seeded B. napus. No 'double low' genes have been found in yellow-seeded B. napus so far. The genes must be transferred from 'double low' black-seeded varieties into yellow-seeded ones. The F_1 is black-seeded and the progeny of the hybrid, it is very difficult to bring the three characters of yellow-seed and lower contents of erucic acid and glucosinolates together because they are controlled by different systems of genes.

By crossing and backcrossing, some yellow-seeded materials which are 'single low' (low erucic acid or low glucosinolate) or 'double low' have been developed, but need be improved in both yield and frequency of yellow-seeded plants in the near future.

Table 3. Difference of chloroplasts in yellowing cotyledons and green cotyledons (1984)

	Yellowing cotyledon		Green cotyledon	
	No. of test	Average	No. of test	Average
Length of chloroplasts (μ)	34	4.90 1.20	30	5.18 0.97
Width of chloroplasts (μ)	34	1.49** 0.35	30	2.38 0.34
Length/width	34	3.38** 0.85	86	2.23 0.34
Number of lamellae per chloroplast	8	17.13** 2.79	7	23.86 2.70
Number of granula per chloroplast	7	20.29** 5.06	7	31.43 5.71

** Significant at 1% level

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EFFECT OF BORON FOLIAR SPRAY ON GROWTH AND THIOCYANATE CONTENT OF GOLDEN BALL TURNIP

H.-Y. Ju

Previous studies (Ju et al. 1982; Bible et al. 1981) indicated that a low boron level in growing media was associated with higher accumulation of thiocyanate in turnips and radishes.

In this study, we examined the effect of boron foliar spray on the growth and thiocyanate content of hydroponically grown Golden Ball turnip.

Two week old seedlings were transplanted to plastic buckets holding 8 l of modified full strength Hoagland's solution with four seedlings in each bucket. A low level of boron (0.1 ppm) was maintained for two weeks until the size of roots reached about 2 cm in diameter. Then boron was removed from the container. Boron was immediately applied as foliar spray with six different concentrations (0, 0.1, 1.0, 10.0, 100.0 and 1000.0 ppm) obtained by varying amounts of H_3BO_3. This experiment was arranged in a randomized block design with four replications. Turnips were harvested a month after treatments were applied. At harvest growth measurements were taken and thiocyanate content was determined.

Turnips receiving 100 and 1000 ppm boron grew equally vigorously until harvest, while those receiving 0.0 to 10.0 ppm boron were substantially reduced in growth of top and root and showed severe boron deficiencies in the roots (Table 1). Thiocyanate contents were higher in plants (top and root) receiving 0.0 to 10.0 ppm compared to plants receiving 100 and 1000 ppm (Table 2).

Table 1. Influence of boron foliar spray on growth of hydroponically grown Golden Ball turnip

Boron (ppm)	Top FW (g/plant)	Leaf number per plant	Top % DW	Root FW (g/plant)	Root % DW
0.0	122±15.3	10±0.6	16.2±1.3	43± 9.8	9.1±0.3
0.1	130±19.5	11±0.6	15.7±1.8	40± 2.2	9.2±0.2
1.0	124±21.0	11±0.6	15.0±1.0	36± 4.9	10.3±1.0
10.0	204±18.0	14±1.1	10.9±0.4	72±11.3	9.9±0.4
100.0	261±17.3	14±0.5	8.6±0.5	149±17.3	10.1±0.4
1000.0	280± 7.9	15±0.0	8.5±0.2	143±11.7	9.7±0.3
LSD (p=0.05)	56	2.6	3.0	27	n.s.

Each datum represents the mean ± SE of four replications, each with four plants.

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Table 2. Influence of boron foliar spray on thiacyanate content of hydroponically grown Golden Ball turnip

Boron (ppm)	Thiocyanate (µg/g DW)	
	Top	Root
0.0	419±172	2791±883
0.1	335± 67	1837±157
1.0	291± 62	1489±218
10.0	301± 77	1289±202
100.0	132± 29	1067±213
1000.0	106± 26	779± 76

Each datum represents the mean ± SE of four replications, each with two subsamples and analyzed in duplicate.

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WINTERHARDINESS AND VIGOUR IN DOUBLED HAPLOID LINES OF SPONTANEOUS AND ANDROGENETIC ORIGIN IN SWEDE RAPE (BRASSICA NAPUS L.)

W. SCHWEIGER

A screening for spontaneous haploids which was conducted in 1983 in swede rape varieties with zero erucic acid and low glucosinolate content (00-varieties) yielded haploide in 10 varieties. They were in vitro propagated and 42 genetically different clones were doubled by colchicin and then selfed for seed production. Simultaneously 40 doubled haploid lines (DH-lines) of androgenetic origin which had been produced via anther culture of six similar 00-varieties were selfed for seed production, too. Both, the 42 spontaneous DH-lines and the 40 androgenetic DH-lines were seeded in 1984 in the field for comparative studies.

There were distinct differences between spontaneous and androgenetic DH-lines, particularly in winterhardiness and vigour. The average surviving ratio of plants was 86 percent in spontaneous DH-lines and only 70 percent in androgenetic ones. In the former 17 DH-lines survived completely and in the latter only 5 DH-lines did so. The spontaneous DH-lines showed an obviously better vigour. The average plant height at the beginning of flowering was 69 cm in the spontaneous DH-lines and only 59 cm in the androgenetic ones. Another striking difference was that 60 percent of the spontaneous DH-lines were of a more leafy type, whereas that was 32 percent of the androgenetic DH-lines.

It is assumed that the above mentioned differences between the both groups of DH-lines are due to natural selection acting during development and growth of spontaneous haploids. These are exposed to a permanent selection pressure caused by environmental stresses and the inter-plant competition in the stand on the field. Any depression of growth and viability caused by homozygosis of letal or subletal genes may result in the elimination of the handicapped plants. The optimum conditions during the anther culture and the following cultivation in the glasshouse make it possible that even plants with homozygous letal genes survive and form seeds. But you find growth depressions here, too, and several genotypes are after successful doubling unable to form seeds. So we found in 221 haploid clones of androgenetic origin 42 ones showing growth depressions of different kinds and not forming seeds or forming only few seeds for this reason.

In the production of DH-lines for breeding purposes one should tharefore eliminate all those clones which show any growth depression during cultivation in the glasshouse.

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TREATMENT OF STIGMA WITH LECTINS AND OF POLLEN WITH SUGARS OVERCOMES SELF-INCOMPATIBILITY IN BRASSICA CAMPESTRIS

Madhu Bajaj and KR Shivanna

Recent studies on Petunia hybrida (Sharma & Shivanna 1983; Shivanna & Sharma 1985) characterized by gametophytic type of self-incompatibility have shown that treatment of the stigma with a lectin or of the pollen with a sugar before pollination was effective in inducing seed set in selfed pistils. Our preliminary studies on Eruca sativa, a sporophytic taxon, showed that treatment of the stigma with a lectin was effective in overcoming self-incompatibility (Sharma et al. 1985). We have extended these studies to another sporophytic system, Brassica campestris subsp. oleifera and have attempted to overcome self-incompatibility by treating the stigma with a lectin (1 mg/ml in 0.015M Tris HCl buffer, pH 8.0), or the pollen with a sugar (100 mM incorporated in the germination medium of Roberts et al. 1983) before pollination (Table 1). The details of the methodologies used were essentially similar to those used in Petunia (Shivanna & Sharma, 1985).

A total of 26 plants were used in experiments involving treatment of the stigma with lectins. All the three lectins were effective in overcoming self-incompatibility in a proportion of the plants tested. Ten of the plants tested did not respond to any of the three lectins. The remaining 16 plants responded to one or more lectins (Table 1). Treatment of the pollen with sugars was also effective in overcoming self-incompatibility (Table 1). Of the 15 plants tested for pollen treatment, six plants responded only to D-glucose, three only to N-acetyl-D-galactosamine and two plants responded to both the sugars; the remaining four plants did not respond.

The results are compatible with the suggestion (Sharma & Shivanna 1983, 1986; Shivanna & Sharma 1985) that lectin-like components of the pollen and specific sugar moiety, presumably of the S-allele-specific glycoproteins, of the pistil are involved in self-incompatibility recognition. Treatment of the pistil with lectins or of the pollen with sugars seems to block recognition molecules and thus overcome self-incompatibility.

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Table 1. Efficacy of treatment of stigma with lectins and of pollen with sugars in overcoming self-incompatibility. Pollinations of only those plants which were responsive to one of the lectins/sugars are included

Treatment	Self-pollinations				Cross-pollinations			
	Number of pollinations	Number of fruits formed	Number of seeds	Number of seeds/ pollination	Number of pollinations	Number of fruits formed	Number of seeds	Number of seeds/ pollination
Treatment of stigma with lectins								
Untreated	176	0	0	0.00	158	155	2507	15.87
Buffer	117	6*	20	0.17	77	75	1003	13.03
Con A	196	117	560	2.86	50	50	692	13.84
PHA	200	138	744	3.72	43	42	527	12.26
SOL	92	72	368	4.00	21	21	312	14.86
Treatment of pollen with sugars								
Untreated	109	0	0	0.00	104	100	1522	14.63
Germination medium	107	0	0	0.00	79	71	1199	15.18
D-glucose	165	129	518	3.14	85	80	1118	13.16
N-acetyl-D-galactosamine	115	94	293	2.55	76	65	966	12.71

*Developed on 3 plants; in none of the other plants did buffer treatment produce any fruits/seeds in selfed pistils.

Con A -- Concanavalin A, PHA -- Phytohemagglutinin, SOL -- lectin from Solanum tuberosum

USE OF CO_2 AND SALT SOLUTION TO OVERCOME SELF-INCOMPATIBILITY OF CHINESE CABBAGE (B. CAMPESTRIS SSP. PEKINENSIS)

Tao Guohua and Yang Rui

In accord with earlier experimental results we chose four Chinese cabbage lines which were treated with CO_2 (1) and salt solution (2,3,4). The results of this experiment were as follows.

Materials and Methods

The experimental material consisted of four lines: 234, 2039 and shuang have strong self-incompatibility and 269 is self-compatible. During the flowering period (April-May), three treatments were used. 1. CO_2; solid CO_2 amounting to 4% of the chamber capacity was put in at 9-10 am immediately after flower pollination, covers were removed at 2-3 pm on the same day. 2. salt solution (NaCl); flowers were pollinated and sprayed with 3% salt solution after 0.5-1.0 h. 3. check; pollinated by hand as normal. The former two lines were treated as above, the other two lines only given treatments 2 and 3. For all lines 20-40 flowers (2-3 plants) were pollinated at each time.

Results and Discussion

Treatments with 4% CO_2 or 3% salt solution were effective in overcoming self-incompatibility in Chinese cabbage and adequate numbers of self-seeds were obtained (Table 1). Lines 234, 2039 and shuang which have stronger self-incompatibility set more self-seeds than the self-compatible 269 line.

But some conditions have to be met, the plants must be growing normally, and the temperature is important. In this experiment, for best self-seed set the maximum temperature was under 27°C, self-seeds were less when the maximum temperature was over 30°C. Nevertheless, in the latter two lines (50-60 days) which were more tolerant to heat than the former lines (90 days), reduction in self-seed set was not obvious.

Previously, our colleagues had treated self-incompatible Chinese cabbage with 3% salt solution in a net chamber, they obtained more self-seeds with the aid of pollinating bees. Further self-incompatible cabbage planted in an isolated field in the suburb of Beijing and sprayed with 3% salt solution at 9 am every second day in the flowering period set 1.3 g/plant self-seeds. It is simple and cheap to use salt solution to overcome self-incompatibility in Brassica, but the concentration of salt solution must be different with different plants and districts.

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Table 1. Number of seeds per flower

Date	234			2039			Shuang		269	
	CO_2	Nacl	CK	CO_2	Nacl	CK	Nacl	CK	Nacl	CK
4.21	0	6.3	0.3	8.2	6.1	0.05				
23	6.1	4.0	0.1	11.2	4.1	1.0				
25	8.7	6.6	0	8.5	5.2	0.2	1.8	0.2	6.5	3.1
27	0.5	0.3	0.06	8.6	4.7	1.4	6.4	0.7	4.0	8.2
30	0	1.6	4.0	0.6	4.9	1.2	5.7	0.5	10.9	7.9
5.2	0	1.3	0.05	0.3	1.3	5.8	5.4	0.9	19.8	11.0
5	0	0.3	0.2	0.1	1.6	0.9	5.5	0.2	17.4	8.5
7	0	0	0.2	0	0	0.09	4.9	0.3	11.7	8.0
9							4.1	0.2	10.7	9.0
@	1.9	2.6	0.6	4.7	3.5	1.3	4.8	0.4	11.6	8.0

SELF-INCOMPATIBILITY REACTIONS IN A SYNTHETIC *BRASSICA NAPUS* LINE

T. Hodgkin

Brassica napus is an amphidiploid species and is commonly self-compatible although rare self-incompatible plants have been detected in some existing cultivars. The parental species, *B campestris* and *B. oleracea*, have sporophytic self-incompatibility systems (Thompson, 1957; Mackay, 1977) and synthetic *B. napus* plants, obtained through interspecific hybridisation, are also self-incompatible and show complex incompatibility interactions (Gemmell, pers. comm.). At SCRI we recently synthesised a *Brassica napus* line from parent plants homozygous at their respective S loci and this paper describes the incompatibility responses of the plants obtained.

B. campestris var *chinensis* plants homozygous for an S-allele designated Sa were pollinated with *B. oleracea* var *alboglabra* homozygous for S29 (as classified by Thompson, 1968) and their ovaries, excised 7 days after pollination, were cultured to obtain haploid *B. napus* plants (Inomata, 1976). One of the plants obtained was treated with colchicine and amphidiploid shoots were bud self-pollinated for seed. Five progeny plants were grown in the glasshouse and tested for their self-incompatibility responses by assessing pollen tube penetration following test pollinations (10 flowers/test) using aniline blue staining and UV microscopy as described by Khc and Baer (1968).

Table 1. Pollen tube penetration in some test-crosses between a synthetic *B. napus* and *B. campestris* and *B. oleracea* lines of known S genotype. Mean tubes/10 flowers (+ 50-100 tubes, ++ 101-200 tubes, +++ 200 tubes, - not tested).

PISTIL PARENT	POLLEN PARENT				
	B. campestris		*B. napus*	*B. oleracea*	
	S_aS_a	S_bS_b	$S_aS_a, S_{29}S_{29}$	$S_{29}S_{29}$	$S_{12}S_{12}$
B. campestris S_aS_a	0	+++	++	-	-
S_bS_b	++	0	++	-	-
B. napus $S_aS_aS_{29}S_{29}$	+	++	0	0	0
B. oleracea $S_{29}S_{29}$	-	-	0	0	++
$S_{12}S_{12}$	-	-	+	++	0

The results from the test pollinations are summarised in Table 1. When the snthetic *B. napus* was used as pollen parent it was incompatible with S29 homozygotes and compatible with *B. oleracea* lines homozygous for S12

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and S23. However, pollen from the synthetics was compatible with B. campestris plants homozygous for Sa as well as with plants homozygous for other B. campestris S-alleles. Used as the female parent the synthetic was incompatible with all B. oleracea testers but gave two distinct incompatible responses. While pollen from S29 homozygotes usually failed to germinate on the stigma surface (as in self-pollinations), that from other B. oleracea S-allele homozygotes germinated extensively with long, highly coiled tubes. Pollen from B. campestris Sa homozygotes was incompatible on the synthetic while pollen from other B. campestris S-allele tester lines was compatible. We have previously noted that B. oleracea pollen frequently fails to penetrate B. napus stigmas although up to 30 pollen tubes have been noted in some crosses.

Test crosses were also made between the synthetic and 2 self-incompatible and 2 self-compatible B. napus lines. Although pollen from the synthetic was compatible with these testers, pollen from the tester lines failed to penetrate the stigmas of the synthetic. In these latter pollinations there was extensive surface pollen germination of the kind observed in the crosses between the synthetic and B. oleracea plants noted above.

These results suggest that both the B. campestris and B. oleracea alleles are expressed in the synthetic B. napus although the expression of the B. campestris allele is masked in the pollen. However, it is also clear that there are additional incompatibility barriers in the B. napus synthetic which operate unilaterally such that it is incompatible with B. oleracea and other B. napus plants when used as female. We are generating further synthetic B. napus plants from parents known to be homozygous for B. oleracea and B. campestris S-alleles in order to investigate the relationship further.

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REMOVAL OF THE SELF-INCOMPATIBILITY BARRIER IN BRASSICA NAPUS

S Gowers

Although selfed seed of self-incompatible lines of brassicas can be produced by green bud pollination, this requires a certain amount of experience and dexterity. Several methods have been examined to overcome the incompatibility reaction and allow pollination of open flowers; these have involved chemical, physical and electrical methods of destroying the reaction on the stigma surface (see Taylor, 1982 for references). The method tested in this report was the complete removal of the incompatible barrier by removing the stigma itself.

