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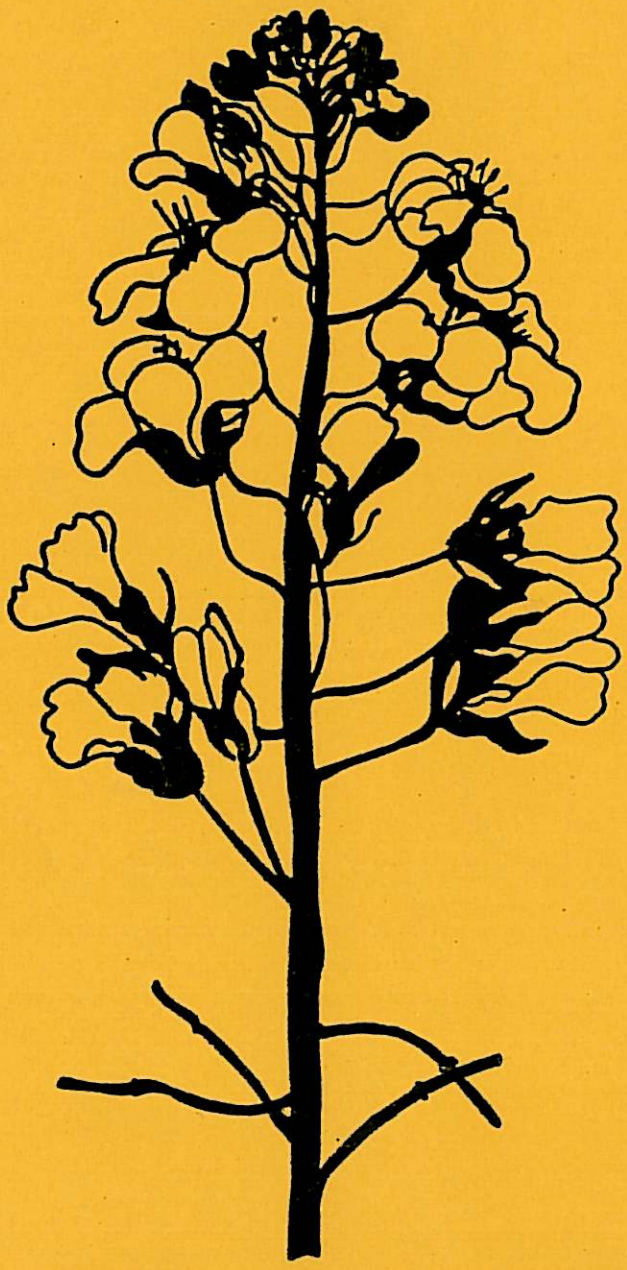

DECEMBER 1984

EUCARPIA

Editorial

Publication of this issue has been delayed by some weeks due to circumstances over which we had little control. This is regretted but there may be some advantage in that the mailing will not now coincide with the busy pre-Christmas postal period.

Additions to the distribution list and other alterations are listed on the loose insert included with the Newsletter. The number of recipients is now 414.

The costs of producing and mailing Cruciferae Newsletter No. 8 amounted to £577, a considerable increase on previous years. The fund had stood at £516 and was thus insufficient to meet the charges. Donations were solicited from all commercial organisations on the distribution list in July and, as a result of the generous response, there is now a credit balance of £1,400.

Dr. A.B. Wills
Scottish Crop Research Institute
Invergowrie
Dundee

Dr. I.H. McNaughton
Scottish Crop Research Institute
Pentlandfield
Roslin, Midlothian

The editors are deeply grateful to the organisations listed below for generous donations to assist in the publication of Cruciferae Newsletter.

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Unilever NV, The Netherlands

Institut de Recherche Vilmorin, France

Zelder BV, The Netherlands

Zwaan en de Wiljes

ACTIVITY OF CRUCIFER GENETICS COOPERATIVE (CRGC) 1982-1984

Paul H. Williams

Since its initiation with the November 1982 issue of this Eucarpia Cruciferae Newsletter, the Crucifer Genetics Cooperative, CRGC (note new name and symbol), has shown steady growth and activity. Most activity has been in the development of and distribution of *Brassica* and *Raphanus* stocks that have unique phenotypes or genotypes that would make them useful for genetics, breeding or biological studies. In 1982, 11 individual requests for seed were made, in 1983, 32 persons requested seeds and in 1984 (as of 9-30-84) 61 requests have been received. Since 1982, 520 packets of seed have been dispensed to researchers and teachers in 13 countries. Research activities with the stocks vary from conventional genetic and breeding applications to a variety of cellular and molecular biology applications including haploid plant regeneration, somatic embryogenesis, protoplast culture, molecular genetics of cytoplasmic organelles and gene vector relations.

Below is a list of the stocks currently available for distribution from the CRGC. Seed is provided without charge in limited quantities normally determined by the general ease of producing the stocks. Usually 50-100 seeds are provided. Repeat requests can be filled if they do not become excessive. Since most traits are placed in the background of a rapid cycling (RC) base population (BP) the seed stocks can be rapidly increased by the recipient. Along with the seed stocks, the CRGC will supply information on increasing the stocks and on evaluating specialized phenotypes such as disease reaction. Cultures of numerous crucifer pathogens are available upon request and with the provision of appropriate permits for transfer of the pathogen.

In addition to the stocks listed below, we are working on an increasing number of stocks containing nuclear marker genes and stocks with particular cytoplasmic phenotypes. When a particular trait is introduced into the rapid cycling background of a base population and when sufficient seed of a stock is produced, the stock is assigned a CRGC number and made generally available. At present we are working on approximately 100 traits and several cytoplasms which will be assigned CRGC stock numbers in the next year or two.

I would like to encourage anyone who has traits (qualitative, quantitative, or cytoplasmic) that they would like to share with others to write me or to send me seed. I am also able to recieve, and prepared to assist you in shipping pollen carrying useful genes. I am also willing to receive genetic material from you on a confidential, proprietory or prepublication basis and to honor your wishes not to make the particular trait generally available through the CRGC until you give me permission in writing. Normally

what I would do with proprietary stocks is to begin introducing the traits into the rapid cycling background, then notify you of when I believed they were sufficiently well developed for release. All persons providing genes and stocks to the CRGC will be recognized as the source of those traits. In the case where the supplier of a trait is not the originator of the trait, I would also like you to provide information on the origins of the trait. A comprehensive file system of stock origins, stock development (lineage), maintenance and distribution is being kept by the CRGC. This information may become increasingly valuable to users of these stocks. If you wish more detailed information on any CRGC stock or have ideas that would improve the operation of the CRGC, I would be pleased to hear from you.

CRUCIFER GENETICS WORKSHOP

A crucifer genetics workshop sponsored by the Crucifer Genetics Cooperative and the Department of Plant Pathology will be held May 29 and 30, 1985 at the University of Wisconsin, Madison. The workshop represents a follow-up of the California Brassica Workshop held at the University of California, Davis, April 12, 1984, sponsored by the Departments of Agronomy and Range Science and by the Department of Vegetable Crops, University of California, Davis, and organized by Daniel Cohen. The 1985 Crucifer Genetics Workshop will have the broad objective of focusing on the development and application of genetic technology for the improvement of crucifer crops. The format will include informal presentations, discussion groups, posters and demonstrations on all aspects of crucifer genetics, cell and molecular biology and their applications. Persons interested in further information or in attending the Crucifer Genetics Workshop should write Paul H. Williams, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, 53706, or call (608-262-6496).

STOCKS AVAILABLE FROM THE CRGC

CRGC#	Genome[a]	Phenotype-Genotype[b]	Source[c]
1	Aaa	RC,BP	WILPAU
2	Bbb	RC,BP	"
3	Ccc	RC,BP	"
4	ABaabb	RC,BP	"
5	ACaacc	RC,BP	"
6	BCbbcc	RC,BP	"
7	Rrr	RC,BP	"
8	Rlaa	RC,CMS	HEYFRI
9	Rlcc	RC,CMS	"
10	Rlaacc	RC,CMS	"
11	ABlaabb	RC,CMS	ANAIJ
12	ACaacc	RC,*Dw/Dw*	THOKEN
13	ABlaa	RC,CMS	ANAIJ
14	ABlaacc	RC,CMS	"
15	Rlrr	RC,CMS	HEYFRI
16	Aaa	RC,Ac2(R)C1	EDWMAR
17	"	RC,Ac2(R)C2	"
18	"	RC,Ac2(R)C3	"
19	Alaa	RC,VG	WILPAU
20	Rlaabb	RC,CMS	HEYFRI
21	Rrrrr	RC,4X	WILPAU
22	Rlaacc(R)	RC,CMS,W	HEYFRI
23	Ccc.c	BI-1,MDR	WILPAU
24	"	BI-2,MDR	"
25	"	BI-3,MDR	"
26	"	BI-4,MDR	"
27	"	BI-5,MDR	"
28	"	BI-6,MDR	"
29	"	BI-7,MDR	"
30	"	BI-8,MDR	"
31	"	BI-9,MDR	"
32	"	BI-10,MDR	"
33	"	BI-11,MDR	"
34	"	BI-12,MDR	"
35	"	BI-13,MDR	"
36	"	BI-14,MDR	"
37	"	BI-15,MDR	"
38	"	BI-16,MDR	"
39	"	BI-17,MDR	"
40	"	BI-18,MDR	"
41	"	BI-19,MDR	"
42	"	BI-20,MDR	"
43	Rlcc.c	BI-21,MDR,CMS	"
44	"	BI-22,MDR,CMS	"
45	"	BI-23,MDR,CMS	"
46	"	BI-24,MDR,CMS	"
47	Ccc.b	BI-25,PB6(R)	"
48	Rlcc.b	BI-26,CMS	"
49	Rlcc.i	BI-27,CMS	"

50	Rlaa.p	BI-28A,CMS,MDR	LEUHEI
51	"	BI-28B,CMS,MDR	LEUHEI
52	"	BI-28C,CMS,MDR	"
53	"	BI-28D,CMS,MDR	"
54	"	BI-28E,CMS,MDR	"
55	Rlaa.c	BI-29A,CMS,MDR	"
56	"	BI-29B,CMS,MDR	"
57	Rlaa.ra	BI-30,CMS,MDR	"
58	Rlaa.r	BI-31,CMS,MDR	"
59	Aaa	RC,*Pb1/Pb1*	JAMVAU
60	"	RC,*Pb2/Pb2*	"
61	"	RC,*Pb3/Pb3*	"
62	"	RC,Ac2(S)CO	EDWMAR
63	"	RC,*ro/ro*	JAMVAU
64	Rlaacc(r)	RC,CMS,*Dw/+*	THOKEN

a) For descriptors of cytoplasms and nuclear genomes see Eucarpia Cruciferae Newsletter No. 2, pp. 40-41 (1977).
 --A = unspecified cytoplasm of *B. campestris.*
 --aa = diploid nuclear genome of *B. campestris* where a=x=10 chromosomes.
 --R1 = *Raphanus,* cms, cytoplasm, Ogura.
 --A1 = *B. campestris,* variegated cytoplasm = VG, Williams.
 --AB1 = *B. juncea,* cms, cytoplasm, Anand.

b) Phenotypic descriptors are in upper case only, genotypic descriptors follow international genetic convention for gene designations.
 --RC = rapid cycling.
 --BP = base population for species.
 --CMS = cytoplasmic male sterile.
 --VG = variegated.
 --W = white flower.
 --BI = Badger Inbred cultivar release.
 --MDR = multiple disease resistance.
 --Ac2(R) = resistant to *Albugo candida,* race 2.
 --Ac2(S) = susceptible to *Albugo candida,* race 2.
 --PB6(R) = resistant to *Plasmodiophora brassicae* race 6
 Genotypic descriptors
 --*Dw* = dwarf, incomplete dominant.
 --*Pb1, Pb2, Pb3* = resistance to Plasmodiophora brassicae race 6, 3 independent dominant genes.
 --*ro* = rosette dwarf, recessive, strong gibberellin responder.

c) Source codes are the three first letters of last name followed by the three first letters of the first name (eg. Paul H. Williams = WILPAU). Source codes are for the provider of the major trait in each CRGC stock and do not always represent the originator of the trait. Most traits have been introduced into the rapid cycling (RC) background of a species base population (BP).