The experiment was carried out on a highly self-incompatible swede line produced by backcrossing cv. Fenix to a self-incompatible artificial B. napus line. Three types of self-pollination were compared: open flower pollination, green bud pollination and pollination of the end of the style after the stigma had been pinched off with forceps. Six plants were used with six inflorescences available on each. Three inflorescences were used for open pollination, and three used for stigma-removed pollination. Bud pollination was carried out on all inflorescences; the four or five largest buds were removed, and the next six to eight buds were opened and pollinated. The time to carry out each set of pollinations was recorded, and the seed set was counted when the capsules had ripened.

Type of Pollination	No. of Polls.	Time Taken	Seed Set	Seed/ Poll.	Polls./ Minute	Seed/ Minute
Open	146	24.2	4	0.027	6.03	0.15
- Stigma	147	30.4	713	4.85	4.84	23.5
Green Bud	285	78.3	1723	6.05	3.64	22.0

The time taken to carry out pollinations with removal of the stigma was 25% longer than for open pollination, and for green bud pollination the time taken was 66% longer. However, the greater seed set per pollination with bud pollination resulted in the seed set per minute

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being only slightly lower than for stigma-removed pollination. From these results, therefore, there would appear to be little advantage with stigma removed pollinations. However, this work was carried out by someone with many years experience in bud pollination. If casual labour was involved in the multiplication of self-incompatible lines, it would be expected that they would take considerably longer to carry out bud pollinations, but should be able to carry out stigma-removed pollinations at a similar speed to the present results.

With swedes there is usually little, if any, inbreeding depression and 10 g of seed per plant would easily be obtained in small scale, intensively managed multiplication plots. Assuming virtually 100% germination, 17 kg of single cross seed would result from 78 minutes of bud pollination. With a double cross hybrid, as intended with swedes, and with a multiplication factor of 1000 for a field-scale crop, 17 tonnes of hybrid seed would be obtained.

The results of this experiment may, therefore, only be of academic interest for swedes. For other brassicas, particularly with inbreeding depression and where casual labour is employed to produce inbred seed, the stigma removal technique may be of greater interest; the main advantage would be its simplicity and lack of complicated or even dangerous equipment.

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A SIMPLE METHOD FOR THE OBSERVATION OF BRASSICA POLLEN GRAIN STAGE

RUFFIO-CHABLE V., PELLAN-DELOURME R., EBER F. et CHEVRE A.M.

Exin appears early during microsporogenesis, at the microspore stage (Knox, 1984). For Brassica species, the thickness of exin prevents a clear observation of microspore and pollen grain nuclei.

This paper describes a rapid and simple method which combines exin clearing and nucleus staining. The treated microspores and pollen grains can then be observed with a light microscope.

METHOD

A) Clearing procedure :

1 - The anthers are collected from buds or flowers.
2 - They are placed in the Herr's solution (Herr, 1971 - Levieil and Huyghe, 1985).
3 - They can be stored in the Herr's solution for a few days.

B) Staining procedure :

1 - The anthers are removed from the Herr's solution and put on a blotting paper to absorb the remaining solution.

2 - They are dissected in a Belling aceto-carmin drop on a slide, the debris are removed and a coverslip is applied. Then the slide is gently heated until the nucleus coloration is sufficient.

This method has been used for anther culture experiments. Usually, the anther culturing stage is determined by bud length and petal/anther ratio which are correlated with microspore stage. This method allows us to define more precisely these relationships for different genotypes of rapeseed and cauliflower. It has been used also to follow the evolution of cultured microspores.

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USE OF RAPID-CYCLING BRASSICAS IN PHOTOPERIODISM RESEARCH

Douglas J. C. Friend

The article on "Rapid-cycling populations of Brassica" by Paul H. Williams and Curtis B. Hill, in the 13 June issue of SCIENCE (p. 1385), points out the potential of these plants in research and teaching and coincidentally appears on the 20th anniversary of the publication in SCIENCE of the isolation of the rapid-cycling Brassica campestris cv. CERES (Friend and Helson, 1966).

There is already a considerable literature on the use of rapid-cycling brassicas in research on photoperiodic responses, beginning with that of a group of Dutch workers (Wassink et al. 1950, 1951; Stolwijk, 1954). The CERES cv. of Brassica campestris was isolated (from the Polish turnip spring rape cv. Arlo) after a number of species were screened for use as long-day equivalents of the rapid-cycling short-day plant Chenopodium (Cumming, 1967, 1969). The photoperiodic reactions of B. cv. CERES were investigated at the phytotron "CERES" of the CSIRO in Canberra, Australia, and the cv. was named accordingly. While not as extreme an example of a short-cycle as Chenopodium, Brassica cv. CERES is photoperiodically sensitive to a single long day as early as 4 days from sowing. When provided with limited nutrients as many as 20 plants can be grown in a 5 cm diameter pot, (Friend 1968a, b, 1969, 1984, 1985).

Use of cv. CERES has provided information on an action spectrum for the high intensity reaction in the photoperiodic responses of long-day plants (Friend, 1968a and b), the promotion of flowering by sucrose (Friend et al, 1984) interactions of photosynthesis and photoperiodism (Friend et al 1979), the anatomy and cytology of flower initiation (Kohli and Seidlova, 1981; Orr, 1978, 1981) the effect of plant growth hormones on flower initiation (Krekule and Seidlova, 1977), and the changing pattern of enzyme activity during floral initiation (Orr, 1984, 1985; Petersen and Orr, 1983). The CERES cultivar has recently provided evidence for the existence of a photoperiodically active pigment with peak sensitivity in the green region of the spectrum, "heliochrome" (Tanada, 1984).

Further investigations of photoperiodic responses in Brassica can build on this accumulated information by working with the CERES cv. I have provided seed to the Crucifer Genetics Cooperative (Williams 1985) and is available as CrGC-101.

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PRODUCTION AND CHARACTERIZATION OF SOMATIC HYBRIDS BETWEEN BRASSICA OLERACEA AND B. CAMPESTRIS.

P. S. Jourdan, M. A. Mutschler and E. D. Earle

Introduction

The purpose of this work is the establishment of novel cytoplasms in the genus Brassica via parasexual hybridization. We have chosen the resynthesis of Brassica napus by fusion of protoplasts from B. campestris and B. oleracea as our system of study because: 1) significant progress has been recently achieved in the in vitro manipulation of these three species, 2) there are well-characterized cytoplasmic traits as markers and mitochondrial molecular probes are available, 3) there are simple nuclear markers which would help identify the hybrids, 4) the parasexual hybrids could be compared with reciprocal sexual hybrids, and 5)novel cytoplasmic combinations have potential agronomic value. The resynthesis of B. napus by protoplast fusion has already been reported by our laboratory and others[1,2,3,4]. Unfortunately there are still no reports on subsequent genetic and agronomic evaluations of the somatic hybrids so far produced. The one hybrid previously synthesized in our lab[4] showed clear evidence of mitochondrial DNA recombination, but has turned out to be female sterile thereby limiting genetic analyses with this material as male parent. In an effort to obtain more hybrids for detailed study of organelle inheritance, we have chosen a different B. oleracea parent which carries the *ogu* cytoplasmic male sterility as a fusion partner with B. campestris carrying atrazine resistant chloroplasts. This B. oleracea is an inbred line of cauliflower which exhibits high regeneration potential[5].

Protoplast isolation and fusion

Mesophyll protoplasts of month-old, in vitro grown seedlings of cauliflower were isolated and cultured as described for Green Comet protoplasts[6]. Etiolated hypocotyl protoplasts from 5-day old seedlings of B. campestris line 114 (atrazine resistant, cv "Candle" background; self incompatible; obtained from Dr. W. Beversdorf, Guelph) were isolated by the same procedure. Fusion was accomplished by mixing ca 7.5×10^5 protoplasts of B. campestris with ca. 3.8×10^5 protoplasts of B. oleracea. The mixture was aliquoted in drops onto 6cm plates and treated with 33% PEG6000 by standard methods. The PEG was slowly diluted with 0.5M Sorbitol, 50mM $CaCl_2$ $2H_2O$, pH 6.5. The protoplasts were cultured while adhered to the plate in the series of liquid media used for broccoli protoplast culture[6]. We have not obtained colonies from B. campestris protoplasts with this media sequence. After ca. 40 days, small colonies were transferred to medium E containing 25 µM atrazine. Calli that turned green on this medium were then transferred to medium F free of atrazine in order to stimulate regeneration. Regenerated plantlets were rooted on medium G and finally transferred to soil. Total DNA was isolated as described by Dellaporta [7] and atrazine resistance or susceptibility was determined by a tetrazolium blue assay developed by D. Robertson of our laboratory[8].

Results

A total of 136 calli were obtained on medium E after fusion, of which 40 were dark green in the presence of atrazine. Control experiments where parental protoplasts were independently treated with fusogen either failed to divide and form colonies (as for B. campestris) or yielded colonies that failed to green in the presence of atrazine (as for B. oleracea). Of the 40 green calli obtained after fusion of both parents, 11 calli regenerated various plants that could be categorized into four groups based on characteristics such as growth habit and overall morphology. The presence of trichomes was an early indication of possible hybridity since any regenerated plant possessing trichomes was not likely to be the B. campestris parent since this material does not regenerate in our culture media. Further characterization of the putative hybrids was done by analyzing isoenzyme patterns as well as by BstEII-generated rDNA restriction pattern after probing with a rDNA clone obtained from pea which has been shown to distinguish between the parents (J. Palmer, pers. comm.). The mtDNA was also analyzed after

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restriction of total DNA with PstI, SalI and HindIII, and probing with B. campestris mtDNA fragments provided by Dr. J. Palmer. The table below summarizes the characteristics of the four groups of plants obtained from a single fusion experiment.

Characteristic	Group I	Group II	Group III	Group IV
Number of plants	4	1	5	5
Overall morphology	Intermediate	Intermediate	Intermediate	Parental B. oleracea
Presence of trichomes	Present	Present	Present	Absent
Atrazine Resistance	Resistant	Resistant	Resistant	Susceptible
Growth Habit	Vigorous, dark green	Vigorous, variegated	Petite, dark green	Parental, B. oleracea
Fertility	Fertile	Feritle	Partial fertile	Male sterile
Seed set[a]	High	High	Very low	n.a.
Pollen viability[b]	95%	95%	<20%	n.a.
Isozyme pattern (PGI)[c]	Hybrid	Hybrid	Hybrid	Parental B. oleracea
rDNA pattern	Hybrid	Hybrid	Hybrid	Parental B. oleracea
mtDNA pattern	Parental B. campestris	Parental B. campestris	Novel	Parental ogu cms

[a]Seed set for Groups I and II based on selfing; for Group III is based on hybridization with B. napus since no self seed was obtained. [b]Determined by acetocarmine stain. [c]Phosphoglucose isomerase.

Conclusions

(1) At least three different types of somatic hybrids of B. oleracea and B. campestris have been obtained from a single fusion experiment. Group IV represent parental cauliflower that escaped the selection on atrazine. (2) Initial investigations reveal that the hybrids have inherited the nuclear genomes of both parents as evidenced by rDNA restriction patterns and PGI isozymes. Preliminary chromosome counts in flower buds from one plant of group 1 indicate the presence of 19 bivalents, but similar counts in buds from Group 3 plants are so far inconclusive. Therefore, the possibility of aneuploidy cannot be ruled out. (3) All somatic hybrids are atrazine resistant, indicating that they have inherited the B. campestris chloroplasts. (4) The fertile and vigourous somatic hybrids of group 1 appear to have mtDNA indistinguishable from B. campestris suggesting that a preferential selection for the fertile cytoplasm has occurred in these plants. However, further analyses of the mtDNA must be carried out before strong conclusions can be reached. (5) The petite and slow-growing plants in group 3 exhibit some evidence of possible mtDNA recombination between the parents. The partial sterility in these plants could be due either to mtDNA changes or to some chromosomal imbalance.

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CELL SUSPENSION CULTURE OF RAPID-CYCLING *BRASSICA CAMPESTRIS* L.

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Our laboratory is interested in the evaluation of alternative methods for creation of novel combinations of organelle-encoded traits in *Brassica* species (Robertson et al., 1985). One such method is development of *Brassica* lines having altered cytoplasms via mutagenesis. Mutants may be selected for resistance to several antibiotics (streptomycin, lincomycin, chloramphenicol), since such resistance is sometimes maternally inherited, or for chloroplast encoded resistance to the herbicide atrazine. Selection for organelle-encoded mutations can be done either using plants grown directly from mutagenized seeds of fast-flowering *Brassica* lines or using protoplasts and/or calli derived from such plants.

We are particularly interested in testing the usefulness of fast-flowering *B. campestris* as a tool for basic research. *B. campestris* is the species with the shortest life cycle of the rapid-cycling *Brassica* populations (10 cycles per year). We can grow this material from seed to maturity *in vitro* maintaining its short life cycle. Large populations of plants can be cultured in a small area (4 cm^2 per plant) and seeds can be obtained *in vitro* (Lentini, 1986). In attempts to develop a somatic cell genetic system, we have studied the tissue culture response of a self-compatible line of rapid-cycling *B. campestris*, SC_1. Here we report a procedure for the induction and the maintenance of rapidly growing suspension cultures derived from hypocotyl calli of this material.

SC_1 seeds (CrGC #66, Crucifer Genetics Cooperative) were surface-sterilized in 30% Chlorox (5.25% NaOCl) + 0.6% (w/v) PEX detergent for 20 minutes and germinated on agar-solidified White's (1963) salts containing 1.5% sucrose. The most prolific callus growth was obtained from 3 to 6 day old hypocotyls grown in light. Explants (1 cm long) were cultured horizontally on agar-solidified Linsmaier-Skoog (LS) (1965) salts + 3% sucrose, naphthaleneacetic acid (NAA) and benzylaminopurine (BA), each at 5.0 mg/l, at 16 h photoperiod and 84 $\mu mol.m^{-2}.s^{-1}$. Calli developed on this hormone combination were pale green and very friable. Calli were maintained in the light on LS salts + 3% sucrose, 1.0 mg/l NAA, 5.0 mg/l BA, 0.1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 200 mg/l enzymatic casein hydrolysate (M medium). The presence of 2,4-D and especially of casein hydrolysate in the medium greatly reduced callus browning, which was often a problem in the preliminary attempts to maintain SC_1 callus.

Freshly induced calli did not initiate cell suspensions. Suspensions were obtained only from hypocotyl calli maintained for 3 to 5 months. Cultures were initiated by placing 10 to 15 friable calli in 50 ml liquid medium in 250 ml flasks sealed with aluminum foil. Flasks were placed on a gyratory shaker at 125 rpm with 16 h photoperiod and constant 25 C. A week later, suspensions were filtered through a double layer of cheese cloth to remove the remaining pieces of callus. Of the media tried, the most actively dividing cell line was established in LS salts + 4% sucrose, with NAA and 2,4-D each at 0.1 mg/l. Suspensions were filtered before

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subculturing to select small size clumps. Transfers were performed by pipetting 20 ml of filtrate in 40 ml medium (1:3 dilution) every 3 weeks. The best culture was selected from 3 replicates for 3 months. Following this procedure, a very fine cell line was developed. It has been maintained on M medium by subculturing every week (1:2 or 1:4 dilution) for 9 months. The presence of 2,4-D in the medium was essential for initiation and maintenance of cell suspensions. Samples pipetted from actively dividing suspensions onto gelrite-solidified M medium formed actively dividing, pale, friable calli that were easily maintained on this medium.

Protoplasts were isolated from cell suspensions approximately 4 days after subculture. Suspension samples were centrifuged and medium discarded. Protoplasts were isolated and cultured following the procedure described for Green Comet broccoli (Robertson and Earle, 1986). Another enzyme solution (WPY) containing 2% CELF cellulase (Cooper Biomedical) and 0.1% Pectolyase &-23 (Seishin Pharmaceutical Co.) in 0.2 M mannitol and 80 mM $CaCl_2 \cdot 2H_2O$), pH 5.6 was also tested. A yield of 3-5 X 10^4 protoplasts/gFW was obtained with both enzyme solutions. After 3 days in culture most protoplasts formed cell walls; however, very sparse first cell divisions were noted. Higher protoplast yields have been recently obtained using callus derived from the cell suspensions (8 days after subculture, 21 days after initial plating). The enzyme solution used contained 1.25% Cellulysin (Calbiochem-Behring Corp.) in 0.5 M sorbitol, 10 mM $CaCl_2{}^2H_2O$ and 3 mM Mes buffer, pH 5.6. Incubation was performed at 25 C, 45 rpm for 18 hours. Under these conditions a yield of 4.7 X 10^5 protoplasts/gFW was obtained (1 experiment). A yield of 3.5 X 10^5 protoplasts/gFW was obtained using WPY enzyme solution diluted 1.5:3.5 with 0.2 M mannitol and 80 mM $CaCl_2 \cdot 2H_2O$. Protoplasts were isolated as described by Robertson and Earle (1986) and cultured in a modified 8p medium (Glimelius, personal communication) in the dark at 25 C. After 3-5 days 5% of the protoplasts had divided once and by day 10 small healthy colonies (8-12 cells) have been observed. The progress of these cultures is currently being monitored.

Plant regeneration from the SC_1 cultures has not yet been noted under our conditions. However, cell suspensions developed from this plant material rapidly generate large homogeneous cell populations that can be utilized as protoplast sources or as starting material for mutagenesis experiments. Evaluation of various enzyme solutions and protoplast-culture media for higher protoplast yield and cell division is currently in progress in our laboratory.

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IN VITRO PROPAGATION OF CAULIFLOWER (Brassica oleraceae var. botrytis cv. Winner Osenia) BY SHOOT-TIP AND CURD TISSUE CULTURE: INFLUENCE OF SOME AUXINS AND BAP ON SHOOT PROLIFERATION

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Cauliflower breeding researches are more difficult compeared with the other species in the same genus. The main cause of this is that it is difficult to maintain the selected curds as breeding material for a long time. Although traditional techniques are used they are still relatively slow. Tissue culture methods are now used in accelarated breeding programmes.

For this purpose, we studied on the cauliflower tissue culture and at first we determined the most appropriate sugar, agar and pH degree that was required in shoot-tip culture (1).

The aim of this study is to investigate the most appropriate auxin-cytokinin combinations and determine the potantial of shoot-tip and curd tissue for rapid multiplication of cauliflower.

In this study four different auxins (IBA,NAA,IAA and 2,4-D) at four different levels (0-0.01-0.1-0.5 and 1.0 mg/1) and BAP at four concentrations (1.0-0.5-1.0 and 2.0 mg/1) were tested. Murashige and Skoog (2) basal media with 30 g/1 saccharose, 6 g/1 agar and 0.1 mg/1 GA_3 were used.

Our results indicate that in shoot culture BAP promoted shoot proliferation on media with high concentrations regardless of auxin type and the best results were obtained from 2.0 mg/1 BAP (Table 1). Auxins, which were used in the study, IBA, NAA and IAA were found more effective than 2,4-D. IBA and IAA gave better results in low concentrations with high concentrations of BAP. The highest shoot production were obtained 0.5 mg/1 NAA and 2.0 mg/1 BAP combination.

On the other hand approximately 30 shoots on no-auxin media and over 50 shoots with auxin media can be produced by using curd portions in 5 mm diameter in one month (Table 2). IBA and IAA gave the best results in all combinations. The more shoots were obtained with the increasing of auxin and BAP concentrations.

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Table 1 The effect of different level of auxins-cytokinin concentrations on the avarage shoot number of cauliflower obtained from shoot-tip culture.

AUXINS		BAP (mg/l)			
Type	Conc.(mg/l)	0.1	0.5	1.0	2.0
IBA	0.01	3.3	26.7	32.2	41.0
	0.1	17.8	21.7	24.0	27.2
	0.5	-	7.0	26.5	31.3
	1.0	-	-	28.5	22.0
NAA	0.01	18.3	31.7	35.7	38.5
	0.1	13.5	24.2	29.5	18.2
	0.5	-	27.0	25.7	66.2
	1.0	-	-	20.0	30.8
IAA	0.01	11.8	26.8	23.7	35.8
	0.1	9.5	18.0	20.2	27.5
	0.5	-	22.2	16.2	23.0
	1.0	-	-	17.8	28.3
2,4-D	0.01	5.2	4.0	7.2	15.7
	0.1	8.0	1.3	3.2	3.0
	0.5	-	11.0	9.3	5.0
	1.0	-	-	8.3	2.3

Table 2. The effect of different level of auxins-cytokinin concentrations on the avarage shoot number of cauliflower obtained from curd tissue culture.

AUXINS		BAP (mg/l)			
Type	Conc.(mg/l)	0.1	0.5	1.0	2.0
	0	29.4	23.3	30.4	22.7
IBA	0.01	31.7	28.5	34.0	25.0
	0.1	35.9	31.2	41.6	27.2
	0.5	-	25.1	35.0	25.0
	1.0	-	-	32.8	42.0
NAA	0.01	25.3	28.2	26.6	22.4
	0.1	32.1	30.1	36.7	17.5
	0.5	-	27.2	27.0	24.5
	1.0	-	-	20.3	31.4
IAA	0.01	37.2	47.0	28.0	19.5
	0.1	31.3	31.7	24.0	16.6
	0.5	-	29.2	25.8	25.2
	1.0	-	-	26.0	30.5
2,4-D	0.01	22.2	20.3	12.5	20.0
	0.1	52.0	33.0	30.5	13.0
	0.5	-	17.5	16.5	8.5
	1.0	-	-	17.0	11.0

THE EFFECT OF GAMMA IRRADIATION ON BUDS OF BRASSICA NAPUS SSP. OLEIFERA PRIOR TO ANTHER CULTURE

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Experiments in which gamma irradiation has been used prior to anther culture in Nicotiana and Datura have shown that, in these species, low doses improved the number of anthers which produced anther embryoids (Sangwan & Sangwan 1986). Experiments have now been undertaken to see whether this response is evident in Brassica napus ssp. oleifera.

Buds of Brassica napus ssp. oleifera were harvested and treated with doses of 0, 1, 3, 5, and 10 K rads of gamma irradiation from a Cobolt 60 source. Anthers were cultured for 6 weeks following the method of Keller & Armstrong (1978) and the number of anther embryoids produced was recorded.

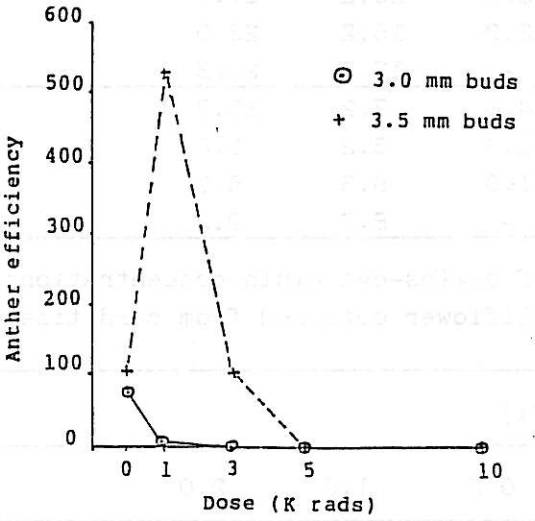

FIG.1. Anther efficiency following gamma irradiation

There was a five fold increase in **anther efficiency** (number of embryoids produced per thousand anthers plated) following the treatment with 1 k rad of irradiation. (Fig 1.) Similar responses were also observed when **anther response** (number of responsive anthers per hundred anthers plated) and **anther productivity** (number of embryoids produced per responsive anther) were calculated. This response was only evident for the 3.5 mm buds and not for the 3.0 mm buds.

The use of gamma irradiation has been shown here to increase anther efficiency in B. napus ssp. oleifera. The mechanisms involved are as yet unclear, but may involve the interruption of the pollen mitotic division, the disabling of either the vegetative of generative nucleus at the early bi-nucleate stage, or the reduction of competition between pollen grains, following the death of some microspores. These possibilities are being investigated further.

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IN VITRO DIFFERENTIATION FROM ROOT CULTURES OF BRASSICA JUNCEA AND B. NAPUS

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Protocols of in vitro plant regeneration from root cultures have both theoretical and practical implications. From the academic angle, since the root emerging from a seed is genetically programmed to give rise only to roots, shoot bud initiation in root cultures provides evidence for true regeneration. From practical view point of meeting crop improvement objectives, root cultures are relevant because: (i) they provide ideal explant in cases where it is desired to monitor the chromosome number of a seed raised plant and use it as a source of unlimited explant material without sacrificing the plant, (ii) they are known to maintain stability of chromosome number on prolonged culture much better than do aerial explants and thus help preserving genetic fidelity of the source material (CHATURVEDI and SINHA 1979), (iii) they retain regeneration potential over extended periods of in vitro culture (LAZZERI and DUNWELL 1984 a&b), and (iv) on account of the earlier listed points, they are good material for genetic conservation using cryopreservation technique.

We have been successful in achieving regeneration from in vitro root cultures of two varieties each of B. juncea and B. napus. Our main findings are:

1. Both the frequency of cultures yielding callus and the regeneration frequency was strongly influenced by the age of the seedling from which root explants were taken. Between 3, 6 and 9 day old seedlings, best response was obtained in 9 day old seedlings (frequencies of callusing and regeneration: 51.0 and 22.0% respectively),

2. On Murashige and Skoog medium, variation in NAA concentrations did not elicit any marked variation in regeneration frequencies. Variations in BAP concentration, however, had a pronounced effect. At a constant NAA concentration of 5 mg L^{-1}, a linear increase in regeneration frequency in the range 4 mg L^{-1} - 7 mg L^{-1} of BAP was observed.

3. Both genomic and genotypic differences in regeneration response have been observed. B. napus (genomic constitution AACC) responds distinctly better than B. juncea (genomic constitution AABB). Within B. juncea, the response in the variety Pusa Bold was markedly better than the response of the variety Varuna. Similarly, in B. napus, the response of the variety B.O.15 was superior to that of B.O. -54.

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GENERATION OF ANTIBIOTIC-RESISTANT BRASSICA CALLUS BY NITROSOMETHYLUREA MUTAGENESIS OF SEEDS

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Nitrosomethylurea (NMU) has been used to produce maternally inherited mutations in a number of species (see R. Hageman in Methods in Chloroplast Molecular Biology, Edelman et al. eds., Elsevier 1982). We are interested in the generation of organellar mutants for use in the study of organelle function and as markers in protoplast fusion and organelle transfer experiments in Brassica. Initial attempts to identify maternally inherited mutations by screening of seed from mutagenized plants and their progeny were hampered by low fertility of plants from mutagenized seed and the low probability of representation of presumptive cytoplasmic mutations in the flowering portions of the plant. A recent report by Fluhr et al. (Proc. Nat. Acad. Sci. USA 82:1485, 1985) described efficient isolation of several different plastome-dependent antibiotic resistances in N. tabacum using NMU mutagenesis of seeds, germination on selective media, and regeneration from cotyledon explants. Although Brassica has not proven as amenable as Nicotiana, we have been able to select streptomycin resistant B. oleracea callus using a combination of NMU treatment of seeds and regeneration of cotyledon protoplasts.

Seeds from a plant regenerated from a hypocotyl explant of Green Comet broccoli (Robertson and Earle, Plant Cell Reports 5:61, 1986) were surface-sterilized 25 minutes in 30% bleach with 5 g/l PEX detergent (Peck's Products Company, St. Louis), rinsed with sterile water, and incubated in 0.35 mM NMU (Sigma) in 50 mM citrate:50 mM phosphate buffer, pH 5.5, 10 ml/10 cm petri dish, on a rotary platform at 50 rpm overnight. Seedlings were grown at 25° with a 16 hr photoperiod of 90 $\mu E/m^2/sec$. Protoplasts were prepared from cotyledons of 2-3 week old seedlings and cultured using the media of Pelletier et al. as described in Robertson and Earle. Small protoplast-derived colonies were transferred to medium E containing 30 µg/ml streptomycin 5-7 weeks after protoplast isolation. After 3-4 weeks callus from protoplasts from cotyledons from non-mutagenized seedlings was completely bleached, while 30% of the callus pieces from the mutagenized seeds contained green sectors. Green sectors were cut out with a scalpel and transferred to fresh selective medium. Most of the selected pieces gave rise to mixed green and bleached callus.

Shoot regeneration in the presence of streptomycin was poor, even on green callus. Green callus pieces with the most uniform appearance after 2-4 transfers on medium with streptomycin were used for induction of shoots on medium without streptomycin. Rooted plantlets generated from these shoots will be tested for the ability to produce green callus on streptomycin medium.

We have not found direct germination of mutagenized seed on medium containing antibiotics as useful for Brassica as for tobacco. Even when seeds of B. oleracea, napus, or campestris are exposed to

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antibiotic immediately after sterilization, bleaching of the cotyledons is slow and "green islands" are not observed despite NMU treatment. This presumably reflects differences in the state of development of cells and plastids in the Brassica and Nicotiana seeds. An initial incubation in the dark in the presence of streptomycin improved the bleaching, but did not reveal resistant sectors.

The dose of NMU used in the experiment described above is about an order of magnitude lower than the dosage used in several other reports. The appropriate dose appears to be influenced by the time of exposure, the type of seed, the type of buffer, and the time of exposure relative to the exposure of seeds to moisture. Some investigators have used pre-imbibed seeds for NMU mutagenesis. In B. oleracea and campestris the effects of NMU on germination and variegation of early leaves are reduced if seeds are pre-soaked for 4 to 5 hours before exposure to NMU. Imbibition in the presence of NMU and the long exposure time may provide more uniform penetration of the mutagen into the relatively large Brassica seeds. NMU is unstable at neutral or alkaline pH, so we have used a buffer similar to that suggested by Hageman. The citrate-phosphate buffer retards germination. This may allow additional time for penetration of the mutagen during long exposures, in addition to stabilizing the NMU. A 10-fold dilution of the buffer has little or no effect on germination.

Fluhr et al. reported that after a 2 hr exposure of dry tobacco seeds to 5 mM NMU 20-90% of the seedlings had antibiotic resistant sectors in the cotyledons, but only 1-2% showed resistant sectors in the first true leaves. This indicates that the effective target size for the meristem is small. The target for the portion of the plant which will give rise to gametes is smaller yet. Selection at the level of true leaves or seeds rather than at the cotyledon level therefore requires much larger numbers of larger plants, which becomes impractical for the Brassica crop species. Direct selection for antibiotic resistance at the cotyledon level has not been possible, but selection of protoplast-derived colonies appears to offer a reasonable alternative. There is time in the initial stages of culture for additional segregation of the mutation to occur, and large numbers of colonies can be examined in a reasonable amount of space.

A NEW APPROACH TO THE IMPROVEMENT OF INDIAN MUSTARD

M V Palmer

Conventional plant breeding and selection has produced several promising new lines of Indian mustard (*Brassica juncea* Coss) with good agronomic characteristics such as drought tolerance, reduced pod-shattering, disease resistance, and yellow seeds with a high oil and protein content and a low content of fibre and erucic acid. If the unacceptably high glucosinolate content of the seed could be reduced, Indian mustard would have a great potential as an alternative oilseed crop, particularly in regions where water stress and high temperatures limit the use of rapeseed (*B.napus* and *B.campestris*) in cereal rotations (2,3).

Two new breeding techniques are being used in the State Chemistry Laboratory, Melbourne, to accelerate the production and identification of low-glucosinolate mustard. Firstly, tissue culture is being used in an attempt to generate low-glucosinolate somaclonal mutants. Cotyledon culture has proved to be the most efficient regeneration procedure for the brown-seeded Indian lines and leaf culture for the low-erucic acid "Zem" mustards (1). In addition anther culture is being used to produce haploid material for callus culture, and pure doubled haploid lines for conventional breeding.

Secondly, an enzyme-linked immunosorbent assay (ELISA) has been developed specifically to screen plant tissues for the two principal mustard seed glucosinolates, sinigrin and gluconapin. The ELISA, which is based on polyclonal antibodies raised in rabbits against a sinigrin-protein conjugate, has a linear response range of 5×10^{-8} to 4×10^{-12} mol sinigrin in crude aqueous plant extracts, and has shown excellent correlation with chromatographic analysis the same samples. This highly sensitive and specific assay will allow hundreds of samples to be analysed per week, and requires only a minute amount of plant tissue such as a single cotyledon. It can therefore be used as a non-destructive test to evaluate seedlings and regenerated plants in the laboratory at a very early stage of development.

In the next year it is hoped that the integration of these two research programmes will accelerate the development of low-glucosinolate mustard as a new oilseed crop in Australia.

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THE TRANSFER OF ATRAZINE RESISTANCE FROM *BRASSICA NAPUS* TO *B. OLERACEA*

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In Ontario, the choice of registered herbicides for weed control in cole crops is very limited and new research and development in this field is unattractive to pesticide manufacturers because of the small area involved. Alternatively, as cytoplasmic atrazine resistance exists in the related species *B. napus*, it should be possible to transfer this resistance into the crop. However, the two species being extremely recalcitrant to hybridization (McNaughton and Ross, 1978), our efforts were directed at finding ways of overcoming the interspecific barriers. Last year, we reported an *in vitro* embryo culture and regeneration protocol capable of producing up to three *B. napus* X *B. oleracea* hybrids/pollination (Ayotte *et al*, 1985).

The F_1 hybrids grew into large and vigorous plants, morphologically intermediate to the parental species. Most plants had 28 chromosomes ($2n = 3x = 28$, A_1C_1C) and a few had 37 ($2n = 4x = 37$, A_1C_1CC), presumably from the union of a normal *B. napus* gamete ($n = 19$) and an unreduced *B. oleracea* gamete ($n = 18$). In meiosis, the most common chromosome associations were $9_{II} + 10_I$ (46.6%), $10_{II} + 8_I$ (11.2%) and $8_{II} + 12_I$ (6.0%). Multivalents, mostly trivalents were found in 31.8% of the PMCs examined. From a breeding perspective, the formation of multivalents is important because it provides the opportunity for recombination between the A and C genomes of the interspecific hybrid.