GENETIC CONSERVATION OF EUROPEAN CRUCIFERS

Anon (on behalf of many, see Notes 2 and 3)

Introduction

With increasing communication, legislation, and objective breeding, genetic erosion of cruciferous crops is accelerating. Concerned European breeders first met to discuss this problem during the Eucarpia "Cruciferae 1979" Conference at Wageningen, the Netherlands, in October 1979. One product of that meeting was a contract from the European Community (via the Programme Committee on Plant Disease Resistance and the Use of Gene Banks) to collect cruciferous genetic resources from EC countries, which at that time consisted of France, West Germany, the Netherlands, Belgium, Luxembourg, Italy, Ireland, Britain and Denmark.

Materials and Methods

The contract was to run for a five year period, until the end of 1983. In practice, it commenced in June 1981, and parts of the work, including its final report (1) were completed during 1984.

A Coordinating Committee was established, consisting of representatives from Dutch and British research institutes (2). They sub-contracted representatives to organise collecting programmes in each country (3).

Early in the programme several decisions were taken as to the kind of material to be collected:

1. Oil-seed crucifers were omitted because these were covered by an initiative taken by the Rapeseed Breeding Subcommittee of the Group Consultatif International de Recherches du Colza, and coordinated by the gene bank of FAL, Braunschweig.
2. Wild crucifers were not collected because of the effort involved, because of their lack of genetic erosion (in comparison with cultivated forms), and because of current work by other persons (4).
3. Landraces, obsolete cultivars and farmers' seed stocks were clearly important, and much effort was to be expended on collecting these.
4. In contrast to much of the genetic conservation currently practised, existing commercial cultivars were also to be collected. The reasons for this approach have already been discussed (1 and 5). Briefly: such material will inevitably form the major part of the genetic base for future breeding; there is a serious threat of erosion; and the acquisition, storage and evaluation of these accessions is cheap and effective.

Results

About 4000 accessions have been acquired, representing all European non-oleiferous forms of cultivated brassicas and radish. Other cultivated species (of Lepidium, Sinapis, Eruca, Rorippa and Crambe) have also been collected.

The accessions have been (or are being) divided into two or more samples. The base collection of each accession consists of the bulk of the seed, and is stored by a gene bank (6), which is responsible for its maintenance and distribution. Duplicate collections are located at other gene banks for insurance against the loss of the base collection. Where possible, duplicates are also located in the country from which the material was acquired.

About 40% of the accessions were of small sample size or low germinability, and will therefore need multiplication before they can be distributed.

A small proportion of accessions ($< 1\%$) would only be donated (or sold) by farmers if a written guarantee was given that seed would not be distributed until the farmer gave his permission. Agreeing to these embargoes was contrary to the general ethos of gene banking; but

the alternative was the probable loss of landraces which had no genetic representation in more readily accessible material.

Collection was reasonably comprehensive in most EC countries, largely because the erosion of landraces had, regrettably, already occurred. In Ireland, France and Italy large numbers of farmers still save their own seedstocks, and collecting in these countries was not fully completed by the end of the contract period.

Conclusions

The programme has been successful in several respects.

Firstly, the number and more importantly the range of accessions represent a major contribution to future plant breeding. Although much current collecting of genetic resources is from developing countries, it is worth emphasising that centres of genetic diversity span Europe. Several European cruciferous crops have become of world-wide importance. The European genetic resources are therefore relevant not only to European agriculture, but to the developing countries, where these crops are increasingly grown.

Secondly, there has been collaboration both internationally, and between breeders and conservationists. This has resulted in a common philosophy of the objectives and potentials of gene banking.

Thirdly, people involved in the programme now understand more of the variation in the gene pools with which they are concerned. More publications on this topic can be expected.

Further collecting is needed, with some urgency, in Ireland, France and Italy, and in the new EC countries, Greece, Portugal and Spain, where much landrace material exists. Many of the accessions require multiplication and evaluation, both phylogenetically and along existing guidelines (7). For these reasons application for a new contract for the five year period 1984-8 was submitted to the EC in March 1983. To date, no decision has been made by EC, but we invite crucifer breeders and genetic conservationists who wish to be involved in further developments to contact members of the Coordinating Committee (2).

References and Notes

1. Report of the EC Research Programme 0890. "The collection of landraces of cruciferous crops in EC countries". Available from P Crisp, NVRS, or Q P van der Meer, IVT.
2. The committee consisted of: H Roelofsen, IVT - chairwoman until March 1983; Q P van der Meer, IVT - chairman from September 1983; H Toxopeus, SVP; W H MacFarlane-Smith, SCRI - until January 1981; I H McNaughton, SCRI - from January 1981; P Crisp, NVRS - until January 1983; D Astley, NVRS - until January 1983. J Doornbosch, SVP, was the financial administrator. For the addresses of institutes, see the list in Cruciferae Newsletter.
3. Programmes were conducted in: Britain (P Crisp, D Astley & I H McNaughton); the Netherlands (H Toxopeus & H Roelofsen); Ireland (R F Murphy); Denmark (V Schelbeck); Germany (P Mattusch & H Toxopeus); Belgium (L van Hee); and Italy (P Perrino & P Crisp).
4. C Gomez-Campo (Madrid) and M Gustaffson (Svalöf), and their colleagues.
5. Crisp, P and Astley, D (1984). Genetic Resources in Vegetables. Plant Breeding Progress Reviews (Ed: G E Russell)., in press.
6. The gene banks at NVRS (Britain), IVT/SVP (the Netherlands), INRA (Rennes, France), Bari (Italy) and FAL (Germany) hold the base collections.
7. Publications of the International Board for Plant Genetic Resources, Rome: Genetic resources of cruciferous crops (a global survey): AGP: IBPGR/80/100, and Descriptor lists of cruciferous crops - in preparation.

RELEASE OF *B. JUNCEA* AND *B. CARINATA* GERMPLASM

D. B. Cohen and P. F. Knowles

Eighteen lines of *B. juncea* and *B. carinata* from the *Brassica* oilseeds program at U.C. Davis are available until seed stocks run out. They are briefly described here and a more complete description will be published in 1985. All lines are adapted to a California, Mediterranean type, winter growing season. They are intermediate to high in erucic acid % and high in glucosinolite content. Most selection lines were grown in 5-row increase stocks in 1983-84 with the center row harvested for distribution. Seeds are untreated; lines known to be disease free will be available in 1985 or 1986 from Dr. P. H. Williams, University of Wisconsin (Madison).

Large seed size was associated with increased resistance to shattering in *B. juncea* lines. Two types of large seeded non-shattering were found. In type (I) seeds can be threshed free relatively easily using a Vogel thresher with a rasp bar cylinder. In type (II) seeds will not thresh free of siliques in a Vogel thresher and are difficult to thresh by hand. Type (II) should be more useful for interspecific transfer of shatter resistance.

Yellow seed coat was associated with higher oil % in *B. carinata* especially.

A useful source of low-glucosinolate *B. juncea* is still not available. No low glucosinolate progeny were found from low glucosinolate seed sent to Australia and Canada; nor could low glucosinolate lines be reisolated at Davis from original plant introduction seed in 1982-83. A single low glucosinolate plant was selected in the 1983-84 UCD nursery from 82r1365-94. Work on characterizing this line will continue one more year.

Brassica Germplasm Release: UC Davis

Release No.	Species	Line Source*	Source UC Intro. No.	1984 Row for Seed Increase	Reason for Interest
UCD-1	B. juncea	Bulk of early PI (India)	--	1039	(Bulk 78) standard check
-2	B. juncea	SPS bulk NU60915	77-1339	1079	large, dark-seeded check
-3	B. juncea	SPS bulk PI 181042	77-920	1119	Seed yield
-4	B. juncea	SPS from NU48110	77-1352	1199	Seed yield
-5	B. juncea	SPS from NU0912	77-1356	1239	Seed yield
-6	B. juncea	SPS from NU60912	77-1356	1279	Seed yield
-7	B. juncea	F5 sel. NU48110 x NU60915		1013	Large dark-seeded, yield (Type I)
-8	B. juncea	F5 sel. NU48110 x NU60915		1017	Large dark-seeded, yield (Type I)
-9	B. juncea	SPS from NU48110	77-1352	1031	Large dark-seeded, yield (Type I)
-10	B. juncea	F5 sel from NU60912 x Leth22A		1325	Large yellow seed (Type I)
-11	B. juncea	F5 sel from NU60912 x Leth22A		1325	Large yellow seed (Type II)
-12	B. juncea	F5 sel from NU60912 x Leth22A		1325	Early, small yellow seed
-13	B. carinata	Bulk SPS PI195, 552	77-1299	1399	Seed yield
-14	B. carinata	Sel from PI193, 761	77-932	1479	Seed yield
-15	B. carinata	SPS from PI 197, 403	77-1304	1439	Earliest carinata sel
-16	B. carinata	Bulk SPS from PI 193,759	77-1285	82-05	Seed yield. Mixed seed colors
-17	B. carinata	SPS from PI 193,759	77-1285	1464	Yellow seed sel
-18	B. carinata	SPS from PI 193,759	77-1285	1469	Yellow seed sel

* PI - USDA plant introduction accession number.
NU - USDA Northern Utilization laboratory accession number.

x *BRASSICORAPHANUS* SAGERET OR x *RAPHANOBRASSICA* KARPECHENKO?

Emiel H. Oost

It seems likely that in a few years' time the first cultivars that are resulting from intergeneric crosses between *Brassica* L. and *Raphanus* L. will be released. By that time registration authorities, plant breeders and all others involved must have reached consensus about the name for this new crop (or crops?).
In papers on the genetics or breeding of the intergeneric hybrid the names x *Brassicoraphanus* as well as x *Raphanobrassica* are being used, mainly depending on the choice of the female parent or the genome formula of the hybrid (AARR resp. RRCC; see a.o. Dolstra, 1982 and McNaughton, 1973).
However, according to the International Code of Botanical Nomenclature (ICBN, 1983), which governs the use of botanical names, there can be only one correct name for a particular hybrid combination, irrespective of the way or direction in which the cross has been made (Art. H.4.). This means that all hybrids between *Brassica* and *Raphanus*, including the reciprokes, can have only one correct intergeneric name, namely the earliest published, legitimate (= in accordance with the rules of the Code) name.
In order to be legitimate, a name of a bigeneric hybrid must have been validly published by means of sufficiently distributed printed matter, stating the names of the parent genera. No description or typification is necessary (Art. H.9.). The name of a bigeneric hybrid is a single word, preceded by a multiplication sign, consisting of both parental genus names, but using the first part or the whole of one, the last part or the last of the other (bur not the whole of both) and, if desirable, a connecting vowel.

x *BRASSICORAPHANUS*

Very likely, the first legitimate name published for the mentioned intergeneric hybrid is *Brassico-raphanus* by Sageret (1826; not mentioned in Index Kewensis!). Under the present rules of the Code this should be corrected to x *Brassicoraphanus* Sageret.
In his paper Sageret states that he has obtained hybrids resulting from a cross of "radis noir" (*Raphanus sativus* L.), fertilized by "chou" (*B. oleracea* L.). He gives a description of the typical, intermediate form of the siliques of these hybrids, which matches well with descriptions of more recent authors (a.o. Dolstra, 1982). His account ends with a statement that the offspring of the hybrids proved to be weak and that he paid no further attention to them.
The same intergeneric name *Brassico-raphanus* was independently used by Fukushima (January? 1929), but only in the title of his paper.

x *RAPHANOBRASSICA*

The first valid publication of the name *Raphanobrassica* is by Karpechenko (August 1929; also not mentioned in Index Kewensis).
Conclusion: The correct name for all hybrids resulting from the cross *Brassica* x *Raphanus* (and the reciproke) is x *Brassicoraphanus* Sageret.

Consequently, x *Raphanobrassica* Karpechenko is a synonym and should not be used.

The question remains, how to denote the different forms/crops which have arisen of may arise in future within x *Brassicoraphanus*. One should wonder if it will prove to be useful to distinguish species within this artificially raised intergeneric hybrid, since still little is known about its variablility.
For the time being, it seems far more adequate to introduce no specific epithets, but to denote the new crops by means of (hopefully internationally accepted) common names. A good proposal has been made by McNaughton (1979) to name the crop resulting from the *Raphanus sativus* L. x *Brassica oleracea* L. cross (with the genomic formula RRCC) "radicole". Plants with the genomic formula AARR (*B. rapa* L. x *R. sativus* L.) have not yet received a common name, but as soon as these plants prove to have commercial value as cultivars, a suitable common name should not be difficult to find.
The author would welcome any remarks or suggestions with regard to this topic.