Backcrosses to *B. napus* were fairly easy, giving, on average, 2.2 seeds/pollination when *B. napus* was the pistillate parent. The interspecific hybrids did not set seed when crossed to *B. oleracea*, but BC_1s and BC_2s were obtained by the same embryo rescue technique used in the first phase of hybridization. Chromosomes were lost in each successive backcross generation and the atrazine resistant cytoplasm was finally stabilized in an 18-chromosome BC_3. The herbicide resistance remained strong throughout the backcrossing process. For convenience, most of the crosses were done with broccoli but resistant BC_3s with cauliflower, cabbage and kale have also been obtained.

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SELECTION OF NaCl-TOLERANT PLANTS FROM CULTURED COTYLEDONS OF BRASSICA JUNCEA

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A major mineral stress of plants involves saline environment. A significant area of the terrestrial environment is affected by high levels of salts. Conventional plant breeding methods have met with some success in improving the response of crops to this stress (Norlyn, 1980). The application of plant tissue culture techniques provide another method to develop salt-tolerant genotypes. Using these techniques, several investigators have reported the selection of salt-tolerant cell lines in several different species (Stavarek and Rains, 1984). However, the regeneration of plants from salt-tolerant cells has been limited (Nabors et al., 1980). Our group is actively involved in the development of salt-tolerant genotypes of Brassica species using in vitro screening techniques. A selection system developed for such an application in B.juncea has been described here.

Seeds of B.juncea cv. Prakash were surface sterilised with 0.1 % $HgCl_2$ solution for 10 min, washed thoroughly with sterilized water and incubated on agar (0.8 %) sucrose (0.5 %) medium. After 7 days the cotyledons were excised and cultured on Murashige and Skoog's (1962) medium supplemented with several different combinations of the growth hormones. The cultures were incubated under constant illumination of 6000 lux at 27 $\pm$ 1°C. Best results were obtained when the medium was supplemented with indole acetic acid (0.2 mg/l) and kinetin (2 mg/l). On this medium the cultured cotyledons expanded and formed callus, usually at the cut ends. This callus proliferated for a few days and then simultaneously started differentiating into nodular structures which subsequently formed shoot buds. In 3-4 weeks, these buds gave rise to multiple shoots. When individual shoots were separated and recultured, they again proliferated to form multiple shoots. This process was repeated subsequently. However, when the shoots were left in the same medium for a prolonged period, they formed roots. These observations which demonstrate that the cotyledonary cells are endowed with a very high morphogenetic potential formed the basis of our selection system.

The cotyledons were cultured on media containing 0, 0.25, 0.5, 0.75 and 1.0 % w/v of NaCl of electrical conductivity 5.0, 7.8, 11.75, 14.75 and 17.15 m mho/cm, respectively. On medium having 0.25 % NaCl, the cotyledons survived and formed callus, though callus growth and subsequent plant regeneration were drastically reduced. On the medium having higher salinity levels (0.5, 0.75 and 1.0 % w/v NaCl), majority of the cotyledons lost chlorophyll, turned pale yellow and eventually died. The medium containing 0.75 % NaCl was used to screen salt-tolerant cells. Out of a total of 2050 cotyledons cultured on above

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medium, 3 survived and showed sustained growth and regenerated shoots. These shoots were micropropagated by using nodal explants on a medium devoid of NaCl. These shoots after 4 weeks incubation regenerated roots and thus gave rise to whole plants. A large number of such plants were washed thoroughly in running water for 1 hr and transferred to pots containing sterilized potting soil. These plants flowered subsequently and produced seed. This seed would be used to evaluate the resultant plants for their salt tolerance in the next crop season.

The technique used in this study is based on isolating and multiplying salinity tolerant cells which might have arisen as a result of spontaneous mutations in the somatic tissues. Since all the sensitive cells died and only the resistant ones grew to form calli and subsequently whole plants, this selection system offers a potential tool for screening somatic mut ations tolerant to salinity and other similar adverse conditions where the stress can be created in the culture vessels.

ACKNOWLEDGEMENT

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DIRECT GENE TRANSFER IN *BRASSICA NAPUS*.

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INTRODUCTION

Direct gene transfer into plant cells by electroporation has been developed in tobacco (1,6,7), carrot (1,3) and maize (1,2). Only in tobacco, have transformed regenerated plants been obtained (6,7). We have applied this technique to *Brassica napus*.

MATERIAL AND METHODS

We have used the plasmid pABDI to transform rapeseed protoplasts. This plasmid (4) consists of a chimeric kanamycin resistance gene cloned in pUC 9. Promoter and 3' regions are from gene VI of cauliflower mosaic virus; the APH(3')II-coding-sequence is from Tn5.

Leaf protoplasts of *Brassica napus* (cv. Brutor) were subjected to electroporation according to 7.

Electroporated protoplasts were plated on culture medium B (5) containing 0,6% w/v agarose. 11 days later, the agarose was cut into small squares. Protoplast-agarose-gel segments were transferred into Petri dishes containing a volume of liquid medium C (5) corresponding to that of the agarose fragments. The selective antibiotic was then added. At about the 20th day, the culture was diluted with a volume of medium D (5) containing the selective antibiotic. Resistant calli were transferred 4 weeks after exposure to DNA onto solid regeneration medium (5). Resistant calli proliferated.

RESULTS

Two presumed transformed plants were regenerated. One of them (PG 20) was analysed at the cellular and molecular levels.

Mesophyll protoplasts have been prepared from plant PG 20 to test kanamycin toxicity (Fig.1). Protoplast-derived cells obtained from this plant were still able to grow in the presence of 100 ug/ml kanamycin, while control cells were killed by 5 ug/ml.

To correlate the kanamycin resistant phenotype to the presence of the APH(3')II-coding sequence, DNA from the resistant regenerated plant PG 20 was extracted and analysed. The genomic DNA of the PG 20 and a control Brutor plant were linearized with SalI, HindIII or EcoRV, analysed by electrophoresis on an agarose gel and transferred onto a hybond-N membrane (Amersham). They have been hybridized with the plasmid pABDI.

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Hybridization was detected in the DNA of plant PG 20, with no corresponding band in the DNA of the control plant. The intensity of the signal suggests that a low number of copies of the plasmid is integrated into the plant genome, but further experiments are neaded to elucidate the exact structure of the transferred DNA.

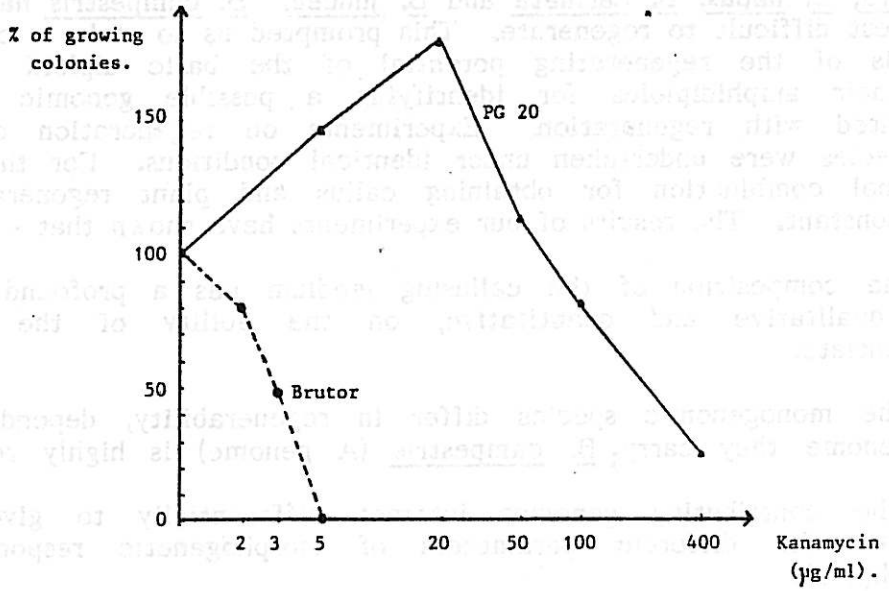

Figure 1: Comparison of the kanamycin toxicity on cells derived from leaves of control Brutor plant or transformed plant (PG 20).

CONCLUSION

The regenerated electropored plant PG 20 is resistant to kanamycin. Electroporation is a suitable technique for direct transfer of foreign DNA in Brassica.

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TISSUE CULTURE STUDIES IN BRASSICA spp.

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In the tissue culture for crop improvement laboratory, attempts are being made to explore the possibilities of improving Brassicas through somaclonally generated variation. Protocols have been developed to differentiate plants from callus cultures with decreasing order of efficacy from B. oleracea, B. nigra, B. napus, B. carinata and B. juncea. B. campestris has remained the most difficult to regenerate. This prompted us to make a comparative analysis of the regenerating potential of the basic diploid progenitors and their amphidiploids for identifying a possible genomic component associated with regeneration. Experiments on regeneration of all the six species were undertaken under identical conditions. For this purpose, hormonal combination for obtaining callus and plant regeneration were kept constant. The results of our experiments have shown that -

1. The composition of the callusing medium has a profound influence, both qualitative and quantitative, on the ability of the callus to differentiate.

2. The monogenomic species differ in regenerability, depending upon the genome they carry; B. campestris (A genome) is highly recalcitrant.

3. The contributing genomes interact differentially to give variable responses for different parameters of morphogenetic response among amphidiploids.

a) Shoot regeneration from primary cultures, an instance of latent bud expression, shows that B. juncea (AB) responded with a frequency higher than the additive response of the donor genomes.

b) The amphidiploids regenerate shoots from dedifferentiated callus cells with a frequency consistently less than the expected mean of the progenitor genomes.

4. The differential response of the regeneration process between the primary explant and the dedifferentiated culture indicates that different mechanisms trigger genetic control of regeneration.

The completed experiments were carried out with natural amphidiploids which have undergone considerable evolution since their origin. To establish the relationships between genomes for their contribution to regeneration, the analysis is being extended to synthetic hybrids between A, B and C genome donors. This approach has yielded significant information on cytoplasmic effects particularly in B. carinata. B. carinata with 'C' cytoplasm shows consistently superior performance over 'B' cytoplasm for shoot morphogenesis. 'B' cytoplasm favours rhizogenesis better than the 'C' cytoplasm. An evaluation of the comparative responses of A, AC and AAC has shown that while C genome can neutralize the inhibitory effect of A in AC, two doses of A genome in AAC is strong enough to suppress the morphogenetic responses of AAC.

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The regenerated plants have been raised to maturity and shall be screened for genetic variation in the coming season. M_2 of chemical and radiation exposed seeds will also be screened for comparison to gain information concerning the efficacy of somaclonal variation vis-a-vis mutagen induced variation.

Acknowledgements:

We thank Scottish Crop Research Institute, Dundee for providing B. napocampestris.

STUDIES OF RESISTANCE TO THRIPS TABACI IN FOUR COMMERCIAL VARIETIES OF CABBAGE

K.A. Stoner and A.M. Shelton

The onion thrips, Thrips tabaci Lindeman, has been a serious problem on cabbage in New York State for the last six years. These insects do direct cosmetic damage to the head, causing rough brown blisters. In previous studies, a wide range of thrips damage to commercial cabbage varieties was observed. We selected four varieties from this range for more intensive study: 'Market Prize' (Harris Moran Seed Co.) and 'Supergreen' (Reed's Seeds), both susceptible varieties; 'Titanic 90' (Ferry-Morse Seed Co.), moderately resistant; and 'Falcon' (Royal Sluis), very resistant.

A two-year field study of the abundance and within-plant distribution of thrips showed that thrips numbers were from 2.8 to 11.5 X higher in the heads of susceptible varieties than in resistant varieties over the period from mid-August to mid-September in both years. To our surprise, however, thrips numbers were lower on the frame leaves of susceptible varieties than resistant varieties in September and not substantially different the rest of the season.

Similarly, in preference tests (conducted in the field by placing uninfested potted plants of the four varieties next to plots of wheat, oats, and alfalfa as thrips adults were moving out of these crops) more T. tabaci adults accumulated in heads of susceptible varieties. However, 'Falcon' frame leaves generally had more thrips than the frame leaves of more susceptible varieties.

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INHERITANCE OF RESISTANCE TO THRIPS TABACI IN CABBAGE

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Based on results from three families (one of which is shown here - Table 1), resistance to thrips damage in cabbage appears to be quantitatively inherited, with susceptibility dominant (F_1 damage ratings approach those of the susceptible parent in all three cases). Tests of generation means also indicate that epistasis may be involved.

In previous work, it has been shown that T. tabaci populations build up in spring on wheat, oats and alfalfa, and colonise cabbage as these crops senesce or are cut. To ensure adequate thrips pressure for selection, we recommend planting one of these crops upwind from the test plot, and planting the cabbage early so that developing heads will be available when thrips leave the grain or alfalfa.

Table 1. Ratings of Thrips tabaci damage to cabbage heads in one family resulting from a cross of resistant and susceptible inbreds (0.0 = no damage, 4.5 = very severe damage)

	Damage Rating												
Generation	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	No.	Mean	Variance
P_1		2	11	5							18	1.08	.096
P_2							5	11	3		19	3.45	.11
F_1(recip.)						1	3	16			20	2.88	.076
F_1				1		7	11	1			20	2.78	.17
F_2		4	8	11	8	18	23	11	16	15	114	2.86	1.28
B_1		4	20	21	7	6	6	6	1		71	1.77	.79
B_2			3	1	6	12	21	20	7		70	2.96	.51

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BASKING BEHAVIOUR OF ATHALIA PROXIMA (KLUG)

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The mustard sawfly, Athalia proxima is one of the serious pests of rapeseed mustard attacking at the seedling stage of the crop in northern India. The grubs are seen damaging the crop during day time and are most active at certain period. In the present study basking bahaviour of this insect was studied.

Brassica campestris var toria crop was sown on 8 th of October, 1983 and observations were recorded on 20 days old crop on the number of larvae in a meter row length starting from 6.00 AM (dawn) to 5.00 PM (dusk). Larvae were counted at half an hour interval at 5 places for 3 days regularly. Average sunshine period was 8 hours starting from 8.00 AM to 4.00 PM with maximum temperature of 23.5 °C.

Grubs started comming out from the crevices and cracks in the soil at 6.30 AM and their number started increasing till 10 to 10.30 AM and then population started declining. At this time most of the larvae were seen on the top of the plant voraciously feeding on the leaves. After 10.30 AM larvae started descending and between 12 to 3.00 PM very few larvae were seen on the aerial parts. Larvae started appearing again after 3.30 PM and reached to maximum number at 4.30 PM and all the larvae disappeared at dusk. The increase in grubs population during 10 to 10.30 AM could be due to rise in temperature of the atmosphere, reaching optimum at 10 to 10.30 AM, but decline in population may be attributed to further rise in temperature and again maximum population when temperature became optimal in the after noon. The number declined again with further lowering of atmospheric temperature. The number of larvae on the plants was more in the fore noon as compared to the after noon. On cloudy days very few larvae were seen.

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INFLUENCE OF SOIL TYPE ON PUPATION OF ATHALIA PROXIMA

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The mustard sawfly, Athalia proxima (Klug)(Tenthredinidae: Hymenoptera) is a well known oligophagous pest defoliating crucifer seedlings in northern India. Full grown grub enters the soil for pupation. The present experiment was conducted to find out the suitability of various soil series of order Mollisol (Deshpande et al, 1971) for its pupation and adult emergence.

Field collected grubs were reared on mustard leaves in glass jars (21 x 15 cm) containing sterilized moist sand. The adults thus obtained were transferred to oviposition cages containing mustard seedlings. Newly hatched larvae were taken out and reared on mustard leaves. The last instar grubs were used in the present investigation. They were transferred to jars containing 1 Kg of respective sterilized test soils having 5 and 10 per cent moisture levels (table 1). Each test soil was replicated three times and 10 grubs were used in each replication. The experiment was conducted under laboratory conditions. Observations were recorded on pupation on 9 th day of descending into soil, adult emergence and pupal period which includes pre-pupal period also. Data were subjected to analysis of variance in completely randomized design.

Perusal of Table 1 indicates that per cent pupation differed significantly in various test soils being maximum on sandy soil and minimum on Phool bagh clay loam and Beni silty clay loam at 5% soil moisture level. Similar trend in pupation was also obtained in various test soils at 10% moisture level. In the same soil type no difference was observed between pupation at 5 and 10% moisture levels. Similarly adult emergence was also highest on sandy soil and lowest on Beni silty clay loam at 5 and 10% moisture levels. Pupal period differed significantly on different soils being maximum on Beni silty clay loam and Phool bagh clay loam and minimum on sand at both the moisture levels.