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FLAVONOIDS IN SOUTH AMERICAN CRUCIFERAE

M. A. Del Pero Martínez and C. Carmona

Many chemical studies have been carried out on glucosinolates in Cruciferae and much is known about them (Kjaer, 1976; Rodman, 1981). It does not happen the same with the flavonoids in the family.

Flavonoids are a group of low molecular weight and biosynthetically related compounds, which are recognized today as being among the most valuable secondary constituents as systematic markers in plants. At present, numerous reports have demonstrated the useful information they provide for taxonomic decisions at species, genera and family levels (see review by Crawford 1978, Young and Seigler, 1981).

The Cruciferae is one of the more easily recognizable family of plants, on morphological grounds. Also from the chemical point of view, it is well characterized by the presence of glucosinolates. However within the family there are some taxonomic difficulties to define genera and species (Heywood, 1976).

Despite the family is best represented in the temperate regions of the northen hemisphere, particularly in the Old World, in Argentine a considerable number of endemisms occurs (Boelcke and Romanczuk, 1984). Most of them belong to the Sisymbreae and Lepideae though other six tribes are also represented in the country.

The purpose of our survey is to find chemical characters to contribute to solve problems of taxonomic arrangements.

Fifty one species from nine genera, mainly Sisymbreae, were analyzed as yet: Sisymbrium (16), Polypsecadium (3), Werdermannia (2), Onuris (5), Xerodraba (3), Rorippa (8), Cardamine (7), Lepidium (2), Menonvillea (5). The results showed that:

1° They are very uniform as far as the aglycones are concerned. They are all flavonols, quercetin is always present together with kaempferol and/or isorhamnetin. Only one exception was found in Sisymbrium irio (introduced in South America), which has the flavone luteolin instead of flavonols. In contrast to the aglycones, a remarkable diversity of glycosides was observed. There is a range from mono to tetraglycosides being the most frequent pattern at 3-7 positions. In addition, acylated glycosides with aromatic acids, or less frequently, aliphatic acids have been identified in various species

(Aguinagalde and Del Pero Martínez, 1982). These structures, of relatively rare distribution in other families, seem to be fairly frequent among the Cruciferae, since they are present in almost all genera analyzed.

2° The data obtained so far have shown that flavonoids are very useful as species markers in Sisymbrium (Del Pero Martínez and Aguinagalde, 1982), Onuris (Del Pero Martínez, 1984) and Rorippa (Carmona and Del Pero Martínez, in prep.), on which intensive studies were carried out. The usefulness of these compounds at higher levels is not clear yet. Further studies in other South American genera are in progress, which will contribute to a better picture on the distribution and taxonomic value of the flavonoids in the family.

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CONSTITUTIVE HETEROCHROMATIN IN SOME MUTANTS OF RADISH

Narshinha Dayal

A study of the amount and distribution of pericentric heterochromatin, represented by chromocentre counts in the interphase nuclei, in four mutants of Raphanus sativus is underway in order to understand the genetics of heterochromatization. Four mutants included in the present report are: 'White Globe' (WG),'Scarlet Long' (SL), 'Dark Scarlet Globe' (DSG) and 'CGS - 7'. The first three mutants have been obtained by high dose gamma rays treatment of dry seeds of the variety 'Scarlet Globe' (SG). These mutants have been characterized morpho-physiologically. WG flowers in 100 days in contrast to SG which takes 120 days on average to flower. Its overall performance is better than SG in conditions here at Ranchi. DSG and SL also differ though insignificantly in flowering time. The mutant CGS - 7, obtained through the courtsey of Professor P. H. Williams, University of Wisconsin, (USA) is a rather weedy plant form and flowers in approximately 18 days from seeding under continuous supplemented illumination. However, under the field condition here, it flowered in approximately 55 days. Interestingly, it was the earliest flowering population, I have studied in Raphanus sativus over the past 15 years (1).

Plants were raised from seeds sown simultaneously in identical field condition. Methods for cytological analysis are same as used earlier (2). Scoring was made in 25 cells per plant. A total of 25 plants, 5 from each form, were examined cytologically.

SG,which served as a control, had a higher mean chromocentre frequency than all the mutants. Individual plants within the population showed negligible variation. The number and distribution of the chromocentres in the nuclei have been presented in Table 1.

Table 1: Number and distribution of chromocentres in the interphase nuclei.

Materials	Number of chromocentres in nuclei 8	9	10	11	12	13	14	15	16	17	Mean° ± SE
SG		3	7	11	22	25	25	20	9	3	13.2 +0.16
WG	1	8	14	24	37	13	13	8	7		12.0 +0.16
DSG		1	1	24	26	20	26	16	6	5	13.1 +0.15
SL	2	13	33	16	36	10	13	2			11.3 +0.14
CGS - 7	10	17	18	24	27	20	9				11.1 +0.15

° Based on the study of 125 nuclei.

There was significant difference in mean chromocentre frequency between SG, WG and SL. The distribution pattern of chromocentre also varied. The rapid cycling mutant CGS - 7 had the lowest mean chromocentre frequency with much changed distribution pattern of chromocentres. Radish can be modelled for studying the cytogenetics of heterochromatin. In recent years there has been a growing awareness about the possible role of constitutive heterochromatin in various genetic functions (2). The present study shows that early flowering mutants, CGS - 7 and WG, have a

lower chromocentre frequency than the late flowering ones. It appears that the number and the distribution of chromocentres, and hence the constitutive heterochromatin, must be some how related with the flowering time. Tanaka (3) has also shown that condensed chromatin influences some physiological characters in radish. The fact that deeply coloured mutant DSG does not differ significantly from SG in mean chromocentre frequency and flowering time is also not less interesting.

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THE INHERITANCE OF PETIOLE CHARACTERS IN PAK-CHOI

Kenneth R. McCammon and Shigemi Honma

Studies were conducted on Pak-Choi (Brassica rapa L., Chinensis group), to develop an appropriate sampling technique for screening petiole width, thickness and angle of adaxial curvature, and to determine the inheritance of these characters. A technique was developed, using the means of the fifth, seventh and ninth petioles, measured at a distance 9 cm from the base of each petiole.

Petiole width was determined to be simple inherited, with narrow petioles being dominant over wide. Petiole thickness was found to be controlled by two major genes, with thick petioles partially dominant over thin petioles. Dominance gene effects appeared most important, although additive gene effects were also significant. A 9:7 recessive suppressor model best explained the observed ratios.

BREEDING OLD AND NEW FORMS OF PURPLE HEADING BROCCOLI

Peter Crisp and Andrew Gray

Introduction

We use the term 'broccoli' to refer to those forms of Brassica oleracea in which the culinary parts are the flower primordia or buds (1, 2). There is a remarkable range of types: from early annuals ('Chinese broccoli', B. alboglabra) to late biennials (winter hardy cauliflowers); and from sprouting to heading forms (with, respectively, many side shoots and a single terminal head). Additionally, the edible floral organ may differ in colour, with white, yellow, pink, orange or green primordia constituting the surface of the head, as in cauliflowers, or with white, yellow, green or purple flower buds. Different combinations of these characters are represented in the various major and minor crop forms of broccoli.

There are two well-established forms which produce single heads composed of purple flower buds. Both are biennials, but they differ genetically and physiologically:

The purple Cape broccoli is a spring-maturing type which became genetically stabilised in Britain about 200 years ago, originating from mixed types introduced from Italy (1). The type no longer exists in Italy, if indeed it ever did. It persists as a poor quality, variable, minor crop grown by amateur gardeners and on a small scale by commercial growers. Genetically, it is undoubtedly close to other biennial broccolis which developed in Britain from the same origins:- the white and purple sprouting broccolis, and the white-curded winter hardy cauliflowers.

The purple Sicilian broccoli is widely grown in southern Italy and Sicily, where it gives a late-autumn/winter crop of pale purple heads. In south east Italy intermediates can be seen between it and the green-curded cauliflowers (eg. cv. 'di Macerata'). It also forms a genetic continuum with the sprouting broccolis of Calabria (in south west Italy), which gave rise to the world-wide crop, 'calabrese-broccoli'.

There are probably other forms of purple heading broccoli which have not yet been described. We know of one, the 'Roxo de Cabela', from Portugal, which is another winter-maturing type giving dark purple heads that are almost loose enough to be termed sprouting.

It seems probable, by the same reasoning as that given in (3), that purple heading broccoli could become an appreciable commercial crop in Britain if improvements were made to uniformity and quality of the existing forms and to continuity of production of the crop type.

We report here our progress in achieving these objectives.

Materials, Methods and Results

Our breeding programme is in three parts.

1) Improvement of purple Cape broccoli

The method is the same as that described for purple sprouting broccoli (4). Briefly, it consists of recurrent selection from a commercially available seedstock, with the objective of producing one or more open-pollinated cultivars maturing in March/April. Levels of self-incompatibility are high, and there is the prospect of further breeding to give F_1 hybrid cultivars. The work started in 1980, and we have progressed through two cycles of selection. Although the new populations have not yet been objectively assessed, there have been clear advances in quality and uniformity.

2) Breeding a new winter-maturing form

Broccoli heads that possess chlorophyll (including the deep purple forms, where the green is masked by anthocyanins) are more frost resistant than the white curds of cauliflowers (5). A winter-maturing purple heading broccoli could therefore be grown in seasons and areas where white cauliflowers cannot.

We constructed a population derived from Cape broccoli (March-maturing), Sicilian broccoli (September) and 'Roxo de Cabela'

(December). The final stages of crossing were made in 1981, and a November/December maturing population with the combination of characters we required was easily selected from this base. We are now assessing the F_4, which intentionally shows considerable variability, but the required phenotype has been fixed. We will treat this in the same way as the Cape broccoli, described above.

3) Breeding a new autumn-maturing form

A population was constructed from autumn cauliflower, Sicilian broccoli, and calabrese-broccoli, with the objective of breeding new green-heading forms of broccoli. An unexpected segregant in an F_4 family was noted in 1979. This was a self-compatible plant which matured about three months after sowing, and bore a deep purple head. Its selfed progeny revealed no further segregation for the type. This material was multiplied and samples distributed to growers in 1983 and 1984; it is currently being further multiplied for release as the cultivar "Rosalind".

Conclusions

The basis of our approach has been that heading character, head colour, and maturity time are highly heritable. Our results have justified this assumption, and it should soon be possible to produce a better continuity of purple broccoli heads in Britain (Table 1).

Table 1 Production sequence for purple heading broccoli in Britain.

Heading time	Crop type	Sowing time
August-September	cv. Rosalind	May-June
September-October	Purple Sicilian	May-June
November-December	Recombinant type (see text)	June
March-April	Purple Cape	June

Breeding new types is, however, only a first step in procuring this continuity: each crop type is likely to have its own peculiarities (good and bad) which growers must learn to accommodate. We already know, for example, that if cv. Rosalind is sown before May, then it is likely to produce heads which resemble poor quality cauliflower curds.

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SELECTION OF F_5 LINES OF WINTER RAPESEED WITH SPECIFIC FATTY ACID COMPOSITION

D. L. Auld, J. E. Crock, R. A. Korus, and C. L. Peterson*

Rapeseed cultivars need to produce oils with a specific fatty acid composition (6). Oils used for human composition require large concentrations of oleic (18:1) or linoleic acid (18:2). Rapeseed oil destined for use in industrial markets needs high concentrations of erucic acid (22:1) (10). A minimal concentration of linolenic acid (18:3) is desired in all oils since this fatty acid undergoes rapid oxidation forming rancid oils in storage and harmful polymers when the oil is used as a fuel for diesel engines (2). Fatty acids in winter rape are formed by a stepwise biosynthetic pathway in which oleic acid (18:1) either undergoes decreasing saturation to form linoleic acid and then linolenic or further chain elongation to form eicosenoic and then erucic acid (8). Edible rapeseed oil usually has less than 2% erucic acid (11). Industrial rapeseed oil contains oleic, linoleic and linolenic acids as well as eicosenoic and erucic acid (7,8,9). Because they are usually esterified at only the one and three positions of the glycerol molecule, a maximum of 66% of the fatty acids in rapeseed can be either erucic or eicosenoic acid (5). Most commercial cultivars of winter rape usually produce oil with less than 60% erucic acid. Only limited work has been done on increasing the concentration of erucic acid (1,3,4,5). The purpose of this research was to screen the F_4 families and F_5 lines of three crosses of winter rape for fatty acid composition. F_5 lines were selected as either edible or industrial oil types based on their fatty acid composition. The selection gain in each cross was calculated.

Seedlings of both F_4 and F_5 lines were grown at 21°C under a 16 hour photoperiod for two weeks. The plants were then vernalized for eight weeks in a cold room at 4°C with a 9 hour photoperiod provided by 40-watt Gro-lux fluorescent lights. The plants were then moved to the greenhouse and grown to maturity at 21°C with a 16 hour photoperiod provided by metal halide lamps.

Seven seeds of each line were ground to a fine powder and mixed with 5 ml of diethyl ether for 30 sec. The mixture was filtered and 3 ml of supernatant of each sample was treated with 0.2 ml of 20% tetramethyl ammonium hydroxide in methanol. Samples were agitated for one minute and then washed with distilled H_2O. Fatty acid analyses were performed on a Hewlett-Packard Model 5790 gas chromatograph equipped with a flame ionization detector and a Model 3390A peak area integrator. The glass column was packed with 3% SP 2310 and 2% SP 2300 on a 100-120 mesh Chrome W solid phase (Supelco Inc., Bellefonte, PA). The column was maintained at 230°C with helium gas as the carrier.

Both Indore and Norde have low levels while Sipal and WW 827 have fairly high concentrations of oleic acid. As expected, the cross between the two parents with low levels of oleic acid (C2) did not segregate for oleic acid concentration (Table 1). Transgressive segregates with over 70% oleic acid were obtained when WW 827 and Sipal

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were crossed to Indore. The selection gains from those two crosses were 94.6 and 61.7%, respectively, which indicates that the concentration of oleic acid is a highly heritable trait which responds rapidly to selection.

Table 1. Fatty acid composition, and selection gain for three crosses of winter rape selected for specific fatty acid compositions.

Indice	Unselected F_4 Pop.	Selected F_4Pop.	Unselected F_5 Pop.	Selection Gain+
	-----% Fatty Acid Composition-----			----%----
Oleic Acid (18:1)				
C1-WW 827 x Indore	35.0	72.0	70.0	94.6
C2-Norde x Indore	--	--	--	--
C3-Sipal x Indore	39.2	69.1	57.7	61.7
Linoleic Acid (18:2)				
C1-WW 827 x Indore	11.0	8.7	16.5	-237.7
C2-Norde x Indore	10.4	7.4	16.4	-198.0
C3-Sipal x Indore	11.0	8.0	21.9	-364.9
Linolenic Acid (18:3)				
C1-WW 827 x Indore	6.2	4.7	4.0	142.2
C2-Norde x Indore	6.1	4.6	3.9	146.7
C3-Sipal x Indore	6.6	5.0	3.4	209.0
Eicosenoic Acid (20:1)				
C1-WW 827 x Indore	12.9	11.0	10.7	113.5
C2-Norde x Indore	12.1	10.8	8.0	350.0
C3-Sipal x Indore	13.9	11.5	10.5	141.8
Erucic Acid (22:1)				
C1-WW 827 x Indore	30.3	48.5	42.5	67.2
C2-Norde x Indore	39.7	50.2	53.4	131.1
C3-Sipal x Indore	24.7	49.5	44.3	79.0

+Selection Gain = $\frac{\text{(mean of } F_5 \text{ pop. - mean of unselected } F_4 \text{ pop.)}}{\text{(mean of selected } F_4 \text{ pop. - mean of unselected } F_4 \text{ pop.)}}$

Because Indore has high levels of erucic acid, all three crosses segregated for erucic acid concentration (Table 1). However, the cross between Indore and Norde (C2) had transgressive segregation for erucic acid concentration. Some F_5 lines had over 60% erucic acid which approaches the biological limits for this fatty acid (5) and exceeded either parent by 6-7%. Selection gain for C2 was 131.1% compared to 67.2 and 79.0% for C3 and C1, respectively. It was interesting to note that C2 also had a 350% selection gain for reduced levels of eicosenoic acid, the biosynthetic precurser of erucic acid. It's possible that the higher levels of erucic acid observed in C2 were partially the result of more efficient conversion of eicosenoic to erucic acid (8).

Selection for low concentrations of linolenic acid reduced the concentration of this fatty acid from an average of 6.3% in the unselected F_4 populations to 3.8% in the F_5 lines (Table 1). The selection gain for

reducing this fatty acid ranged from 142.2 to 209.0% in the three crosses. However, as the concentration of linolenic acid was reduced, the level of linoleic acid more than doubled. The unselected F_4 families had an average of 10.8% and the selected F_4 familes had an average of only 8% linoleic acid. The F_5 lines derived from this selection had an average of 18.3% linoleic acid. Trying to reduce the level of polyunsaturated fatty acids in these crosses was apparently futile since reducing the synthesis of linolenic acid more than doubled the level of linoleic acid. Lines need to be identified that produce low levels of both linolenic and linoleic acids to significantly reduce the level of polyunsaturated fatty acids in rapeseed oil (8). These results indicate that hybridization of existing cultivars of winter rape seed and subsequent selection could produce lines with improved fatty acid composition for a wide range of specific markets.

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TURNIP ROOT-FLY RESISTANCE IN SWEDES

S. Gowers, I.K. Munro and D.J. Gemmell

The new SCRI swede cultivars Angus and Melfort have higher dry matter contents than previous cultivars and they have also been found to possess good resistance to turnip root-fly (TRF, Delia floralis L.). A trial was carried out to test the hypothesis that resistance was associated with high dry matter content and to examine the mode of inheritance of TRF resistance.

The swede lines sown in the trial were: Angus and Melfort; their parental cultivars Bangholm Wilby (common parent), Wilhelmsburger Danila and Gullacker III; crosses of Angus and Melfort with Ruta Otofte, Vogesa and Merrick, and five commercial cultivars as given in Table 1.

The trial was sown in 1981 at Crichton Royal Farm, the experimental ground of the West of Scotland College of Agriculture, where attacks by TRF are often quite severe. Four replicates were sown in randomised blocks, with a plot size of 10 x 0.7 m. Twenty plants from each replicate were sampled and the number of TRF tunnels in each bulb were scored. The incidence of TRF was low, but the results were reasonably consistent over replicates. The number of plants attacked and the total number of TRF tunnels in each line are given in Table 1, along with dry matter contents for the commercial lines.

Table 1. The number of infested plants and total number of TRF tunnels and the dry matter content of the commercial cultivars in trial

Cultivar/line	DM%/	No. infested plants	Total no. tunnels	Cultivar/line	No. infested plants	Total no. tunnels
Angus	12.7	3	3	Bangholm Wilby	4	13
Melfort	12.5	6	9	W. Danila	3	14
				Gullacker III	9	20
				Angus x Ruta	0*	0*
Sator Otofte	12.2	19	66	Angus x Vogesa	4	8
Vogesa	10.9	11	31	Angus x Merrick	4	9
Merrick	9.8	15	29	Melfort x Ruta	8	9
Doon Major	9.5	14	25	Melfort x Vogesa	4	5
Magres	11.4	11	36	Melfort x Merrick	5	8

/ - National List Trial data

* - 2 replicates only: for comparison, Angus and Angus x Merrick also had no attack in these two replicates.

Considering the somewhat sporadic attack, three conclusions can be drawn from the data:

1. TRF resistance is not consistently associated with high dry matter content and, if anything, it would appear to be negatively correlated with dry matter content. The regression of number of tunnels on DM% for

the commercial cultivars has a slope of - 2.0 ± 7.0 when Angus and Merrick are included, but when they are excluded the slope is + 12.6 ± 4.4.

2. TRF resistance appears to be dominant: the six hybrid progenies from crosses with Angus and Melfort all have equal or lower scores than Melfort for total number of TRF tunnels.

3. The incidence of natural infection is uncertain and could not be relied upon for the breeding and selection of TRF resistant lines. More reliable sites may be available, but a method of indirect selection or a technique for artificial infestation may be required before a breeding programme for resistance to turnip root-fly could be undertaken.

Thanks are due to Valerie Heppel, A.R. Whitelaw and R.M. Stewart for their help in organising, sowing and scoring the trial.

'BASANT' A NEW PLANT TYPE IN YELLOW SARSON (BRASSICA CAMPESTRIS L.) SPP. OLEIFERA (METZG.) SINSK.

H.C. Saini and Upander Kumar

Rape and mustard as a whole has maximum yield potential of 15-20 q/ha against the National average of 6.5 q/ha under normal fertility and irrigated conditions. Further improvement in yield can be possible if varieties are developed with the following characteristics.

1. Resistant to aphids and disease like Alternaria and white rust.

2. Early flowering (30-35 days) and early maturing (125-135 days) so that they can escape from the infestation of aphids and infection of diseases and can help the farmer to adjust sarson crop rotation.

3. A suitable genotype which may fit well under high plant density as has been done in wheat and rice for achieving higher yield levels. This type of plant material will naturally be responsive to a high level of fertility also.

In view of the above facts, a detailed account of recently developed strain having the above mentioned characteristics is given here and named as 'Basant'. The performance of this strain was observed under close spacing at 30 cm row to row distance in 9 sq metre unthinned plots replicated thrice in a randomised block layout with 27 other strains during 1983.

Major Characteristics

This strain flowers in 32 days and matures in 129 days with a plant height of 135 cms. The average numbers of primary and secondary branches observed on a single plant basis were 11.5 and 10.4 respectively. The main fruiting axis attained a height of 61.4 cms with 40 siliquas and the average length of the first and last siliqua was 6.6 cms. Each siliqua contained 34 well filled seed. The harvest index taken as the percentage seed yield of biological yield, was 29.17 per cent and seed weight was 6.7 gm/1000 seed. The oil percentage from a bulk sample showed 47.7 per cent recovery of oil as estimated by NMR. A seed yield of 29.5 q/ha with 50-52 plants/sq metre was obtained in this strain. However, its maximum yield potential of 47.7 q/ha could be realised under high density with 63 plants/sq metre compared with 24.8 q/ha from the control variety DYS1 at its optimum level of plant density of 17 plants per square metre at 60 cm row spacing.

SELF-INCOMPATIBILITY IN INDIAN CAULIFLOWER

S.S. Chatterjee and V. Swarup

The self-incompatibility status of different Brassica oleracea specially in case of Kale, Cabbage, Brussels sprouts and sprouting broccoli, has been studied in more detail than in cauliflowers. In cauliflower, Watts (1963; 1965) found that the winter and autumn types of Europe are moderate to strong in their self-incompatibility while it is rather weak in summer cauliflowers. Jensma (1957) and Nieuwhof (1963) also found a high degree of selfing in Erfurt and Snowball types of European summer cauliflowers.

In recent years studies have been made in the Indian cauliflowers as well. Early in 1961, Chatterjee and Mukherjee reported that individual plants in a variety of early Indian cauliflower, vary in their degree of self-incompatibility and it is possible to develop self-compatible lines in them. The Indian cauliflowers have been developed mainly from the European winter types and hence it is but natural that these should show moderate to strong self-incompatibility. From the studies in the different maturity groups in these types of cauliflower, Swarup and Chatterjee (1972) reported that the early types (maturity group I) show much stronger self-incompatibility than the others while Ram (1975) and Singh et al. (1981) found somewhat strong self-incompatibility in all the maturity groups. Hoser-Kranze (1979) working on Pusa Katki, an early Indian variety, concluded that the Indian cauliflowers are comparatively strongly self-incompatible.

Considering all these reports, studies were conducted from 1976 to 1982 in the Indian Agricultural Research Institute New Delhi to find out the level of incompatibility reaction in the different maturity groups more intensively undertaking inbred lines and open pollinated varieties developed in the agricultural universities and also from the seed companies which may not have gone into thorough selection under the present condition of seed production in India. In all 260 individual plant progenies were studied from eleven inbred lines, nine open-pollinated selected varieties from agricultural universities and nine varieties from the seed companies representing all the three maturity groups, classified by Swarup and Chatterjee (1972). These were studied in the open field condition in five pollination treatments eg natural cross-pollination, artificial cross-pollination, bud-pollination, self-pollination in freshly opened flowers and natural self-pollination. The results were analysed from the average seed set data under each pollination treatment and the fertility indices were computed from these data as suggested by Watts (1963) and Nieuwhof (1974). These fertility indices were used as the measures of self- and cross-incompatibility/ compatibility of the individual plants. The S-alleles isolated earlier were used to study the interactions of the reciprocal crosses between each S-allele heterozygote and its two corresponding homozygotes. S-allele activity was quantified according to the method advocated by Wallace (1979).