Based on above parameters, it is clear that light soils are more suitable for post larval development of this insect as compared to heavy soils. Various soils tested can be arranged in order of their suitability as follows

Sandy Khamia sandy soil Patharchata sandy loam Haldi loam Nagla loam Phool bagh clay loam Beni silty clay loam

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Table 1. Per cent pupation, adult emergence and pupal period of A.proxima at 5 and 10 per cent moisture level in different soils

Treatments	% pupation		% Adult emergence		Pupal period (days)	
	Moisture levels		Moisture levels		Moisture levels	
	5%	10%	5%	10%	5%	10%
Khamia sandy soil	86.66	90.00	66.66	70.00	11.66	11.83
Nagla loam	76.66	80.00	60.00	53.33	11.50	11.66
Haldi loam	73.33	76.66	53.33	56.66	11.66	11.00
Patharchata sandy loam	83.33	80.00	63.33	60.00	11.50	11.50
Phool bagh clay loam	66.66	70.00	46.66	50.00	13.66	13.16
Beni silty clay loam	66.66	66.66	43.33	43.33	13.66	13.16
Sand	90.00	96.00	80.00	76.66	12.00	11.33
SEM ±	2.85	3.19	2.33	2.64	0.21	0.24
LSD at 5%	8.66	9.68	7.07	7.91	0.61	0.73

These are soil series of order Mollisol
Deshpande, S.B., Fehrenbacher, J.B. and Ray, B.W. 1971. Mollisols of tarai region of Uttar Pradesh, Northern India, 2. Genesis and classification. Geoderma 6(3): 195-202.

PREFERENCE OF BRASSICA SPECIES INFLORESCENCE BY ALATE LIPAPHIS ERYSIMI KALTENBACH

G.C. Sachan and Sumati Sharma

The mustard aphid, Lipaphis erysimi Kaltenbach, is an important sucking pest of rapeseed mustard in northern India. Since the inflorescence of these crops is the main site of aphid settlement on arrival, the present study was conducted to find out the preference of various Brassica species to alate aphid.

Vials containing inflorescence of different species/varieties of test plants, the cut ends of which were immersed in water, were arranged at random and placed at equidistance in the circumference of a circle having a radius of 70 cm. In the centre a vial containing a bunch of stalk inflorescence of B. campestris var toria completely covered with matured apterous/alate aphids was kept for regular supply of winged aphids. Inflorescence of the same size were used in the experiment. The entire set up was covered with an 80 mesh wire cage. After 24 hrs the number of alate aphids resting on each stalk was counted and the inflorescence was replaced with fresh one. This was continued for six days. The treatments were replicated three times.

The number of winged aphids recorded on different Brassica species and their varieties differed significantly (Table 1). It is evident that B. juncea and Sinapis alba were the most preferred and B. napus, B. carinata, B. nigra and B. alba the least preferred species. Amongst the varieties of B. juncea, Porbiraya was most preferred followed by PR 18 and Varuna. In B. carinata and B. napus there was no difference amongst the varieties.

Table 1. No. of Lipaphis erysimi (alate) on inflorescence of different species/varieties of Brassica

Species/varieties	Cumulative no. of winged aphids (total of six days)	Species/varieties	Cumulative no. of winged aphids (total of six days)
Brassica alba	6	Brassica napus	
Brassica carinata		Altex	3
PC 1	3	Olivia	2
PC 2	5	Pant n 1	2
Brassica juncea		Pant n 2	4
Blaze	11	Tilde	4
Lethbridge	14	Trawse	2
PR 15	9	W W 1313	2
PR 18	19	Brassica nigra	5
Porbiraya	23	Sinapis alba	22
Stoke	7		
Varuna	18		
	SEM±		1.3
	LSD at 5%		5.9

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EFFECT OF SOME FUNGICIDES AND BORIC ACID SPRAYS ON THE CONTROL OF IMPORTANT DISEASES OF TORIA

Vishwanath and S.J. Kolte

Toria (Brassica campestris var toria) is affected by Alternaria blight, white rust and downy mildew diseases when the toria is sown late beyond second week of October in the Nainital Tarai region of Uttar Pradesh. The efficacy of spray of six fungicides viz, mancozeb (0.2%), captafol (0.2%), mancozeb + metalaxyl (Ridomil MZ, 0.05%), ziram (0.2%), iprodione (.2%), topsin-M (0.05%) and that of boric acid spray (0.53%) were tested under field conditions for the control of the above diseases using toria cultivar 'PT 303'. The sowing was done on 1 November 1985 and randomised block design with four replications was followed. The gross plot size was 3m x 2m. The row-to-row and plant-to-plant distance was 30 cm and 15 cm, respectively. Each fungicide and the boric acid were sprayed three times beginning from 45 days after sowing at 10 days interval using the foot-sprayer.

The results revealed that iprodione was superior to mancozeb, captafol and ziram in the control of Alternaria blight and gave significant increase in yield as compared to check (Table 1). Mixture of metalaxyl + mancozeb gave complete control of staghead phase of white rust and downy mildew. It was interesting to note that boric acid spray also controlled Alternaria infection on pod and resulted in significant increase in yield as compared to unsprayed plants (Table 1). Thiophanate-methyl was not effective.

Table 1. Effect of some fungicides and boric acid sprays on the control of Alternaria blight, white rust and downy mildew diseases of toria

Spray treatment	Alternaria blight: Disease index (Arcsin values)		Staghead: (Arcsin values)		1000 seed wt (g)	Yield/ plot(g)	Yield/ha (q)
	On leaves	On pods	Incidence	Severity			
Mancozeb (.2%)	27.82	43.04	8.46	11.10	3.22	500	13.33
Captafol (.2%)	27.76	33.17	16.48	19.86	3.20	460	12.26
Mancozeb + metalaxyl (Ridomil MZ.05%)	28.88	45.44	0.00	0.00	2.86	503	13.41
Ziram (.2%)	27.10	43.01	15.36	17.72	2.87	503	13.41
Iprodione (.2%)	21.32	13.39	9.56	13.55	3.26	590	15.73
Thiophanate methyl (.05%)	31.92	62.82	17.53	22.19	2.84	345	9.20
Boric acid (0.53%)	27.63	27.25	14.69	21.77	3.18	433	11.54
Check (unsprayed)	31.62	52.93	17.78	24.26	2.19	315	8.40
CD at 5%	4.75	13.26	8.43	10.35		117	

Net plot size = 3.75 sq m

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ROLE OF ENVIRONMENT AND CULTIVAR INTERACTION ON THE EPIDEMIOLOGY OF WHITE RUST OF MUSTARD.

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White rust caused by Albugo candida (Pers.ex Lev.) Ktze has become wide spread and most destructive disease of mustard (Brassica juncea) in India. The incidence and severity of the disease on foliage and floral parts has increased tremendously during the last few years taking heavy toll of the crop. Environmental factors such as relative humidity, temperature, rainfall, dew and wind velocity play important role for the initiation, development and spread of white rust on radish and spinach (Glaeser, 1971, Raabe and pound, 1952). The present paper includes studies on the progress of disease in relation to prevailing environmental factors such as humidity, temperature and rainfall on different cultivars of mustard grown under field conditions.

To study the progress of white rust in relation to weather variables, mustard cultivars namely 'Varuna','RH-30', 'Prakash', 'RC-781' and 'YRT-3' were selected. The experiment was conducted in three replicated trial in 1.5 x 4 m plot size during rabi, 1983 at Haryana Agricultural University Farm, Hisar, India. Five leaves from each 10 randomly selected plants were tagged in each plot just before the appearance of the disease. The initiation and progress in the number of pustules were recorded at an interval of 7 days. The weather variables Viz. mean relative humidity, temperature and rainfall prevailing during the period of observation were recorded to correlate with the white rust development on different cultivars of mustard.

The data in fig-1 indicate that in all cultivars number of white rust pustules increased with the passage of time. The increase in number of pustules was maximum from January, 27th to February 10, 1984. The weather conditions during this particular period were, mean temperature 11.23 to 12.5 ^{o}C, mean relative humidity 57 to 60 per cent and wind velocity at the rate of 4.5 to 8.0 km/hr. On the other hand when the observations were recorded on 17th February, 1984 the increase in number of pustules was very less because of increase in mean temperature during the last week. However, again there was sharp increase in the number of pustules when the observations were recorded on 24th February, 1984 since there was high relative humidity and rainfall during the last week. After 2nd March 1984, number of pustules decreased since there was increase in temperature. The average increase in number of white rust pustules was highest in case of cultivar 'Prakash' (16.0) followed by Y R T -3 (3.2) and was minimum in cultivar RC-781 (1.1). It indicates the inherent susceptibility/resistance level of these cultivars against white rust.

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It is evident that last week of January to first fortnight of February, 1984, the weather variables were most congenial for the progress of white rust. Saharan et.al. (1980) reported that white rust pustules on Indian mustard increased at faster rate during 2nd and 3rd week of January when mean temperature was less than 12°C with mean relative humidity of 68 percent and 2.7 to 3.4 km/h wind velocity. The present findings are in accordance with the findings of Saharan et.al.(1980).

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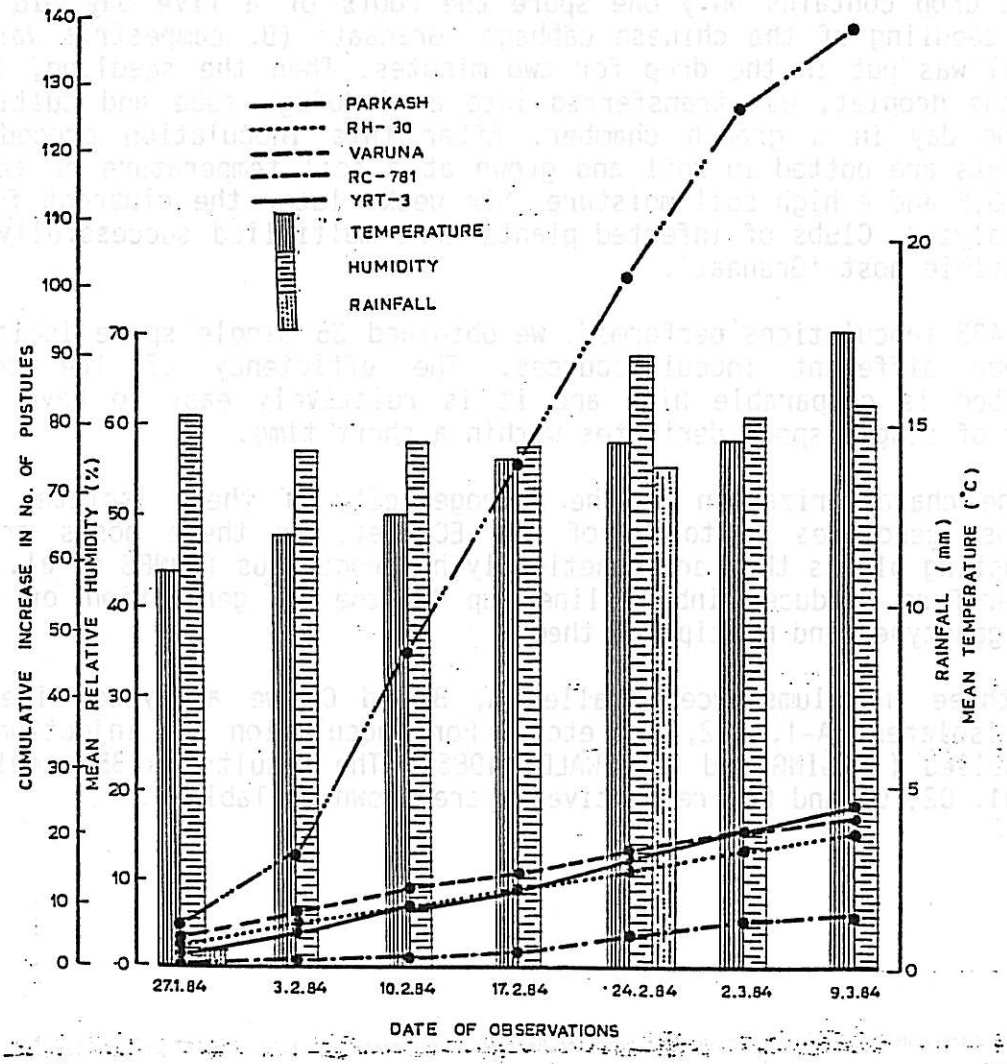

Fig.1. INCREASE IN NUMBER OF WHITE RUST PUSTULES ON RAYA CULTIVAR IN RELATION TO WEATHER VARIABLES

Production and characterization of single spore derived lines of P. brassicae Wor.

M. Schoeller and J. Grunewaldt

Clubroot, the most dangerous disease of crucifers, is caused by Plasmodiophora brassicae Wor. Populations of P. brassicae consist of a mixture of pathotypes in the soil, and in the roots of plants, as well (JONES et al. 1982a). These pathotypes show a differential pathogenicity when tested on a differential set.

The commonly used differential set is the ECD-Set (BUZACKI et al. 1975). These differentiation can only yield comparable results when both, the homogenicity of pathotypes and of hostgenotypes is given. The homogenicity of P. brassicae can be achieved with single spore derivates. Methods described so far for producing such single spore isolates of P. brassicae are laborious and with low efficiency (BUCZACKI 1977, JONES et al. 1982b, SCOTT 1985, TINGGAL and WEBSTER 1981).

In this paper an easier and more efficient technique for producing single spore derivates and the characterization of some of these isolates are described.
The following method for the inoculation was used:
One drop of a spore suspension, containing about 10^2 spores/ml, was given on a cover slide using a micropipette. The cover slide was put on a plexiglas slide with a hole in the middle. With an inversion microscope and a magnification of 1.000 the number of spores per droplet can be observed. If the drop contains only one spore the roots of a five day old sterile grown seedling of the chinese cabbage 'Granaat' (B. campestris var. pekinensis) was put in the drop for two minutes. Then the seedling, together with the droplet, was transferred into a microfuge tube and culti- vated for one day in a growth chamber. After this inoculation procedure the plantlets are potted in soil and grown at a soil temperature of +23° C, a pH of 5,5 and a high soil moisture. Six weeks later the clubroot formation was analyzed. Clubs of infected plants were multiplied successfully in the susceptible host 'Granaat'.

From 435 inoculations performed, we obtained 35 single spore isolates out of four different inoculumsources. The efficiency of the technique described is comparable high and it is relatively easy to have a large number of single spore derivates within a short time.

For the characterization of the pathogenicity of these isolates we used the host genotypes 01 to 10 of the ECD-Set. As these hosts are cross pollinating plants they are genetically heterogenious (JONES et al. 1982b). We therefore produced inbred lines up to the S_4 generation of each of these genotypes and multiplied them.

From three inoculumsources, called A, B and C, we analyzed nine single spore isolates (A-1, A-2, A-3 etc.). For inoculation the injection method was applied (THESING and GRUNEWALDT 1985). The results in 35 seedlings of host 01, 02, 03 and 04, respectively, are shown in Table 1.

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Table 1: Percentage of diseased plants in inbred lines of ECD hosts 01-04 and P. brassicae-single spore isolates A-1, A-2, A-3 etc. from the original populations A, B and C.

Inbreedline of ECD-host	A	A-1	A-2	A-3	B	B-1	B-2	B-3	C	C-1	C-2	C-3
01	65,0	47,4	15,0	57,9	30,0	15,0	76,5	57,9	89,5	85,0	90,0	52,9
02	0,0	0,0	0,0	0,0	0,0	20,0	94,8	18,7	100,0	5,3	57,9	5,0
03	30,0	31,6	30,0	70,6	44,0	38,9	73,3	47,6	100,0	72,2	63,1	56,2
04	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	80,0	0,0	0,0	0,0

There are great differences between the reaction of the orginial population and the single spore isolates and within the single spore isolates. Some of the isolates differ considerably from the orginal host and from each other. For example, isolate B-2 and C-3 react with a higher or lower infection as compared with the original population.

The population C is able to overcome the resistance of the host 04. None of the single spore lines tested contains the respective virulence gene. Population B could not infect the host 02 but all single spore isolates did infect this genotype. To explain this it might be possible that the clubs used for the production of single spore lines contained the pathotype able to overcome the resistance of host 02. This pathotype was then not present in the spore suspension of the original isolate used in this test. The inbred lines of the genotypes 06 to 10 and the host 05 were susceptible to the three original populations A, B, C, and to all single spore isolates derived from these populations.

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INTERRELATIONSHIP BETWEEN SAND AND MANURE MEDIA TO REDUCE CLUBROOT DISEASE

I Djatnika

Clubroot caused by Plasmodiophora brassicae W. on cruciferae is one of the important disease in Indonesia. The development of the disease is affected by enviromental conditions, as soil moisture, soil structure, pH, and concentration of inoculum.

This research was carried out from January 1986 until April 1986 in glasshouse of Segunung Horticultural Research Station, West Java, Indonesia. The research used the Completly Randomized Design with 5 treatment combinations of mixture of sand and manure, in 6 replication. The mixture was artificially inoculated with P. brassicae spore (3×10^5 cell/g of dry mixture) and planted with K-K Cross cabbage.

Observations consist on measuring of the clubroot disease index, number of diseased plants, and the leaf wet weight.

The degree of infection was rated on a 0 to 3 scale, where 0= no infection, 1= very slight swelling, usually confined to lateral roots, 2= moderate swelling on lateral and/or tap roots, 3= severe swelling on lateral and/ or tap roots (Buczaki et al., 1975).

The treatments with 100% sand + 0% manure (A) and 75% sand + 25% manure (B) reduced disease intensity, and % diseased plant, and the leaf wet weight the B treatment was the heaviest (Table 1).

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Table 1. Intensity of clubroot disease, % diseased plants, and weight of cabbage leaf (8 weeks)

Sand : manure (v/v)	Intensity of disease (%)	% diseased plant	Weight of leaf (%)
100 : 0	11.1 a	16.7 a	2.9 a
75 : 25	33.2 b	56.7 b	17.4 b
50 : 50	67.7 c	83.3 bc	14.0 bc
25 : 75	72.0 c	100.0 c	12.4 c
0 : 100	78.3 c	100.0 c	12.4 c

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

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A TECHNIQUE FOR ARTIFICIAL INOCULATION OF CURD STAGE OF CAULIFLOWER WITH SCLEROTINIA SCLEROTIORUM

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Sclerotinia sclerotiorum (Lib.) de Bary has caused significant losses to cauliflower seed growers along the northern part of India since at least 1970. In recent years there has been a drastic decrease in cauliflower seed production because of the erratic success of protective chemicals and lack of resistant varieties. The high cost and threat of development of fungicide-resistant strains of the pathogen indicates that resistance should be investigated. However, the success of a breeding programme depends upon having a successful artificial inoculation technique. A classical technique to create infection at seedling stage to screen germplasm against S. sclerotiorum has been reported by Kapoor et al., 1985. A technique to screen the material at curd stage, however, has not been available so far. As reported here, a technique has now been developed to screen germplasm successfully at the curd stage in order to substantiate the results of seedling stage screening.

In this technique partial colonisation of petals of a member of the cruciferae was accomplished as described by Kapoor et al., 1985. Such pre-colonised petals, with the help of a drop of sterilised water, were placed on a fresh cauliflower curd kept in a humid chamber at 25 $^{+}$-1°C. Inoculated curd was colonised within 7-10 days and subsequently served as a primary source of inoculum for screening the standing crop in the field. Cauliflower curds undergoing screening were inoculated in the centre each with a small bit (1x1 mm) of previously colonised curd and were mist sprayed immediately after inoculation. Sufficient humidity was also maintained by irrigation plots before and after inoculation. Loosening of curds followed by the silvery white appearance of hypertrophic branches was evident after 7-10 days of inoculation under cool and humid conditions. After 15-20 days the entire curd was putrified and failed to bolt exhibiting numerous black sclerotial bodies. This simple technique will greatly help to screen the germplasm at adult stage and will confirm the resistance exhibited at seedling stage.

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ABSCISIC ACID (ABA): A NEW GERMINATION INHIBITOR FOR TESTING CRUCIFER SEED INFECTED WITH PHOMA LINGAM

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Blackleg of cruciferous crops is caused by P. lingam (Tode ex Schw.) Desm. teliomorphic stage: Leptosphaeria maculans (Desm.) Ces. and de Not. Its importance, epidemiology and control have been discussed by Gabrielson and Maguire (1977) and more recently by Gabrielson (1983).

The recommended International Seed Testing Association (ISTA) procedure for determining the incidence of P. lingam in crucifer seed calls for examination of seed and blotters for typical pycnidia and conidial ooze after 10-11 days incubation at 20°C on blotters wetted with 0.2% 2,4-D solution. The new proposed test is similar to the ISTA procedure, except ABA is used. A naturally infected cabbage seed lot previously assayed at 5% P. lingam was used in this trial.

The relative proportions of cabbage seed yielding P. lingam when tested on 2,4-D and ABA substrates are presented in Table 1. Abscisic acid at 100 mg/l gave higher P. lingam incidence than the sodium salt. Thiram applied together either with ABA or 2,4-D substantially reduced the percentage of P. lingam but did not eradicate it, indicating that there is no antagonism between fungicide and ABA.

Increased concentration of ABA up to 200 mg/l did not reduce the incidence of P. lingam and at any ABA concentration the percentage of P. lingam was higher than 2,4-D treatment (Table 2). However, no germination was obtained with ABA at 200 mg/l while 2,4-D treatment was not effective in inhibiting seedling emergence. The data suggest, therefore, that the most feasible concentration of ABA is 100 mg/l.

The advantages of this new seed assay are: a) ABA is totally soluble in water and using low concentration eliminates the risk of laboratory contamination; b) seed pre-treatment with sodium hypochlorite to reduce saprophytic contaminating fungi is not necessary; c) ABA is not toxic to P. lingam and d) ABA is available worldwide with no difference in formulations.

Suggestions have been made to the working group on seed-borne diseases of crucifers of the ISTA Plant Disease Committee to compare this method with the presently recommended 2,4-D procedure.

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Table 1. Percentage of cabbage seed with P. lingam using different seed assay treatments

Seed assay treatment	Inhibitor conc. mg/l	Percentage of P. lingam at 6 days	at 11 days
ABA	100	6.8 b	7.2 b
2,4-D	2000	5.4 b	5.4 ab
ABA + .2% Thiram	100	2.6 a	3.1 a
2,4-D + .2% Thiram	2000	2.2 a	2.6 a

Table 2. Percentage of cabbage seed with P. lingam and percentage of germination with different concentrations of ABA and the standard 2,4-D assay

Seed assay treatment	Inhibitor conc. mg/l	Percentage of P. lingam at 6 days	at 11 days	Percentage germination
2,4-D	2000	4.2 a	5.0 a	98 a
ABA	50	5.0 a	6.2 a	24 b
ABA	100	4.6 a	6.0 a	10 c
ABA	200	4.8 a	6.2 a	0 d

Means within columns followed by the same letter are not statistically different at 5% level by Duncan's Multiple Range Test

THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON INFECTION OF OILSEED RAPE LEAF TISSUES BY ALTERNARIA BRASSICAE AND ALTERNARIA BRASSICICOLA

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Severe leaf spot disease of brassicas caused by *Alternaria brassicae* or *A. brassicicola* is associated with humid conditions or wet years. However, it is generally reported that the optimum temperature for *A. brassicicola* infection is higher than for *A. brassicae*. In this paper the results of one experiment to study the effect of temperature and another to study the effect of atmospheric humidity on *Alternaria* infection of oilseed rape are reported. In the two experiments leaf discs (12 mm diameter) were sampled from the 6-7th leaf of 7-8 week old plants of two cultivars, Jet Neuf and Rafal, and maintained on 0.5% water agar containing 80 ppm benzimidazole. Five separate isolates of each *Alternaria* species were used to provide inoculum, and spore dosage rates were about 1250/leaf disc. The experiments were designed in a randomised block lay-out with four replicates. In the first experiment five different temperatures over the range 5° to 30°C were tested. Inoculum was applied in a 0.025 ml droplet of water, containing 0.1% Tween 21, to the centre of each disc: the discs were incubated in darkness in plastic chambers which were closed to maintain a high humidity. Following incubation for 5 days disease development was assessed on a 0-5 scale (1 = scattered specks; 5 = large spot covering most of disc). In the second experiment inoculum was applied either as a 0.025 ml water droplet ('wet' spores) or as a similar droplet which was carefully taken up in blotting paper leaving the spores ('dry' spores) dispersed on the disc. The inoculated leaf discs were incubated in desiccators at five different humidities over the range 56 to 98% RH. The incubation temperature was 20 ± 2°C, with a 12 hour light period alternated with 12 hours darkness for 5 days, after which disease assessments were made. In both experiments no significant effects were associated with cultivar or isolates within species.

Both *Alternaria* species showed their greatest lesion development at 25°C when large spots were formed: infection occurred to a declining level at lower temperatures down to 5°C when disease indices were very small, while at 30°C intermediate levels of infection occurred. Below 25°C, *A. brassicae* produced larger lesions than *A. brassicicola* but at 25°C and above *A. brassicicola* showed the higher indices. Observations by other workers have shown temperature optima of 19-23°C and 23-25°C (Degenhardt et al., 1982) or 15 and 25°C (Humpherson-Jones and Hocart, 1983) for *A. brassicae* and *A. brassicicola* infection respectively. In earlier reports Van Screven (1953) had noted that 20-24°C gave maximum disease development for *A. brassicae* and Domsch (1957) considered 21-27°C as the optimum temperature range for *A. brassicicola* infection. Differences in the findings of different workers may be in part explained by the different hosts used, and it was observed in this work that temperatures affected rate of leaf disc yellowing independently of inoculation. However, the results are in keeping with a general impression that at temperatures below 25°C conditions are more favourable for *A. brassicae* than for *A. brassicicola*, which was more favoured at temperatures of 25°C or above.

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Although several workers (Rangel, 1945; Domsch, 1957; Louvet, 1958; McDonald, 1959; Jorgensen, 1967; Humpherson-Jones and Hocart, 1983) have reported that *Alternaria* pathogens require wet or humid conditions for disease development, in these studies it was observed that leaf spotting, while slight at low humidities, was seen over the full range of humidities tested down to 56% RH: Karwasra and Saharan (1983) reported a progressive development of *A. brassicae* leaf spotting on *Brassica pekinensis* over a 3 month period where the relative humidity fluctuated within the range of 55-75%. In comparing the two *Alternaria* species on oilseed rape leaf discs it was found that the form of inoculum influenced their responses to differences in humidity. With 'dry' spores both fungi produced substantially greater lesion development at higher humidities, the maximum, in the form of large spots, occurring at 98% RH. However, with spores applied in undisturbed droplets the two species showed a differential response: with *A. brassicae*, disease indices increased slightly with increasing humidity but lesion development of *A. brassicicola* was less at higher humidities. Leaf spotting in general was more restricted with 'wet' spores and this may be associated with self-inhibitory factors which accumulate when spores are clustered together in a small droplet. Mukadam (1982) reported that self inhibition of spore germination of *A. brassicicola* was associated with increased spore load; it is possible that spores of *A. brassicicola* exhibit greater self inhibition than those of *A. brassicae* from the results of this present study. A further factor may relate to physical properties of spores which impede their effective contact with leaf surfaces. Royle (1976) indicated that the capacity of the inoculum itself to be wetted and the effect of spores on the surface tension of water drops containing them can influence the degree to which plant surface wettability contributes to disease resistance. The differential response of *A. brassicae* and *A. brassicicola* inoculum in water drops to increasing humidity may be due to higher humidities prolonging the period of intact droplets, intact droplets being assumed to interfere with infection, and the two species possessing spores with different abilities to lower the surface tension of water drops.

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ALTERNARIA OLERACEA MILBRAITH, A PROBLEM FOR THE SEED PRODUCTION OF EARLY CAULIFLOWER VARIETIES

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Introduction

Cauliflower is one of the important winter vegetables in Turkey. Total production area and amount are 4000 ha and 65,000-70,000 tonnes per year, respectively (1). Curds are susceptible to extreme climatic conditions like low temperature and heavy rains in the periods when they complete their maturation for market. This event is also of great importance for seed production. Obtaining good seed yield is difficult because of curd rotting caused by low temperature and heavy rain. This problem is observed clearly on the early varieties maturing in the autumn months.

During some experiments on the control of this curd a lot of brownish-black, dry and sunken, scattered lesions were observed in December 1985 on the curds of variety 'Brio osenia'. The curds showing these symptoms formed no flower stalks.

This paper reports studies to identify the causal organism of the symptoms on the cauliflower curds.

Materials and Methods

Isolations were made from the variety 'Brio osenia' and pathogenicity tests were done on plants of 'Matra'. A filter paper technique was used to induce sporulation (2). Petri dishes were then kept at 24°C in the dark for 3 days. The inoculum concentration used in the pathogenicity tests was 80,000 spores/ml. After inoculation the curds were covered by polyethylene bags and kept at 24°C in a growth chamber.

Results

In pathogenicity tests the first symptoms occurred on 'Matra' curds 4 days after inoculation. Isolations from these lesions revealed the presence of the same fungus. The pathogen was identified as Alternaria oleracea Milbraith, by the morphological characteristics of the spores and of the colonies on agar medium.

The conidia averaged 22.7 µ long, 9.67 µ thick with a beak length of 4.2 µ, i.e. approximately 1/5 the length of the conidium.

Discussion

As reported before (3,4,5) the conidia of A. oleracea are 18-130 µ long, 8-30 µ thick and have a beak 1/6 the length of conidium. The colonies of the fungus are dark olivaceous brown to dark blackish brown in colour, velvety, smooth and effused. These morphological features were also recorded on our isolate used in this study.

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In the next studies it is planned to investigate the relation between disease severity and growing technique in the field and also in greenhouses with or without chemical control of the pathogen.

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CORRELATION AND REGRESSION ANALYSES OF MAIN QUALITY CHARACTERS OF RAPESEED (BRASSICA NAPUS L.)

Zejing Tang and Guangwei Yang

Correlation and regression analyses of major fatty acid components, oil content, protein percentage, glucosinolate content, sum of oleic and linoleic acid and sum of oil and protein were investigated in 51 strains with low erucic acid (less than 3.5%)

The results obtained showed that the percentage of erucic acid and glucosinolate content had no significant correlation with other characters, which indicated that further selection for double low and double zero strains did not affect other quality characters. Linoleic acid showed a strong negative correlation with oleic acid ($r=-0.8046$), but it had a strong positive correlation with linolenic acid ($r=0.5258$). Selection for sum of oleic acid and linoleic acid can improve oil quality effectively. Although the oil content showed a strong negative correlation with protein content ($r=-0.5357$), selection for sum of oil and protein can change the association between them and increase their contents simultaneously.

In low erucic acid population, selection for sum of oleic acid and linoleic acid and sum of oil and protein may be more effective in breeding programme than selection for any character alone.

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EFFECT OF PHYLLODY ON YIELD AND YIELD COMPONENTS OF TORIA

C.D. Kaushik, P.P. Gupta and G.S. Saharan

Toria (Brassica campestris var toria) being a short duration and low cost input for cultivation is an important catch crop suitable for multiple cropping situation in India. Although phyllody has been reported on most of the Brassica crops but its damage is more on toria crop (Kaushik et al., 1978). In the present paper an attempt was made to assess the effect of different intensities of phyllody infection (mycoplasma like organism) on the yield and yield components of toria.

To find out the losses caused by this disease with different intensities of infection, 25 plants of each i.e. 25, 50. 75 and 100% disease intensity along with healthy plants were selected from recommended toria variety 'Sangam'. The data on different yield components like plant height, number of primary and secondary branches, number of pods/plant, pod length, number of seeds/pod, seed weight of 25 plants and oil percentage were recorded. It is evident from Table 1 that plant height was reduced from 124 to 110 cms due to phyllody infection. Number of secondary branches increased in diseased plants with the increase in intensity of infection. The number of pods was reduced from 46.4 in healthy to 7.7/plant in plants where disease intensity was 100%. There was drastic reduction in pod length also. No seed was formed in 100% infected plants in comparison to 13 seeds/pod harvested from healthy plants. Seed yield/plant was 8.3 gm and 0.0 gm in healthy and 100% diseased plants respectively. Oil percentage was reduced from 42% in healthy to 24% in plants with 75% infection due to small size of the grains in diseased plants. In grain yield the losses were recorded as 7.2, 57.8, 65.0 and 100% at disease intensity levels of 25, 50, 75 and 100% respectively.

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Table 1. Effect of phyllody on yield and yield components of Toria

Yield components	Phyllody Intensities (percent) (0)	(25)	(50)	(74)	(100)	CD at 5%
Plant height (cm)	124.0	118.7	114.0	109.7	110.0	
No. of primary branches	7.2	7.3	6.8	7.2	6.8	
No. of secondary branches	9.5	16.8	15.0	16.7	21.7	5.24
No. of pods/plant	46.4	37.0	34.8	28.8	7.7	6.95
Pod length (cm)	4.2	4.0	3.4	3.4	0.6	
No. of seeds/pod	13.0	12.7	11.0	11.0	0.0	
Seed wt/plant (gm)	8.3	7.7	3.5	2.9	0.0	
Oil percentage	42.4	35.3	29.0	24.0		

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NITRATE CONTENT IN GREEN MATTER OF SOME SELECTED BRASSICA FORMS

Waleria Młyniec, Halina Blaim, Michał Płoszyński

Investigations of thirty different forms of Brassica napus, B.campestris /ssp.oleifera, rapifera, chinensis, pekinensis, japonica/ and B.juncea obtained thanks to the curtesy of Dr.R.T.Opeña from the Asian Vegetable Research and Development Centre Shanhua, Taiwan, were carried out. The aim of this work was the consideration of their value as initial material for breeding of new fodder forms. As the forms investigated are used in Far East as food for man - it was expected that their feeding value will be high and it would be reasonable to include them to the programme of breeding forms used as fodder for animals.

The pots and field experiments were carried out. The quality examinations concerned such features as: green matter yield, DM yield and per cent of DM in green matter. The determination of glucosinolate content in green matter was conducted by Wetter and Astwood analytical method. Nitrate content, most important quality feature investigated was determined in green matter of plants collected in two harvest dates: beginning of buds formation and full flowering stage, by the methods with phenylotwosulphinide acid and colorimetry /the results were similar/.