From the results it was found that irrespective of inbred selected open-pollinated varieties of the universities or of the seed companies, those of Group I maturity showed stronger self-incompatibility reaction while those of Group III were rather weak in self-incompatibility reaction (Murugiah, 1978;1982, Vidyasagar, 1981; Saha, 1983). The Group II occupied an intermediate position specially in the inbred lines (Vidyasagar, 1981; Murugiah, 1982) while the open-pollinated varieties showed moderate to strong self-incompatibility reaction (Saha, 1983). Homozygous S-allele lines have been isolated from both Group I and II though more in number from the former. Under field conditions, it was not possible to isolate any S-allele from the Group III maturity (Vidyasagar, 1981; Murugiah, 1982). All the four types of interaction between the sexual organ and S-allele were noted (Saha, 1983) though type III was very few as reported by Hozer-Krauze (1979). The activity of the S-alleles was found to vary from zero to 100 percent in both pollen and stigma. The S-alleles isolated were also studied for their dominance relationship and their stability (Murugiah et al., 1983).

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CROSSABILITY BETWEEN DIFFERENT POLYPLOIDS AND CHROMOSOME NUMBERS OF THEIR PROGENIES IN RAPE (*BRASSICA NAPUS* L.)

S. Tokumasu

In rape, tetraploids and triploids are decidedly inferior to diploids with regard to seed fertility, the amount of their seeds being only about 10% of diploids (Tokumasu 1984*). Therefore, tetraploids and triploids are of no practical use in breeding. However, it is interesting from a cytogenetical viewpoint to examine crossability between different polyploids and to clarify chromosome variation among their progenies. Results of crossing experiments are shown in Table 1.

Table 1. Results of crossing between different polyploids

Combination (♀) × (♂)	No. of pollination	No. of siliques	No. of seeds	No. of seeds per pollination	
3x × 2x	205	202	727	3.55	No. tri-
2x × 3x	107	90	181	1.69	ploid
4x × 3x	77	71	90	1.17	seeds
3x × 4x	61	34	17	0.28	√
4x × 2x	178	154	165	0.93	(0.72)
2x × 4x	177	162	70	0.40	(0.01)
2x × 2x	99	99	2399	24.23	
4x × 4x	97	81	254	2.62	

Fertility is always better when plants with higher chromosome numbers are used as female parents than when used as male ones (3x × 2x > 2x × 3x; 4x × 3x > 3x × 4x; 4x × 2x > 2x × 4x). When comparison is made among the crosses in which plants with higher chromosome numbers are used as female parents, the sequence of their seed fertility is 3x × 2x > 4x × 3x > 4x × 2x. Among the crosses involving female parents with lower chromosome numbers, the same sequence is indicated, i.e., 2x × 3x > 3x × 4x > 2x × 4x (in 2x × 4x, only triploid seeds are considered).

The progenies from these crosses had chromosome numbers as shown in Table 2.

Table 2. Chromosome numbers found in the progenies from crosses between different polyploids

Combination	Chromosome numbers in progenies
4x × 2x ⟶	2n=41 ~ 50, 53 ~ 58.
2x × 4x ⟶	2n=29 ~ 31, 38, 56.
3x × 2x ⟶	2n=37 ~ 52.
2x × 3x ⟶	2n=38 ~ 48.
4x × 3x ⟶	2n=54 ~ 69.
3x × 4x ⟶	no viable seeds.

Crosses between tetraploids and diploids gave not only triploids but also aneuploids whose chromosome numbers were below triploids. The occurrence of aneuploids which had smaller chromosome numbers than diploids was peculiar. These plants were perhaps originated parthenogenetically from eggs which underwent irregular meiotic division. Crosses between triploids and diploids as well as between tetraploids and triploids produced plants whose chromosome numbers were located between their both parents.

Details will be published in future (Mem. Coll. Agr., Ehime Univ. 29, 1984 in press).

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INTERGENERIC HYBRIDS BETWEEN RAPHANUS SATIVUS AND BRASSICA NIGRA

Y. MATSUZAWA and M. SARASHIMA

Interspecific and intergeneric hybridization have great potential in breeding programs of cruciferous crops. In our studies, some species of Brassica, Raphanus, Sinapis and Eruca have been crossed to breed hybrid progenies, through which it may be accomplished to synthesize de novo species and to transfer the useful characteristics to leading varieties. Moreover it may be possible to rear not only chromosome addition and substitution lines but cytoplasm substitution ones via hybrid progenies.

Low cross-compatibility is , however, the fundamental barrier in these works. This problem is now in part being overcomed by culturing in vitro of excised or extracted embryos, ovules and ovaries. Recently reciprocal hybrid plants were obtained between R. sativua and B. nigra. The data in hybridization are as follows.

Table 1. Hybridization in R. sativus x B. nigra by embryo culture

Female R. sativus	Male B. nigra	P G I* mean (range)	Flowers pollinated	Pods developed	Ovules enlarged	Embryos extracted cultured	F1 hybrids
24 varieties of Japanese radish	Ni 110	0.7 (0-3.3)	2560	131	157	16	3

*Pollen Germination Index = $\frac{b + 2c + 3d + 4e}{a + b + c + d + e}$

a: No. of pistil in which no pollen grain was recognized, b: No. of pistil in which pollen grains showed no germination, c: No. of pistils in which pollen grains germinated on the stigma, d: No. of pistils in which pollen tubes reached to the stylar tissue, e: No. of pistils in which pollen tubes penetrated near or to the ovule.

Table 2. Hybridization in B. nigra x R. sativus by ovary culture

Female B. nigra	Male R. sativus	P G I mean (range)	Ovaries cultured	Ovules enlarged	Embryos extracted cultured	F1 hybrids
Ni 110	6 varieties of Japanese radish	1.2 (0.8-2.1)	954	42	18	4

Among them an amphiploid (B. nigra x R. sativus) showed seed fertility and following progenies are under investigation. As far as we know Brassicoraphanus with BBRR genome constitution have not been bred although that of AARR and CCRR were reported. We would greatly appreciate receiving informations as to the synthesis and utilization of this hybrid.

PRODUCTION OF INTERSPECIFIC HYBRIDS BETWEEN BRASSICA CAMPESTRIS AND B. OLERACEA IN COMBINATIONS OF DIPLOID AND AUTOTETRAPLOID BY OVARY CULTURE IN VITRO AND THEIR PROGENIES

Nobumichi INOMATA

Production of interspecific hybrids between B. campestris and B. oleracea was comparatively easily by ovary culture (Inomata 1977, 1978a, 1978b, 1979, 1983b). In the previous papers, hybrids were obtained in many cultivars between B. campestris and B. oleracea, and the cytogenetical studies on the F_1 hybrids and their progenies were also reported (Inomata 1980, 1983a, 1983b). The present paper deals with the production of interspecific hybrids between B. campestris and B. oleracea in combinations of diploid and autotetraploid.

The materials used in the experiment were two diploids and two autotetraploids of B. campestris, and one diploid and one autotetraploid of B. oleracea. Ovaries were obtained from the cross of B. campestris x B. oleracea, and they were excised from the plant and cultured in vitro. The culture method was the same as a previous paper (Inomata 1978b). The medium used in the present experiment was Nitsch and Nitsch's (1969) minerals with 50g/l of sucrose and 8g/l of agar.

The results are shown in Table 1. The production of interspecific hybrids was observed in all cross combinations. The frequency of hybrid production was high. All plants obtained showed the hybrids. The first meiotic division and pollen fertility were examined in the F_1 hybrids.

In the cross of diploids B. campestris x B. oleracea, all hybrids had 19 chromoosmes and 5 out of them were examined cytogenetically. Pollen fertility was 6.8% in 500 pollen grains were counted in each hybrid, and mean frequency of PMCs showing $9_{II}+1_{I}$, $1_{III}+8_{II}$, $8_{II}+3_{I}$ and $1_{III}+7_{II}+2_{I}$ was 70.5%, 18.7%, 9.6% and 0.06%, respectively, in 156 cells observed. No seed was obtained in 204 self-pollinated capsules examined and 6 seeds were obtained in 25 open-pollinated capsules examined.

In the cross of diploid B. campestris x autotetraploid B. oleracea, all hybrids had 28 chromosomes and 5 out of them were examined cytogenetically. Mean pollen fertility was 15.4% and mean frequency of PMCs showing $9_{II}+10_{I}$, $10_{II}+8_{I}$, $1_{III}+8_{II}+9_{I}$ and $2_{III}+7_{II}+8_{I}$ was 31.3%, 20.0%, 6.9% and 2.6%, respectively, in 115 cells observed. No seed was obtained in 313 self-pollinated capsules examined and 10 seeds were obtained in 148 open-pollinated capsules examined.

In the cross of autotetraploid B. campestris x diploid B. oleracea, all hybrids had 29 chromosomes and 4 out of them were also examined. Mean pollen fertility was 20.6% and mean frequency of PMCs showing $10_{II}+9_{I}$, $1_{III}+9_{II}+8_{I}$, $13_{II}+3_{I}$ and $2_{III}+8_{II}+7_{I}$ was 70.8%, 21.6%, 1.7% and 1.7%, respectively, in 120 cells observed. Fifteen seeds were obtained in 255 self-pollinated capsules examined and 48 seeds were obtained in 107 open-pollinated capsules examined.

In the cross of autotetraploids B. campestris x B. oleracea, three hybrids had 38 chromosomes and one out of them was examined. Pollen fertility was 90.8% and no PMCs were examined. Many self-pollinated seeds were obtained.

No seed was obtained in all crossing experiment under natural con-

dition except in the cross of diploid B. campestris x autotetraploid B. oleracea. Twenty capsules were examined and 32 seeds were obtained and they had 28 chromosomes.

Ovary culture in vitro for hybrid production was useful between B. campestris and B. oleracea in combinations of diploid and autotetraploid.

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Table 1. Production of interspecific hybrids between B. campestris and B. oleracea in combinations of diploid and autotetraploid by ovary culture

Cross combination (B. campestris x B. oleracea)	No. of capsules examined (A)	No. of hybrids obtained (B)	B/A x 100
ssp. chinensis (2x) [1] x var. acephala (2x)	45	3	6.667
ssp. pekinensis (2x) [2] x var. acephala (2x)	43	9	20.930
Total or mean	88	12	13.636
ssp. chinensis (2x) [3] x var. acephala (4x)	43	77	179.070
ssp. pekinensis (2x) [4] x var. acephala (4x)	45	15	33.333
Total or mean	88	92	104.545
ssp. chinensis (4x) [5] x var. acephala (2x)	21	0	-
ssp. pekinensis (4x) [6] x var. acephala (2x)	45	11	24.444
Total or mean	66	11	16.667
ssp. chinensis (4x) [7] x var. acephala (4x)	23	1	4.348
ssp. pekinensis (4x) [8] x var. acephala (4x)	43	2	4.651
Total or mean	66	3	4.545

1: cv. Seppaku-taina x cv. Portuguese kale. 2: cv. Kyoto-hakusai No. 1 x cv. Portuguese kale. 3: cv. Seppaku-taina x cv. Tema kale. 4: cv. Kyoto-hakusai No. 1 x cv. Tema kale 5: cv. Seppaku-taina x cv. Portuguese kale. 6: cv. Chifu-hakusai x cv. Portuguese kale. 7: cv. Seppaku-taina x cv. Tema kale. 8: Chifu-hakusai x cv. Tema kale.

SOLUBLE SUGAR CONTENT AND WINTER HARDINESS IN SWEDES

S. Gowers and D.J. Gemmell

Recent work by Millard, Bain and Chesson (1984) examined the effect of overwintering on the chemical composition of nine cultivars of swede, which were classed as having 'low', 'medium' or 'high' winter-hardiness. The main differences in overwintering response involved the soluble sugar contents. The high winter-hardiness cultivars had the lowest soluble sugar levels in November, but the highest levels in March, with a mean increase of over 20 per cent. The opposite result was obtained with the low winter-hardiness cultivars, and the medium ones had intermediate levels of soluble sugars at both sample dates. These results would suggest that soluble sugar levels were related to winter-hardiness, and that selection for high sugar content could lead to selection for winter-hardiness as a correlated response. MacDearmid (1978), however, reported a decrease in soluble sugar content overwinter in one of the high winter-hardiness cultivars used by Millard et al.