Nitrate content in DM in the first harvest was much higher than in the second one. Mean content in DM in the first harvest for B.campestris forms was 0.190 and for the second - 0.099, while for B.napus 0.403 and 0.130 per cent respectively. There were great differences among forms investigated within species. As the experiments were conducted in the controlled environmental conditions one may assume, that the differences were mainly of genotypic character. Differences in nitrate content in green matter between two harvest dates depends /as it is known/ on the enzyme /nitrate and nitrite reductase/ activity. It was found, that the smallest differences between two harvest dates in the amount of nitrate was found in the plant forms which rate of growth was very slow /they did not come to flower after 91 days of vegetation/. The greatest differences were found in forms which at that time come to the stage of full ripeness.

On the example of forms investigated this relationship was remarcably simple and it was found of great importance from the point of view of evaluation of initial material for breeding new Brassica fodder forms.

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FACTORS AFFECTING VARIABILITY OF SMCO IN KALE

R.C. McDonald

High S methyl-cysteine sulphoxide (SMCO) levels are a major problem in kale (Brassica oleracea). Kale is often the highest yielding brassica during autumn and winter, yet its use is restricted by SMCO poisoning or kale anaemia which reduces animal growth and often causes animal deaths. SMCO levels can vary markedly between fields and in different years (Whittle et al 1976). This was confirmed in eight recent field trials by the author in south east Scotland, where whole plant SMCO levels ranged from 6.6 to 20.6g/kg DM at different sites. This inter-trial variation was much greater than within trial variations between various treatments. These recent trials were part of a study examining factors affecting SMCO in kale, and the purpose of this article is to summarise the important aspects causing the large variability in SMCO between kale crops.

1. CLIMATE

Effects of Frosting:

As SMCO levels generally increase throughout the season at the same time as temperatures become lower and frosts more frequent, it has been a widely held view that frosting enhances SMCO toxicity (Whittle et al 1976).

The effects of temperature on SMCO were examined in two trials in different years using Maris Kestrel kale plants transplanted from the field. In trial A plants were either frosted at -6°C in growth cabinets for 1 or 6 days; kept at 12°C for 1 or 6 days; or frosted for 1 or 6 days followed by 1 day at 12°C. In trial B plants were kept for 6 days at either -6°C, 2°C or 15°C. In trial A, only young leaf samples (within 2.5cm of growing point) were harvested and analysed, while in trial B, entire leaf and stem samples were processed.

The following table shows the effects of the treatments on SMCO levels (g/kg DM).

Trial A (Young Leaves)				Trial B			
	Length of Treatment				Plant Component		
Treatment	1d	6d	Mean	Treatment	Leaf	Stem	Whole
Frost	21.8	22.7	22.2	Frost	9.4	6.9	7.9
12°C	26.2	19.1	22.7	2°C	7.8	8.1	7.9
Frost/Thaw	25.2	24.0	24.6	15°C	8.2	7.6	7.8
SED (n = 24)	6.62	6.62	4.68	SED (n = 18)	1.64	1.67	1.52

Neither temperature, length of temperature or thawing frosted kale significantly affected SMCO. These results suggest, therefore, that frosting is not responsible for higher SMCO levels in mature kale. Previously observed anaemic symptoms in animals grazing frosted kale (Evans 1951, Dunbar et al 1963) were probably not caused by increased SMCO levels, but more likely due to colder temperatures either putting further stress on already debilitated animals or causing animals to ingest more kale and SMCO.

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Soil Moisture Effects:

Maris Kestrel plants from plots were transferred into pots and grown at 15°C in a glasshouse. After a two week settling-in period, 40 plants were watered daily and another 40 given no water for 11 days before being harvested. The average fresh weights of the wilted and non-wilted plants were 87 and 144g respectively, showing the severity of wilting achieved.

The mean SMCO levels of the wilted and non-wilted plants were 9.6 and 8.5 g/kg DM (SED = 1.36) respectively. This non-significant difference between treatments suggests that moisture stress, as with temperature, has no effect on kale SMCO levels; hence neither of these climatic factors explain the large variations in SMCO levels between kale crops.

2. SOIL FERTILITY (soil nitrogen and sulphur status)

The effects on SMCO of cultural methods, such as sulphur (S) and nitrogen (N) fertilisers, plant populations, and length of growing period, were examined in eight field trials. The detailed effects of these factors will be published elsewhere, but the effects of soil fertility on the large variation in SMCO levels (6.6-20.6g/kg DM) between the trials are summarised.

Maris Kestrel kale was sown between the end of April and early July in each trial and adequate phosphate and potassium fertilisers applied to each. The following table gives for each site, the mean SMCO and plant N levels on the low N fertiliser treatments at harvest >120 days from sowing, plus the average soil sulphate levels from samples taken in October (autumn) each year. The %SMCO responses to N fertiliser at seven of the sites are also included.

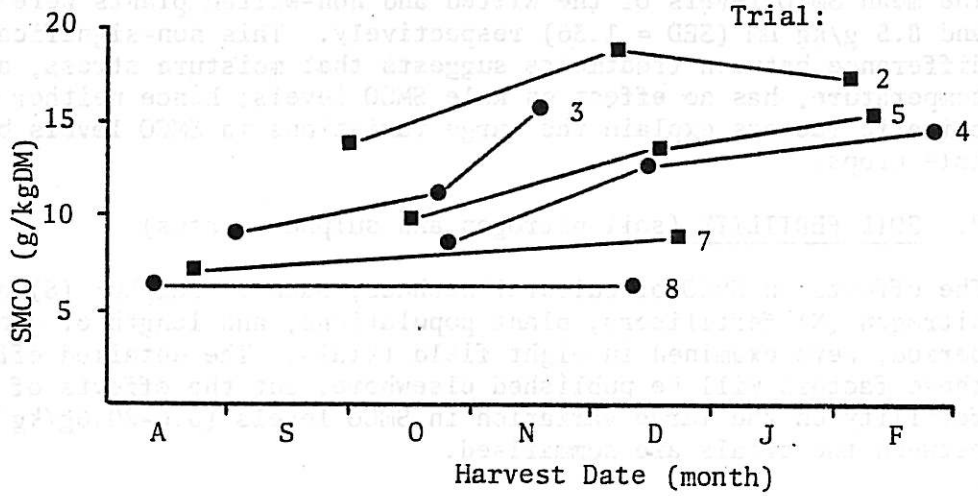
Site (crops since pasture)	SMCO (g/kgDM)	Plant N (%DM)	Soil Sulphate (ppm)	% increase in SMCO when 100 kg N/ha applied
1. (0)	20.6	2.93	18.8	0
2. (5)	17.1	2.83	2.7	10
3. (5)	15.4	2.65	0.5	-
4. (5)	13.0	2.16	4.3	11
5. (6)	11.7	1.82	4.5	18
6. (4)	9.7	1.99	8.6	19
7. (3)	9.0	1.77	10.8	20
8. (7)	5.3	1.23	6.8	39
Correlation with SMCO		r=0.97**	r=0.21	r= -0.92**

There was a highly significant ($p<0.01$) inter-trial correlation between SMCO and plant N levels in the crops (r=0.97) but no significant relationship between SMCO and soil sulphate levels (r=0.21). As plant N is a function of available soil nitrate, SMCO levels were more dependant on soil N status than on soil sulphate levels, even though available sulphate can affect kale SMCO in some soils (McDonald et al 1981). There were also significant responses in SMCO to N fertiliser, except where kale followed immediately after pasture, and these responses increased as plant N levels decreased (r= -0.92), thus confirming the relationship between SMCO and available N.

These results show that most of the differences in SMCO levels between kale crops can be explained by variations in soil fertility and in particular soil N status.

3. HARVEST DATE

In six of the eight trials, kale was harvested at more than one date during autumn and winter. The effects of the varying harvest dates on whole plant SMCO levels are shown in the following figure.



In all trials, except trial 8, SMCO increased between the first and last harvests, with greater increases occurring during autumn up to December, rather than after December during winter. Within trials, later harvests increased SMCO levels by up to 6g/kg DM. This variation in SMCO caused by harvest date was significant and important but much less than the inter-trial variation caused by soil N status.

4. CULTIVAR

SMCO levels in several kale cultivars were measured in two trials. In trial A four cultivars were examined, each replicated twice, while in trial B 6 cultivars were grown in each of three blocks. Cultivars were sown in May in each trial with adequate levels of NPK fertiliser, and harvested in November (trial A) and December (trial B). SMCO concentrations (g/kg DM) in leaves, stems and whole plants are shown in the following table.

	Trial A			Trial B		
	Leaf	Stem	Whole	Leaf	Stem	Whole
Maris Kestrel	8.2	6.8	7.5	8.1	10.3	9.3
Merlin	8.9	9.1	9.0	8.2	9.4	8.6
Vulcan	11.3	9.0	8.9	10.6	9.8	10.1
Canson	11.3	4.8	8.8	13.4	6.5	10.4
Chrysol				14.3	11.0	13.1
Bittern				7.7	7.9	7.8
SED	2.14	0.43	1.21	1.62	2.16	1.54
F Test Sign	NS	**	NS	**	NS	NS

Whole plant SMCO levels were not significantly different between cultivars in either trial, although in trial B Chrysol, a French bred cultivar, tended to have a higher level than the other cultivars. There were some differences between cultivars, however, within leaves and stems. In trial A, Canson, a thousand-head type kale, had lower stem SMCO levels than the marrow-stem cultivars. In trial B, Canson and Chrysol, which were also the two leafiest and lowest yielding cultivars, had the highest leaf SMCO levels which would cause them to be very toxic if animals grazed them at a low utilisation.

The variation between cultivars (up to 5g/kg DM) was slightly higher than reported previously (Bradshaw and Borzuki 1981), probably due to higher errors caused by less replication. There was, however, less variation between the marrow-stem cultivars than between Canson or Chrysol and the marrow-stem cultivars, as found previously by Bradshaw and Borzuki (1981).

5. PLANT POPULATION

The effects of plant population on SMCO levels were examined in the eight field trials and detailed results are being prepared for publication. Increasing plant density consistently reduced kale SMCO, and within sites whole-plant SMCO levels varied by up to 4g/kg DM at different populations.

CONCLUSION

Soil N Status is the main factor responsible for the large variation in SMCO between kale crops. Other lesser important factors are harvest date, cultivar and plant population; while soil sulphate levels and climatic factors, such as temperatures and moisture, appear to have little or no effect on SMCO.

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RELATIONSHIP BETWEEN GLUCOSINOLATE CONTENT AND YIELD COMPONENTS IN RAPESEED

A.M. Olivieri, P. Parrini

In two published studies carried out on a diallel cross set involving winter and summer varieties, we analyzed some yield components (Olivieri and Parrini, 1983) and the glucosinolate content in the seed and its inheritance (Olivieri et Al., 1982).

Here, , in about the same material, we report correlations between yield components (i.e. no. of siliquas per plant, no. of seeds per siliqua and 1000 seed weight) and glucosinolate content in progenies coming from recurrent parents.

Table 1 shows that for all progenies, except for those coming from Dolora and Brink, glucosinolate content affects significantly 1000 seed weight, whereas do not influence the other yield components.

Regression coefficients indicate that for 10 unit of glucosinolate, expressed as μ mole x g meal^{-1}, there is an increase of 0.10 to 0.14 g in the 1000 seed weight.

It seems that genes controlling glucosinolate content are pleiotropic or in linkage with filling stage. The mechanism of this relationship could be analyzed starting from crosses involving Bring and Dolora, varieties which appear very interesting also for their yield performance.

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Table 1. Correlation coefficients between glucosinolate content and yield components for each summer (S) or winter (W) recurrent parent. In parentheses mean values.

Recurrent parents		Glucosinolate content	No. siliquas/plant	No. seeds/siliqua	1000 seed weight
			(1)		
Midas	S	(121)	0.2 (17.4)	0.2 (21.6)	0.7 ** (4.5)
Dolora	S	(75)	0.4 (17.0)	-0.0 (21.8)	0.4 (3.6)
Vanda	S	(89)	0.4 (17.3)	0.1 (23.1)	0.6 ** (4.2)
Cresor	S	(100)	-0.2 (16.7)	0.0 (24.9)	0.5 * (4.3)
Eurora	W	(114)	-0.0 (16.0)	-0.1 (22.8)	0.6 ** (4.5)
Primor	W	(126)	-0.4 * (16.2)	0.3 (23.6)	0.6 * (4.3)
Sedo	S	(75)	0.3 (13.5)	0.2 (21.2)	0.8 ** (3.8)
Status	W	(131)	-0.3 (14.3)	-0.0 (22.9)	0.5 * (4.3)
Brink	W	(123)	0.1 (18.6)	-0.0 (22.2)	0.1 (4.4)
Ramses"o"	W	(119)	-0.2 (13.3)	-0.3 (23.6)	0.7 ** (4.4)

(1) x 100

Level of significance: * = $P \leq 0.05$; ** = $P \leq 0.01$.

COMPARATIVE STUDY ON DETERMINATION OF GLUCOSINOLATES IN RAPESEED

W. Brzeziński, P. Mendelewski and B.G. Muuse

In comparative studies on total glucosinolate (GSL) content carried on by Thymol method (Brzeziński,W. and Mendelewski,P., 1984: Z. Pflanzenzüchtg. 93, 177-183) and ISO 5504 gaschromatographic (GLC) method usually differences were observed. The results obtained with Thymol method have been always higher than with GLC. Most probably, indolyl GSL were responsible for this disagreement. Under GLC procedure very unstable indole aglucones are partly destroyed and difficult to quantificate.

In this study content of GSL was determined by HPLC (Spinks E.A. et al., 1984: Fette Seifen Anstrich. 86, 228 - 231) and Thymol method. For the HPLC procedure a molar extinction coefficient of the indolyl GSL relative to sinigrin of 5.0 at 226 nm has been established and applied. The results of the studies are summarized in the table and HPLC patterns of tested varieties are shown. The samples originated from cultivar plot trials of the Research Centre for Testing Cultivars, Słupia Wielka, Poland.

Table of GSL content and composition in some lines and cultivars of Brassica napus L. rapeseed, determined by two analytical procedures.

Cultivar or line	GSL content (µmole/g defatted meal)					
	Thymol	HPLC				
	Total	Total	PRO	GNA	4-OH GBC	Rest
BOH 484	9.7	11.4	4.0	1.5	5.1	0.8
BOH 183*/	13.3	15.5	6.9	2.7	4.7	1.2
BOH 384	17.5	17.6	9.2	3.5	3.8	1.1
Jantar	19.8	19.3	10.2	4.0	4.0	1.1
BOH 183*/	25.1	24.5	12.6	4.9	5.1	1.9
Liglandor	40.3	40.3	21.0	8.0	5.3	6.0
Darmor	49.9	54.5	36.8	12.2	3.9	1.6
Tandem	59.3	59.2	40.4	13.0	3.3	2.5
Lingot	94.6	100.9	71.3	22.7	0.6	6.3

*/ from different soil and climate regions.

Abbrev.: PRO - Progoitrin, GNA - Gluconapin, 4-OH-GBC - - 4-hydroxy Glucobrassicin

Based on the results, a good compatibility between Thymol and HPLC method has been found. The methods do not differ significantly and standard deviation of the differences between the paired results was 2.5 µmole/g.

Indolyl GSL content in the rapeseed cultivars is found to be rather constant and therefore relatively high in low - glucosinolate cultivars. The content of the main indolyl GSL in rapeseed 4-hydroxy Glucobrassicin vary from 3.3 to 5.1 µmole/g and can reach as much as 50% of total GSL.

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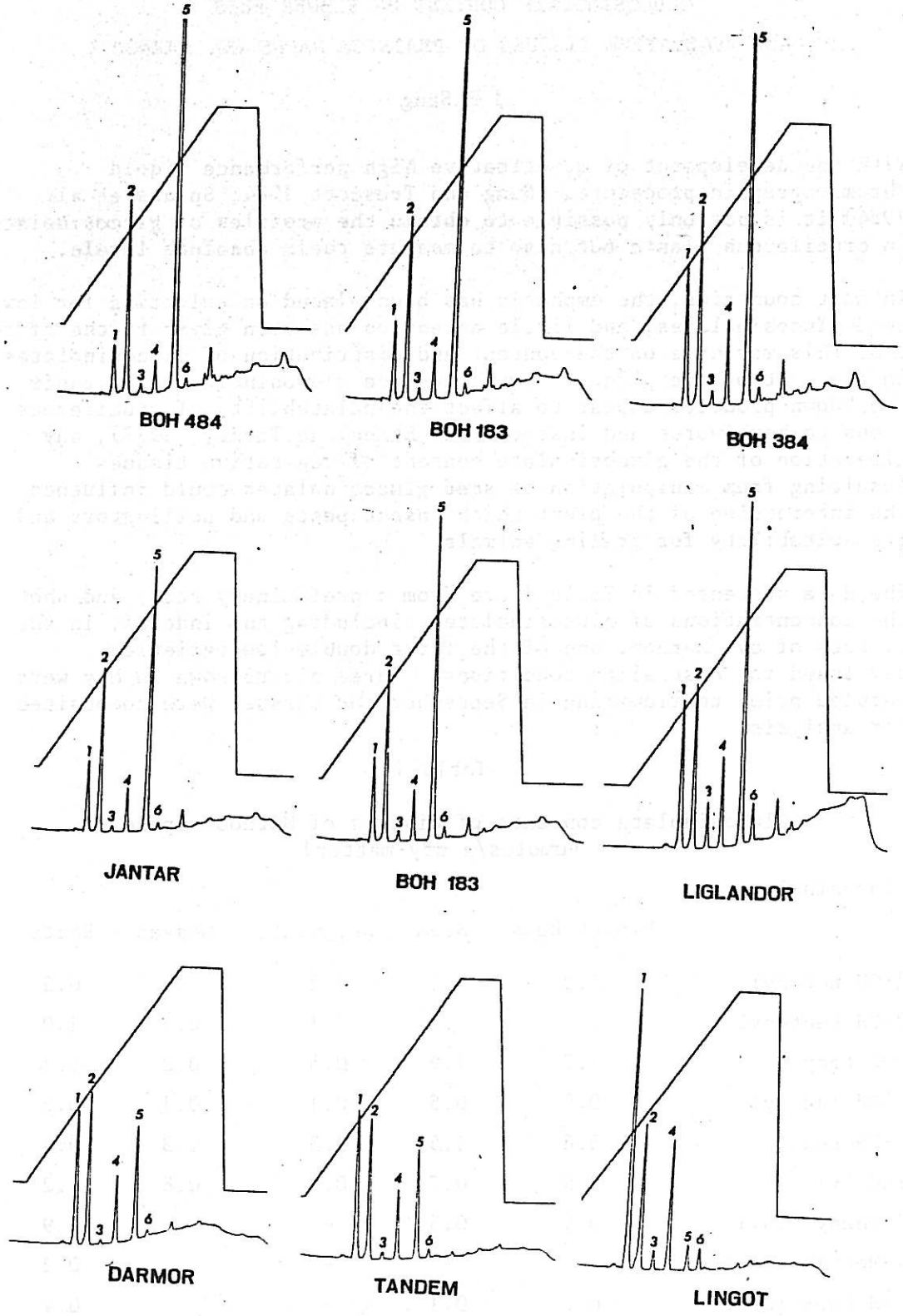

HPLC patterns of tested varieties.
Peak identification: 1 - Progoitrin, 2 - Sinigrin (Internal Standard), 3 - Gluconapoleiferin and unknown, 4 - Gluconapin, 5 - 4-hydroxyglucobrassicin, 6 - Glucobrassicanapin.

GLUCOSINOLATE CONTENT OF FLOWER BUDS AND VEGETATIVE TISSUES OF *BRASSICA NAPUS* CV. MARNOO

J P Sang

With the development of quantitative high performance liquid chromatographic procedures (Sang and Truscott 1984; Spinks et al. 1984) it is not only possible to obtain the profiles of glucosinolates in cruciferous plants but also to measure their absolute levels.

In most countries, the emphasis has been placed on selecting for low seed glucosinolates, and little attention has been given to the effect that this may have on the content and distribution of glucosinolates in the rest of the plant. However, since glucosinolates and their breakdown products appear to affect the palatability of cruciferous crops to herbivores and insects (Van Etten and Tookey, 1979), any alteration of the glucosinolate content of vegetative tissues resulting from manipulation of seed glucosinolates could influence the interaction of the plant which insect pests and pollinators and its suitability for grazing animals.

The data presented in Table 1 are from a preliminary study and show the concentrations of glucosinolates, including the indoles, in the tissues of cv. Marnoo, one of the first double-low varieties developed for Australian conditions. Three plants sown in May were sampled prior to flowering in September and tissues were composited for analysis.

Table 1

Glucosinolate contents of tissues of Marnoo rapeseed (μmoles/g dry matter)

Glucosinolate	Flower buds	Stem	Stipulate	Leaves	Roots
2-OH Butenyl	5.2	4.1	1.2	-	0.5
2-OH Pentenyl	2.0	1.5	1.1	0.3	1.0
3-Butenyl	1.7	1.9	0.5	0.2	0.1
4-OH Indolyl	0.9	0.5	0.1	0.1	0.2
4-Pentenyl	5.6	1.5	2.5	0.3	0.4
Indolyl	2.9	0.7	0.9	0.8	1.2
2-Phenylethyl	0.4	0.5	-	-	12.9
4-Methoxyindolyl	-	-	-		0.2
1-Methoxyindolyl	0.2	0.1	-		0.4
Totals	18.9	10.8	6.3	1.7	16.9

Concentrations of glucosinolates in the various tissues will certainly change with plant development but the high contents of 2-phenylethyl

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glucosinolate in the roots and 4-pentenyl in the buds are evident at this stage. The study should provide information on glucosinolate accumulation during this phase of growth. Comparisons between high and low varieties should provide data to determine whether the tissues of the low glucosinolate varieties compensate for low seed glucosinolates or whether the distribution of glucosinolates in them is changed.

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COMPARISON OF THE THYMOL METHOD OF GLUCOSINOLATE ANALYSIS WITH GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES FOR PRECISION, SPEED AND COST

D. I. McGregor

Plant breeding programs usually require the testing of large numbers of seed samples in order to make advances in seed quality characteristics. Ideally methods of analysis must not only be accurate and precise, but also simple and rapid. When these requirements can not be met by one method, a two method system for breeding is often adopted. One method sacrifices some accuracy and precision in order to facilitate rapid screening of large numbers of samples. If more than one component is measured, as in the case of glucosinolates in rapeseed, compositional information may also be sacrificed. The second method, which is often more involved and time-consuming, is sufficiently accurate and precise to verify composition or content of the initial selections. Such two method systems have been extensively used for glucosinolate analysis (McGregor et al. 1983).

Recently Brzezinski and Mendelewski (1984) published a method for determining the glucosinolate content of rapeseed in which the glucosinolates were purified on a DEAE-sephadex A-25 column, reacted with thymol in sulfuric acid and measured colorimetrically. Although the method does not provide compositional information it was reputed to have the advantage over existing method in that it was simple, rapid and sensitive.

The thymol method was compared with gas chromatography of trimethylsilyl derivatives (Daun and McGregor 1981), a method widely used for determination of the glucosinolate content of rapeseed. Samples used were drawn from 1985 Saskatoon, Canada Brassica napus L. breeding nursery and represented both the rapeseed range and the narrower canola range of glucosinolate content. Of particular interest was to determine if the thymol method could be used to screen large numbers of samples in plant breeding programs at reduced cost, without sacrificing precision.

Analysis of thirty rapeseed samples in duplicate by gas chromatography yielded standard errors of 4.0 and 3.7 and corresponding coefficients of varibility of 5.9 and 4.8 for the sum 4 (3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl and 2-hydroxy-4-pentenyl) and 6 (3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl and 2-hydroxy-4-pentenyl, 3-indolylmethyl and 4-hydroxy-3-indolylmethyl) glucosinolates, respectively. Duplicate means ranged from 9.3 to 124.6 and 17.3 to 132.9 umoles/g meal for the sum 4 and 6 glucosinolates, respectively. Analysis of the same samples in duplicate by the thymol method yielded a standard error of 6.4 and coefficinet of variability of 7.5 indicating that the thymol method is capable of precision comparable to the gas chromatography method. Duplicate means for the thymol method ranged from 19.1 to 158.8 umoles/g meal.

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Regression of the means of the duplicate thymol analyses with the means of the duplicate gas chromatographic analyses for the 6 glucosinolates yielded a slope of 1.17 and an intercept of -4.27. Thus thymol analyses were comparable to gas chromatography at the lower end of the range but 18.3 umoles/g meal higher at the upper end of the range.

Analysis of twenty eight canola samples in duplicate by gas chromatography yielded standard errors of 0.86 and 2.0 and corresponding coefficients of varibility of 5.6 and 8.7 for the sum of 4 and 6 glucosinolates, respectively. Duplicate means ranged from 7.4 to 27.9 and 10.5 to 37.2 umoles/g meal for the 4 and 6 glucosinolates, respectively. Analysis of the same samples in duplicate by the thymol method yielded a standard error of 1.5 and a coefficient of variability of 6.1 indicating that the thymol method is also capable of precision comparable to the gas chromatography method over the canola range of glucosinolate content. Duplicate means for the thymol method ranged from 12.7 to 38.3 umoles/g meal.

Regression of the means of the duplicate thymol analyses with the means of the duplicate gas chromatographic analyses for 6 the glucosinolates yielded a slope of 0.99 and an intercept of 0.90. Thus thymol analyses were comparable to gas chromatography of the 6 glucosinolates being only 0.6 to 0.8 umoles/g meal higher over the range.

Equipment for the thymol method is less expensive relative to gas chromatography of trimethylsilyl derivatives. Thymol analysis is simpler, technically less demanding, and less expensive. Reagent costs for a thymol analysis were estimated to be approximately one tenth that of gas chromatography of trimethylsilyl derivaties. One technician in an eight hour period was able to perform 30 to 40 thymol analyses starting with oil-extracted meal and measuring color development in a cuvette. If cuvette test tubes are used to eliminate rinsing between samples, it is estimated that throughput could be doubled to 80 samples. Simplicity, lower cost and comparable precision indicate that thymol analysis is a suitable alternative to gas chromatography of trimethylsilyl derivatives where compositional information is not essential. Higher throughput facilitates screening large numbers of samples in plant breeding programs.

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HIGH ERUCIC OIL FROM WHITE MUSTARD (SINAPIS ALBA L.) FOR TECHNICAL USE.

C. Persson

Rape-seed oil with a high content of erucic acid can be used for technical purposes as a lubricant. Since oil for production of margarine must have low erucic acid content, special cultivation of high erucic cultivars for this purpose is necessary. However, if both high- and low-erucic cultivars are grown in the same area, problems may occur due to crossing and mixing between the two types.

White mustard (Sinapis alba L.) is an interesting alternative for production of high erucic oil since no crossing can occur between Sinapis and Brassica. Older swedish cultivars such as Sv Trico have about 40 % erucic acid in the oil but for technical use the content should be 50-60 %. In 1976 selection work started at Svalöf to increase the erucic acid content. Both selection of single plants and selection of single seeds by half-seed technique was used. In six selections the erucic acid content was increased from about 40 % to about 55 %.

Selection for yield, oil content and agronomic performance has resulted in the cultivar Sv Mustang which was released in Sweden in 1985. The erucic acid content in the oil is about 54 %, the yield is similar to Sv Trico but the oil content is higher and the stalk is stiffer. In table 1 a comparison is made between Mustang, Trico and Kirby.

Table 1. Comparison between Mustang, Trico and Kirby. 11 trials 1983-1985.

	Seed yield		Oil	Oil yield		Stalk
	kg/ha	% of check	content	kg/ha	% of check	stiffness
Mustang	1725	100	37.6	556	100	77
Trico	1747	101	36.6	549	99	67
Kirby	1717	100	32.9	483	87	79

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POLLEN GERMINATION, POLLEN TUBE GROWTH AND SEED REDUCTION IN WINTERRAPE IN RELATION TO LIGHT INTENSITY AND CULTIVAR

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Seed reduction under intra- and interplant competition is a well known phenomenon in rape. Supply of assimilates plays a major role in this context.

We analysed pollen germination, pollen tube growth and seed set of the zero erucic cultivars Jet Neuf and Garant and the 0-0 lines 154/81 and 1657/79 grown under different light intensities.

Four variants were tested in an experimental nursery: normal planting (NP), reduced density (RD), shadow 1 (S1) and shadow 2 (S2). Except of variant RD with 25 plants /m ^{2} 55 plants per m^2 were grown. Light intensity above the crop varied from 107 klx (NP,RD), 67 klx (S1) to 40 klx (S2) during bright sunshine, but was reduced under cloudiness to 37 klx, 25 klx and 14 klx respectively.

The highest rate of pollen germination in vitro (78 %) was shown by Jet Neuf, but no significant differences between cultivars and light variants could be observed for this character in vitro as well as in vivo.

In contrast pollen tube growth was influenced remarkably by reduced light intensity. In comparison of the two 0-0 lines 154/81 showed stronger reactions than 1657/79.

On the stigma normal pollen germination was observed in both lines, but after reduction of light we found a reduced number of pollen tubes in 5 days old siliques of 154/81 in variant S1 compared with 1657/79. Under variant S2 conditions very often in 5 days old siliques of 154/81 no pollen tubes could be seen in the basal part of the silique.

Line 1657/79 showed just another type of reaction. In variant S1 numerous pollen tubes grew in all parts of the silique. There was clearly a lower reduction of pollen tube growth in variant S2 than was found in line 154/81.

Reduction of seed set starts already in the very beginning of fruit development caused by the failure of fertilisation. Seed reduction increased remarkably by competition on supply. The final number of seeds is the result of both these effects.

Seed set was calculated by relating the number of normal developed seeds to the number of ovules (seed potential). We analysed siliques

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of the terminal inflorescence and of the axillary branch 5. The number of ovules varied significantly between varieties. No insertion effect could be detected by comparing the results of the terminal inflorescence and the axillary branch 5 respectively from variant RD.

We observed significant differences in the final number of seeds between cultivars as well as between light variants. Specific cultivar x light interactions were found. This was due for the terminal inflorescence as well as for the axillary branch 5.

Tab. 1 F values, number of normal developed pods

Variant	cultivar effect	insertion effect	interaction cultivar x insertion
NP	22,14***	33,77***	4,72***
RD	25,47***	2,46	0,08
S1	15,84***	31,49***	5,58***
S2	21,00***	84,69***	7,86***

Under normal planting conditions line 1657/79 showed the highest number of normal seeds, both on the terminal inflorescence and the axillary branch 5. Jet Neuf exceeded this line by 2 seeds per silique in variant RD only, the variant with a high light intensity in lower parts of the crop. In general the 0-0 line 1657/79 showed a higher seed set caused by a lower rate of seed reduction over all variants.

Under conditions of light reduction in a crop by intra- and interplant competition 1657/79 can be characterized as remarkably stable in seed production compared with the other three varieties tested. This could be demonstrated by calculating the fertility of the terminal inflorescence as well as of the axillary branch 5 on the basis of seed potential. The amount of seed reduction during the early fruit development is comparably less than in the other cultivars, especially when light intensity decreases. This might be due to a better net assimilation rate or more effective translocation of assimilates. It seems, that already at the very beginning of fruit development more assimilates are available in the style and in the ovary as can be concluded from the analysis of pollen tube growth.

COMMERCIAL SEED PRODUCTION OF EARLY CAULIFLOWER VARIETIES

Dursun Esiyok

Commercial seed production of early cauliflower varieties is difficult in open field conditions because of the adverse effects of heavy rains and low temperatures (Singh et al., 1960, Gill and Singh, 1973).

Cauliflower production is mostly done in the middle west coastal part of Turkey. The production cycle begins in July and goes on until March for market and June for seed production. In that part of Turkey, the weather conditions are similar to the Mediterranean climate but are not suitable for commercial seed production in open fields.

The curds of early cauliflower varieties mature during October and November. Following maturation, the adverse effects of low temperature and particularly heavy rains begin to be effective and seed yields drop significantly.

Faulkner and Jackson (1980,1981a,b) and Gowers (1984) used polyethylene tunnels for seed production and obtained good results in commercial mean.

We observed that the major cause of low seed yield is wet conditions and we thought, if we can control the bad effect of rain it will be possible to obtain good seed yields. For this reason, we carried out our experiments under coverings. The shape was like a conventional glasshouse and the dimensions were: width = 4.5 m, length = 20.0 m, middle height = 3.5 m, side height = 2.0 m. We covered only the top of this construction with hard plastic material and left open all sides to maintain good ventilation and insect activity for pollination. We used the early cauliflower cv. Brio osenia. Seeds were drilled in open seed beds at the beginning of July and seedlings were transplanted into the covered area and open field at 0.9 x 0.6 m about 30 days later. Flowering occurred in February and March, seed set and fruit maturation were seen between March and May and seed harvests were done in June.

The results of the experiments in 1983 and 1984 are seen in Table 1.

The first interesting result is the difference between the covered area and the open field. In both years, these differences were significant and seed yields were increased about thirty-fold in the covered area as compared with the open field. The mean seed yields of both years was 243 kg/ha from the covered area and 8 kg/ha from the open field.

For us, the main cause of this situation is firstly the amount of rain between the months November-April. In the first year the total amount of rain was about 780 mm during the sensitive period of the curds and flowers to rain and humidity. Most of the plants rotted

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and this seed-set capability was decreased due to the heavy rain in the open field. Under coverings, the percentage of rotted plants was less and there was no significant damage to the flowers. Temperature did not drop below zero and low temperature caused no adverse effects.

Table 1. Seed Yields in Both Places and Years

	Seed Yields					
	Open Field			Covered Area		
Years	g/plot($20m^2$)	g/plant	kg/ha	g/plot($20m^2$)	g/plant	kg/ha
1983	30	5.2	15.6	582.0	20.8	303.1
1984	0	0	0	350.5	15.8	182.6
Mean of both years	15	2.6	7.8	466.2	18.3	242.8

In the second year the total amount of rain was about 330 mm, but the temperature during the flowering stage (February) dropped below zero for 14 days. In the open field all the plants froze and died, although most of the plants under coverings were alive and gave good seed yield.

These results show us that it is possible to control the bad effects of rain and low temperature and to obtain satisfactory seed yield in commercial mean.

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