Gowers, Borzucki and Gemmell (1982) gave the soluble sugar contents of several lines of swede sampled in November and March. No direct comparisons were made between soluble sugars and winter-hardiness, primarily because there appeared to be little relationship. In view of the results of Millard et al., however, further comments now appear necessary. Winter-hardiness scores for the lines tested are given in Table 1, along with their soluble sugar contents at the two harvest dates. The swede lines used were partially inbred lines derived from commercial cultivars.

Table 1. Soluble sugar contents of thirteen swede lines in autumn and late winter and their winter-hardiness scores (L = low; M = medium; H = high)

Swede line code name	Winter-hardiness	Soluble sugar contents 14.9.81	29.3.82	% change overwinter
BR a	L	36.4	41.5	+ 14
b	M	20.2	38.2	+ 89
c	M	24.7	41.5	+ 68
EK a	L	35.4	42.4	+ 20
b	L	39.2	39.9	+ 2
c	L	41.0	42.7	+ 4
BW a	H	31.5	39.7	+ 26
b	H	28.0	40.3	+ 44
c	H	35.2	35.5	+ 1
CR a	L	37.5	49.7	+ 33
b	M	40.3	52.3	+ 30
BM a	M	33.4	47.2	+ 41
BD a	M	32.2	45.2	+ 40

These results were taken after a very severe winter, and the winter-hardiness is given relative to the BW lines, which are extremely winter-hardy. Reclassification of the lines as 'medium', 'high' and 'very high' may make the results more comparable with those of Millard et al. There is also another problem with classification, however, which involves the samples taken. To obtain samples in March, the plants sampled must have survived the winter. With low winter-hardiness lines, therefore, this means that the sampled plants may be atypical and possess a greater than normal degree of winter-hardiness.

Irrespective of classification difficulties, some of the results do not support the main findings of Millard et al. Of the three lines showing little or no increase in soluble sugar content overwinter, there were two lines with low winter-hardiness (EKb, EKc) and one with high winter-hardiness (BWc).

Two of the BR lines (BRb, BRc), classified as medium winter-hardy, gave the largest increases in soluble sugars; this, however, was more a reflection of the low sugar levels in September rather than the production of higher levels in March. Obviously there is a great deal of variation in soluble sugar levels over the winter, and the results obtained with any particular line will depend upon the sampling times taken in relation to the increasing or decreasing levels of soluble sugars.

The lines with the highest soluble sugar contents (CRa, CRb) are not the highest winter-hardy lines; in fact the CRb line has 30% more soluble sugars than the highly winter-hardy BWb. This result is from comparisons made of sugar contents taken as a proportion of the dry matter; for correlations with winter-hardiness it should be more appropriate to take the soluble sugars as a proportion of the fresh weight. Making comparisons on this basis, however, the soluble sugar content of CRb is still 14% higher than BWb.

In conclusion, these results show that there is considerable variation between and within cultivars for soluble sugar contents, and that levels of soluble sugars could probably be increased by selection. Soluble sugars may have an important role to play in winter hardiness, but the relationship is by no means a simple one.

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PRELIMINARY STUDIES ON GRAFT HYBRIDIZATION IN BRASSICA

Surinder S. Banga and K.S. Labana

Graft transfer of mendelian characters in tomato (Glavinic, 1975) and red peppers (Ohta & Chuong, 1975) and of cytoplasmic male sterility in Petunia (Frankel, 1956) and alfalfa (Thompson and Axtell, 1978) has been reported. Contrarily, negative graft transfer results are also available in wheat, tobacco and faba beans. We initiated some experiments to test the efficacy of this system in Brassica.

Materials and Methods

Four important species of Brassica eg B. Juncea, B. napus, B. carinata and B. campestris were used as the experimental material. Direct as well as reciprocal grafts in all combinations were attempted. Cleft grafting technique as suggested by Bernardo et al (1970) was utilised. Grafts were carried out on the main shoot before flowering shoot emergence. Young scion was invariably grafted on to more mature stock. Immediately after grafting, the plants were covered with polythene coverings and irrigated heavily. New buds or branches emerging on the stock crown were periodically removed.

Results and Discussion

The intraspecific grafting (control) was highly successful and had normal flowering, pollen fertility, open pollinated fertility and well developed seeds (Table 1). On the other hand, interspecific combinations gave varied responses. In some cases, the frequency of successful grafts was low whereas in some, the subsequent growth of the scions was slow ultimately having less and irregular flowering. In general, pollen fertility was very low and seed setting was absent or aborted seeds were produced. An exception was the B. campestris graft on B. juncea which had normal flowering and seed setting. A few normal seeds could also be obtained from B. napus grafted on to B. carinata but only after pollinating with stock pollen.

A significant observation was the early flower initiation of B. napus grafted on to B. juncea. Grafted B. napus was 7-10 days earlier than the control indicating the transfer of flowering hormones across the graft from the B. juncea stock. The present experiments have demonstrated that grafting is possible in Brassica. Further studies are needed and are being carried out to assess the possibility of inducing graft transformation.

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Table 1. Results of graft hybridization in Brassica

STOCK \ SCION	B. juncea	B. napus	B. carinata	B. campestris
B. juncea	Compatible, normal pollen and seed fertility.	Compatible, low pollen fertility aborted seeds.	Compatible, very low pollen fertility, no seed setting.	Compatible, normal pollen and seed setting
B. napus	Poorly compatible, no seed setting.	Compatible, normal pollen and seed setting.	Not compatible	Not compatible
B. carinata	Compatible, very low pollen fertility, aborted seeds.	Compatible, low pollen fertility, aborted seeds.	Compatible, normal pollen fertility and seed setting.	-
B. campestris	-	-	-	Compatible, normal pollen and seed setting.

SYNTHETIC *BRASSICA CARINATA* - A PRELIMINARY REPORT

Shyam Prakash, Sarla Gupta, R.N. Raut and Anita K. Kalra

In recent years, Ethiopian mustard *Brassica carinata* has been given considerable attention as an oilseed crop. In India, it has shown a high seed yield potential of up to 36 q/ha. Additionally, it is very suitable for growing under moisture stress conditions and possesses a high degree of resistance to diseases like *Alternaria* blight and white rust besides being highly tolerant to aphids which cause heavy loss to Indian Brassicas. In spite of these advantageous features, its cultivation is not possible on a commercial scale mainly because of its very long duration (around 180 days). The growing period for late maturing Brassicas in India is restricted to roughly 150 days due to an abrupt rise in temperature at the time of harvest.

To generate new variations with the major objective of evolving early and productive types, we obtained various artificial alloploid forms of *B. carinata* by hybridizing different ecotypes of *B. oleracea* such as vars *botrytis*, *italica*, *alboglabra* and *capitata* with two strains of *B. nigra*. Hybrid frequency was very low either by hand pollination or resorting to culturing *in vitro* of pollinated ovaries. In all these cases, *B. oleracea* was the female parent as against earlier reports where *B. nigra* was used as female. This may also result into some new forms.

The important variations obtained were dwarf types (90 to 120 cm tall), and very close arrangement of a large number of pods on the long fruiting branches - the pod number ranged from 80-117. Some of the synthetic alloploids, particularly those involving cauliflower were of early maturity. These constitute useful genetic variants which are being exploited in breeding and evolving high seed yielding and early maturing *B. carinata* at the Indian Agricultural Research Institute, New Delhi.

INTERSPECIFIC SOMATIC FUSION BETWEEN *BRASSICA NAPUS* AND *BRASSICA HIRTA*

Catherine Primard

Interspecific hybridisation is one of the various ways to increase genetic variability when required for plant breeding. Within the genus *Brassica*, hybrids between two cultivated species, *B. napus* and *B. hirta* (or *Sinapis alba*) were wanted for two purposes:

1 - The first was transferring the *B. napus* nuclear genome to the *B. hirta* cytoplasm, in order to test whether this combination could produce a cytoplasmic male sterility system or not.

2 - The second was obtaining nuclear hybrids to transfer interesting traits from one species to the other.

In the 1940's, F1 hybrids were obtained but were completely sterile (in 'Brassica crops and wild allies', Tsunoda *et al* ed.); we have no information about chromosome doubling of these F1 plants.

The quickest way to achieve these objectives is to use the protoplast fusion and plant regeneration techniques (as used by Pelletier *et al*, 1983). Fusion experiments were performed between protoplasts of *B. napus* cv. Brutor and those of *B. hirta* cv. Carine.

B. hirta protoplasts could develop into actively dividing colonies but were unable to differentiate shoots in our culture conditions; this constituted a means of selection against this parent at the last *in vitro* step.

Three successive fusion experiments (October 1983) allowed us to regenerate about 1800 plants; 1500 could be transferred to soil culture in the greenhouse. Even during *in vitro* growth, 14 of these plants could be distinguished by morphological traits intermediate between those of the parents cultivated in the same conditions (colour, leaf shape, presence of hairs). All the others were of the *B. napus* type and were planted in the field.

In June-July, all but one plant flowered. At that time, the 14 plants presented various morphologies but always had some traits of both parents (inferior epidermis cell shape, pistil shape, inflorescence habit, odour ...); one plant did not open its flowers; the other 13 produced pollen more or less abundantly.

Several studies are now under way to characterise these plants:

1 - Pollen viability: pollen is put to germinate on a solidified medium (Guerche pers. comm.). For 7 plants analysed so far, about 1/5th of the pollen is able to germinate, compared to the parental pollen. Lots of small aborted pollen grains are observed in all cases.

2 - Meiotic behaviour of the chromosomes: preliminary results on the first plants indicate that their chromosome number is approximately the sum of the parental ones.

3 - Comparison of the restriction patterns of the nuclear DNA coding for ribosomal RNA, identified by Southern hybridisation with a labelled probe.

4 - Analysis of chloroplastic DNA: it has been already performed by Vedel (C.N.R.S. Gif sur Yvette): 2 of the 14 plants contain B. hirta chloroplasts and the remaining ones have those of B. napus. This confirms that at least for these two plants a fusion event took place, followed by plant regeneration.

Self pollination and crosses with the parents were done on each flowering plant. All of them developed siliquae, having an intermediate shape; however, only a few of these contained one or two mature seeds; nevertheless, these seeds could germinate and give little seedlings. We have begun in vitro embryo culture in order to maximise offspring numbers.

Knowing the number of somatic hybrids we obtained, it is highly probable that among the regenerated plants several tens should be the result of a transfer of a B. napus nucleus to B. hirta cytoplasm (except in the case of incompatibility between the two). But only 5 to 6 plants grown in the field exhibited reduced pollen shedding; this may indicate that B. hirta cytoplasm does not induce a useful male sterility in B. napus.

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REGENERATION OF GREEN COMET BROCCOLI FROM MESOPHYLL PROTOPLASTS

D. Robertson, E. Earle and M. Mutschler

Protoplast fusion makes possible the combination of different cytoplasmically encoded traits, such as atrazine resistance and the male sterility in Brassica that is derived from the radish cytoplasm (Ogura). This cytoplasmic male sterility trait suffers from a sensitivity of the plants to temperatures below about 12°C. The problem may be alleviated by the introduction of Brassica chloroplasts (carrying the atrazine resistance trait) into the radish cytoplasm. Pelletier et al have used protoplast fusion to achieve this goal in B. napus[1]. Brassica oleracea currently does not carry the atrazine resistance trait although it does contain lines which are male sterile. A prerequisite for using protoplast fusion to combine these traits in B. oleracea is a reliable procedure for the regeneration of plants from protoplasts. Mesophyll protoplasts offer many advantages in that they are easy to isolate in large numbers, they are mostly diploid and they provide a visual means of selection for protoplast fusion hybrids when combined with protoplasts from either hypocotyls or cell cultures, which lack green chloroplasts. Presented here is a method which produces large numbers of regenerated plants from mesophyll protoplasts and some initial observations of the variability of these plants.

Green Comet hybrid broccoli, B. oleracea cv. italica (Joseph Harris, Rochester, NY, USA) was used in all experiments. The detailed procedures for protoplast isolation and culture will be published elsewhere. Both greenhouse-grown plants and plants grown in vitro have been used as protoplast donors. Sterilized leaf material was incubated overnight in an enzyme mixture containing .5% cellulysin, .01% driselase, .2% macerozyme, .2M mannitol, .08M $CaCl_2$, 1mM KH_2PO_4 and 1mM MES, pH 5.6. The protoplasts were filtered through a 167µ mesh, pelleted in .5M sorbitol + .01M $CaCl_2$, floated in .5M sucrose + .01M $CaCl_2$ and re-pelleted in .5M sorbitol + .01M $CaCl_2$. They were then resuspended in nutrient medium and plated at a concentration of 5.10^4/ml in 0.5ml aliquots in 24 well multiwell plates. The protoplast culture media used were developed for Brassica napus by Pelletier et al[1]. Although modifications of these media were tested (hormone concentrations and types), no improvements were noted in protoplast or plant development. In some cases plating efficiencies and the percentage of calli that gave rise to shoots were very high, indicating that this series of media was capable of supporting good development of B. oleracea protoplasts.

The proportion of cells that divided to cells that had formed walls ranged between 0-70% after 8 days of culture. Variability in the response of protoplasts from different plants in the same experiment suggested a genotypic effect on protoplast development. Browning of the media due to the production of polyphenols has been a problem in a small proportion of the plants tested. Protoplasts from other plants occasionally showed prolific budding but no divisions. Protoplasts from at least 10% of the plants began to divide after 4-5 days and typically showed division frequencies of between 40-70% by day 8. Optimization of environmental factors (temperature and condition of the donor plants) has increased the percentage of plants which produce vigorously dividing protoplasts. Multiple shoot formation occurred on medium E after 2-6 weeks in up to 80% of the calli. Vitrification[2] of the shoots occured but the plants developed normal morphology when the concentration of

hormones and carbohydrates was reduced, on medium G. Transfer of plants to pots covered with plastic bags was successful only if the plants had developed a normal morphology.

Plants from two experiments have been hardened and analyzed for variability. Four plants (from a total population of about 250) are variegated. One of these plants produces light green leaves which become white, with occasional green sectors, as they mature. White explants from this plant developed chlorophyll when cultured on LS medium with cytokinin (10mg/l BA), auxin (1mg/l NAA) and 3% sucrose. The explants remained white on medium containing only auxin and sucrose. Variegation in the three other plants is sectored in one plant, at the edges of the leaves in another, and mottled throughout the leaves in the third. Crosses to determine the mode of inheritance of the variegated trait have been made.

100 protoplast-derived plants have been tested for their response to field conditions and 60 plants have been grown to maturity in the greenhouse. Variations in days to flowering, plant height and head development were noted. The vegetative characteristics of plants grown in the greenhouse were not as variable as those grown in the field but in both populations variations in floral morphology were observed. Fusion of the filament with the ovary occured in one sterile plant; petalloid anthers with viable pollen were noted in another plant. A third plant had flowers with white petals that were about half the normal size. Plants which show interesting characteristics are being selfed to test for possible heritable variation due to the tissue culture process.

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A SIMPLE METHOD FOR SEED PRODUCTION FROM SELF-INCOMPATIBLE LINES OF CABBAGE (BRASSICA OLERACEA VAR. CAPITATA F. ALBA)

Guelly Vitanova

The production of sufficient quantities of seeds from parent lines which have shown a high combinative possibility is an important problem in the heterosis breeding of cabbage (Brassica oleraceae var. capitata f. alba). In the Research Station, Negovan, Sofia, Bulgaria, trials with solid CO_2 were done to establish the influence on pollination and to obtain more seeds from self-incompatible lines of cabbage which were homozygous for the self-incompatibility factor S.

Plants vernalized in the field were planted in December in a glass-house. During the flowering period (February-March) on cloudy days, at 3-4 pm, cabbage plants with enough flower buds were marked and hand-pollinated with pollen from the same line. The plants (2-3) were covered with pyramidal polythene isolation chambers. Uncovered Petri dishes containing solid CO_2 equivalent in amount to 6% of the chamber capacity were immediately placed in the top part of these chambers. The plants were uncovered at 9 am on the next day.

The results show that the proportion of pods set on plants treated with CO_2 was 78-85% in comparison with 35-52% on the untreated plants. However the top buds on the treated plants set also.

The treatment with CO_2 of new buds on the same plants is possible without any phytotoxic action. The quality of the seeds from the treated plants is not affected.

USING INSECT POLLINATORS IMPROVES SEED YIELD IN BRASSICA JUNCEA UNDER ISOLATION CAGES

Ryo Ohsawa and Hyoji Namai

In conserving crop varieties and producing hybrid seeds in Brassica species, a major problem is to prevent frequently occurred contamination brought about through the cross pollination with different varieties of the same species or the various allied species. This contamination can be overcome by separating one species or variety from the others using isolation cages. Seed growing method using isolation cages has been widely investigated in outcrossing Brassica crops, and it has been shown that the use of isolation cages with insect pollinators promise to improve their seed yields.

In B.juncea which has been considered self-fertilizing plant,Free and Spencer-Booth(1963) studied the relationship between the presence of insect pollinators and seed yield and showed no connection between them. On the other hand, Olsson(1960) reported the wide intervarietal and intravarietal variations in self fertility of B.napus. Nevertheless, seed growing under isolation cages is usually done without insect pollinators.

Then, we have been tring to estimate the insect pollinators'effect on seed yield in digenomic Brassica crops which have been considered self-fertilizing plants. In this paper we describe the intimate relationship between the number of insect pollinators and seed yield in a caged B.juncea cultivar in comparison with a typical outcrossing B.campestris cultivar.

Each caged plot was L3.4m x W1.4m x H1.6m and covered with clean saran screen(#300) containing 12 plants,with three replications in randomized blocks. Eristalis cerealis artficially reared was used as pollinators and released into each cage at the stage of all plants flowered. The number of insect pollinators were provided five levels being 0,1,2,3, and 4 per plant.

Table 1 shows the relations between the number of insect pollinators and seed setting. Pod set percentage of B.juncea in the cages without insect pollinators was 93% and was not improved even if the number of insect pollinators was increased. But,pod set percentage of B.campestris was only 19% in the cages without insect pollinators and was improved significantly as the number of insect pollinators increased. For B.campestris the number of seeds per flower meaning seed yield was very low in the cages without insect pollinators and was improved as the number of insect pollinators increased. However the number of seeds of caged B.juncea without insect pollinators was considerably higher than that of B.campestris, increasing the number of insect pollinators tended to improve seed yield in the same manner as B.campestris.

Correlation between the number of insect pollinators per

plant and seeds per flower was highly significant in B.juncea and B.campestris(Fig.1 and 2). Because B.juncea has about 21 plasentae in a pod and about 26 in B.campestris,seed set percentage of plasenta was about 90% in B.juncea and about 75% in B.campestris of caged plants with four insect pollinators per plant. Hence more number of insect pollinators may improve seed set percentage not only in B.campestris but also in B.juncea.

It appeared that using insect pollinators improved seed yield in B.juncea considered self-fertilizing plant. Therefore,we suppose that using insect pollinators to caged plants will promise to improve seed yields in various digenomic Brassica crops considered self-fertilizing plant.

Table 1. The relations between number of insect pollinators and seed setting in B.juncea cv.Kikarashina and B.campestris cv.Nozawana under isolation cages

No. of pollinators per plant	Brassica juncea		Brassica campestris	
	Pod set (%)	No. of seeds per flower	Pod set (%)	No. of seeds per flower
0	92.6 a[1)]	11.9 c	18.5 c	0.1 c
1	97.7 a	15.9 b	58.6 b	10.9 b
2	98.3 a	16.7 ba	73.2 ba	12.3 b
3	99.4 a	17.8 ba	91.2 a	17.4 a
4	96.7 a	18.8 a	95.3 a	19.4 a

1) Means within a colum followed by the same letter are not significantly different at 5% level of probability according to Duncan's multiple range test.

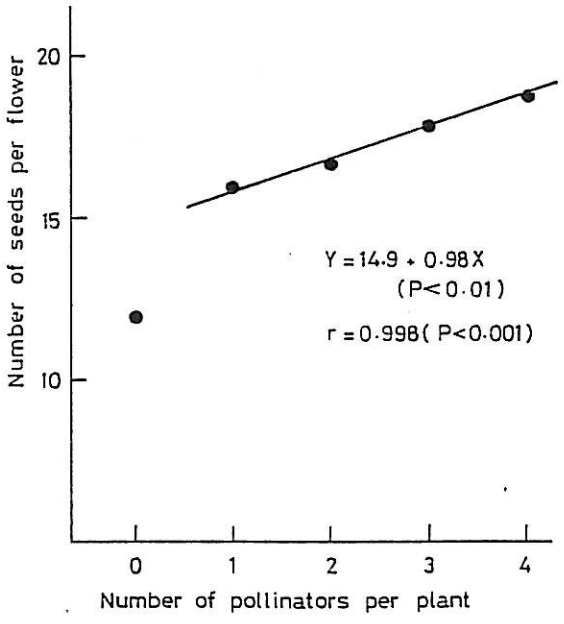

Fig.1. B.juncea cv.Kikarashina.

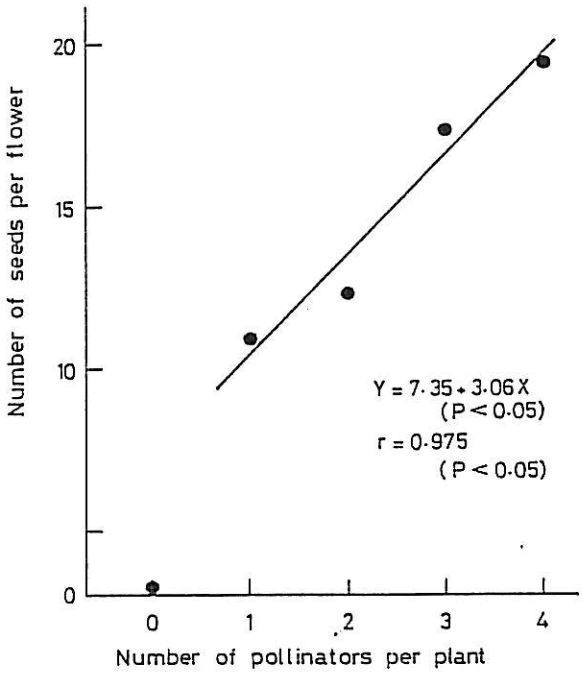

Fig.2. B.campestris cv.Nozawana.

MULTIPLICATION TUNNELS FOR BRASSICA BREEDING PROGRAMMES

S. Gowers

During Brassica breeding programmes, different sizes of multiplication cages are required to produce increasing quantities of seed as the lines progress towards cultivar status. At SCRI, flat-topped cages made of 'Tygan' mesh were used, but these suffered several problems particularly under the weather conditions at Pentlandfield. Late falls of snow sometimes caused severe damage; wet conditions in late summer gave poor ripening; older cages needed frequent repairs; styles could grow through the mesh and allow contamination; and access to maintain the cages whilst containing the blow-flies used as pollinators was difficult.

Polythene tunnels have been used successfully at NVRS for large scale multiplication of Brassicas and other vegetables (Faulkner and Jackson, 1981). Adaptations to this type of structure have been made to provide a range of sizes. Using 4.2 m wide framework, different sizes are produced by adding 1.5 sections as required. As few as 10 or 12 plants in a 1.5 m tunnel and up to 200 or more in a 12 m tunnel have been grown. With the smaller sizes, a 2 m x 1 m panel made of 'Tygan' mesh secured with 'Velcro' fastening strip allows access and ventilation. For sizes over 1.5 m, a fixed panel 2 m x 2 m of 'Tygan' at the other end provides through ventilation.

With clear polythene, as usually used for covering tunnel structures, the temperatures often become far too high for the plants and the blow-fly pollinators. Other types of covering have, therefore, been examined to try to find a more suitable material. White polythene, 'Lobrene B35' white shade material and a white woven polypropylene material were compared with 'Tygan' and clear polythene, using single section 1.5 m tunnels with four plants each of four lines of swede. The temperatures were monitored at weekly intervals throughout the summer with max-min thermometers and the readings, on a monthly basis, are given in Table 1.

Table 1. Maximum-minimum temperatures recorded in tunnels covered with five different materials

	Clear polythene		White polythene		White woven polypropylene		B35 shade material		Tygan mesh	
Month	min	max	min	max	min	max	min	max	min	max
June	1	32	1	31	2	29	1	27	1	24
July	6	31	4	28	7	30	6	28	8	25
August	5	39	5	38	5	33	5	29	5	26
Mean	5.0	31.5	6.2	29.5	5.0	28.9	5.1	24.4	7.0	23.6

The maximum temperatures were much lower in the two porous materials, B35 and Tygan, and the reflectance of the white pigmentation appears to have caused a slight decrease in temperature in the white polythene and the polypropylene tunnels compared with the clear polythene tunnel.

Of the four lines of swedes used in these tests, two did not establish well on transplanting to the tunnels and, with such small samples, this caused erratic effects in the results. The total seed sets are given in Table 2, but the results from the lines which established well may give a better indication of the effects of the covering materials on the variations in seed set.

Table 2. Seed sets from tunnels covered with five different materials

	Clear polythene	White polythene	Woven propylene	Lobrene B35	Tygan mesh
Total seed set, g	315	475	387	462	379
(no. plants)	(15)	(14)	(12)	(13)	(15)
Mean, g/plant					
Line A	32.3	47.3	46.1	55.1	52.2
Line B	22.9	31.3	23.7	25.9	22.6
Mean of A and B	27.6	39.3	34.9	40.5	37.4

The only statistically significant results were that the seed yield of Line A under clear polythene was lower than the mean, and that the seed yield of Line B under white polythene was higher than the mean. However, the total seed yield under white polythene was 50% higher than under clear polythene, and this is unlikely to be due to slight differences in temperatures. The most likely cause appears to be the behaviour of the pollinators. On hot sunny days the blow-flies in the clear polythene tunnels sheltered in the shade and carried out little pollinating activity. In the tunnels covered with the white materials there was little shade as light was diffuse, and the flies remained constantly active.

Although Lobrene B35 provided a cooler environment and gave similar seed yields to white polythene, the polythene cover satisfies the requirement of keeping the crop dry during ripening. The woven polypropylene gave slightly lower yields than white polythene and, although it lasts longer if sufficient UV inhibitor is included, it is difficult to obtain the correct quality and width for covering tunnels. White polythene, in comparison, is cheap and easily available, and using this instead of clear polythene for tunnel covers gives a useful increase in the efficiency and cost-effectiveness of this method of seed production.

Thanks are due to Deborah Page and Dorothy Gemmell for assistance with scoring and harvesting.

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RELATIONSHIP BETWEEN FILLING PHASE AND YIELD PER PLANT IN RAPESEED

A.M. Olivieri and P. De Caneva

As a general rule longer is the crop duration higher is the yield. However, some phases play a major role than others in crop yield and some of them are genetically controlled. Addomestication process has changed deeply the crop duration in many species.

In rapeseed the phases of flowering and filling are important in regulating the seed yield for photosynthetate supply (Tayo and Morgan, 1979; Morgan et al., 1983; Horodyski and Tobola, 1983), but there are indications that the amount of temperature in winter season affects the grain yield, too (Toniolo and Mosca, 1983).

In this note we show that it is possible to break the relationship between crop duration and yield by breeding programs, so that improvements for both yield and earliness can be achieved. A variety early in ripening allows a catch crop sowing in Northern Italy.

Data were gathered in F_2 single plants coming from a diallel cross combination involving the following winter varieties: Eurora, Primor, Status, Brink, Ramses "O". Seed was sown by machine in four row plots according to a two randomized block design and observations were made on the plants of the two central rows. Flowering ended around May 1st for all material and filling phase lasted 20 to 37 days.

Fig.1 shows the relationship between parental and segregating generations. Parental varieties appear to be less yielding than their progenies in spite of their longer filling period.

This behaviour is possibly due to a residual heterotic effect that appears as increase in earliness and yield per plant. As it has been postuleted for protein evolution in ripening phase (Voltan et al., 1982), heterosis can be an increase in the speed of metabolic processes.

From a practical point of view breeders have to try to maintain a high heterosis. For this hybrid and synthetic varieties are advisable to develop.

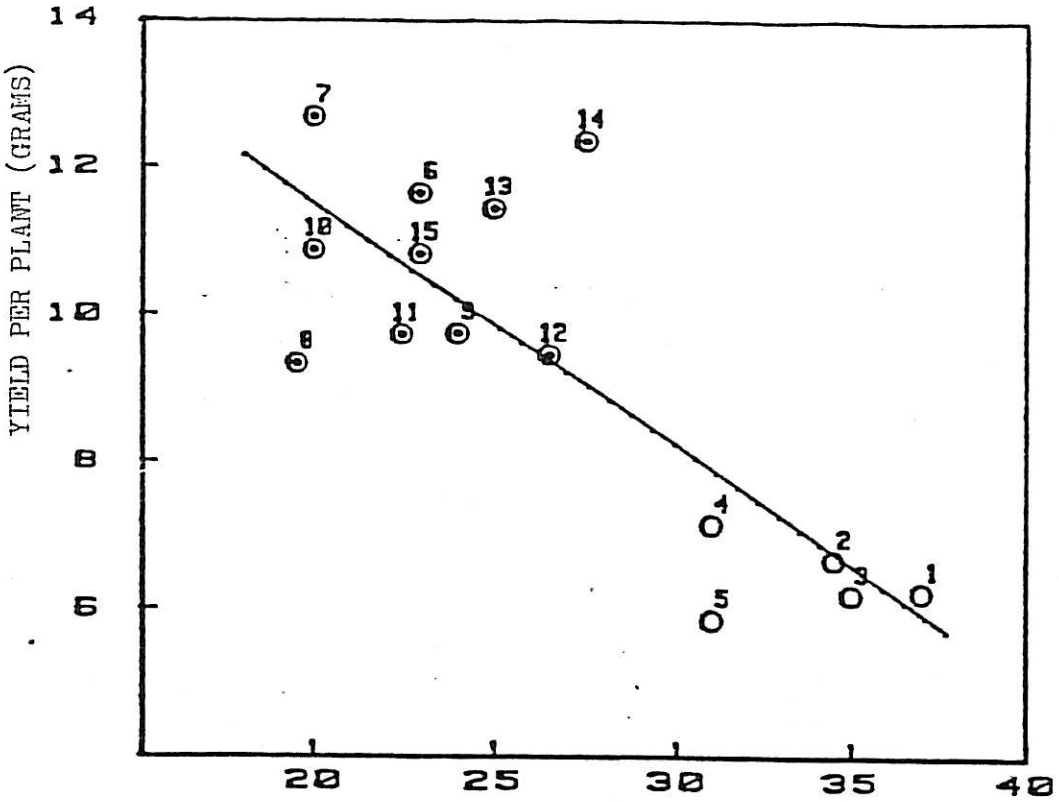

FIG.1

◎ F_2 generations.

O Parental varieties.

1. EURORA 2. PRIMOR 3. STATUS 4. BRINK

5. RAMSES "O" 6. EURORA X PRIMOR 7. EURORA X STATUS

8. EURORA X BRINK 9. EURORA X RAMSES "O"

10. PRIMOR X STATUS 11. PRIMOR X BRINK

12. PRIMOR X RAMSES 13. STATUS X RAMSES "O"

14. BRINK X STATUS 15. BRINK X RAMSES "O"

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POD INTENSITY, YIELD POTENTIAL AND OIL CONTENT OF EXOTIC COLLECTIONS OF RAPE AND MUSTARD

S.S. Dhillon, K.S. Labana, K.L. Ahuja and S.K. Banga

Although Indian mustard (Brassica juncea (L.) Czern & Coss) and turnip rape are the predominantly cultivated species of Brassica in India, recently rape (B. napus L.) and Abyssinian mustard (B. carinata) have also been found growing with some farmers of Punjab. The present study was planned to test these lines for their yield potential, oil content and other agronomic characters at the Regional Research Stations (Faridkot and Abohar) of Punjab Agricultural University, situated in the potential areas for rape seed and mustard production.

Materials and Methods

Materials comprised 13 lines of four Brassica species, six of B. napus, five of B. juncea and one each of B. campestris and B. carinata. These were collected from the farmers of Faridkot and Bhatinda districts of Punjab state who had procured these seeds from European and other countries through their relatives and visitors. Sowing was done in a randomised complete block design with four replications at both the sites as per the local recommendations. Yield data were recorded on a plot basis and converted to kg/ha from the mean of the respective entry. Number of pods on the main shoot and length in cm were recorded from five competitive random plants per replication. Pod intensity was calculated as the number of pods per cm length converted into percentage. Oil content was determined by the NMR technique. The data for the three characters were subjected to analysis of variance.

Results and Discussion

Analysis of variance revealed significant differences among the experimental material for all the three characters. Quite encouraging results were obtained (Table 1) for B. napus collections. On average, the yield levels of B. napus introductions were quite comparable to the recommended varieties of B. juncea. The best yields of B. juncea types obtained at Abohar (3444 kg/ha) and of napus types (2861 kg/ha) are among the highest yield levels obtained in the country. These yields are about 4-5 times the national average and about 3-4 times the state average. Rape types were also superior for oil content and per cent pod intensity. Up to 45% oil content was recorded (range 41.80-45%) in rape types whereas the highest value in juncea types was only 40.60% (range 39.24-40.60%). Abyssinian mustard had the lowest oil content (34.84%). Pod intensity of napus types was much higher (74-101%) as compared with other Brassica types (37-70%), with B. carinata having the lowest pod intensity (37.0%). This is an important trait and should be given due importance in breeding programmes. The campestris entry had good oil content (43.75%) and pod intensity (70.0%), although its seed yield was lower than the rape and Indian mustard types.

Of all the entries, Abyssinian mustard performed poorly with respect to the three traits, therefore, precluding its direct distribution and cultivation under Indian conditions. However, some rape (B. napus)

collections seem to be quite promising, although they have a high degree of shattering and stem breakage. So far, no source of resistance is available for these two characters in napus. Improvement for these characters may be sought through interspecific hybridization with B. juncea or B. carinata which can reciprocally be improved for oil content and pod intensity. Intensive investigations are being made and will be reported subsequently.

Table 1. Mean values for three characters

Sr No.	Entry	Seed yield (kg/ha)			Oil content (%)			Pod intensity (%)
		Faridkot	Abohar	Mean	Faridkot	Abohar	Mean	
1	V_2	1353	2791	2071	42.50	42.43	42.47	92.0
2	V_3	1320	2528	1924	4273	42.20	42.46	81.0
3	V_6	1167	2611	1859	45.78	44.23	45.00	90.0
4	V_{10}	1055	1583	1319	42.75	43.27	43.01	74.0
5	$GS_{(B)}$	1139	2564	1854	44.68	43.43	44.15	101.0
6	$GS_{(D)}$	1625	2861	2243	42.63	40.79	41.80	100.0
7	V_7	1139	2715	1927	41.33	38.33	39.83	68.0
8	V_{11}	1278	3444	2361	41.95	39.23	40.60	60.0
9	RLM 198	1055	3055	2055	37.55	40.93	39.24	70.0
10	RLM 514	1444	3444	2444	40.30	39.30	39.82	61.0
11	RLM 619	1083	3222	2153	3953	39.90	39.71	59.0
12	B_C	1070	1854	1462	36.15	33.63	34.89	37.0
13	B camp 931	2305	1618	45.30	42.20	43.75	70.0	
	C.D. 5%	463	504		1.33	1.88		11.10

B. napus (1-6), B. juncea (7-11), B. carinata (12), B. campestris (13)

ENHANCEMENT OF FROST TOLERANCE IN BRASSICA CAMPESTRIS L. BY CHEMICALS

A.K. Dhawan

Low winter temperatures cause injury to tender herbaceous plants such as rape and mustard in north Indian regions. Screening of a large number of Indian Brassica lines has revealed little frost tolerance in this genus (Yadava and Bhola, 1977; Dhawan et al, 1983). It was, therefore, considered worthwhile to attempt to alleviate freeze injury to this crop by chemical sprays. An earlier communication from this laboratory (Dhawan et al, 1983) reported that dimethyl sulphoxide and maleic hydrazide prevent the decrease in seed yield caused by frost, while cycocel, glucose, sucrose and sulphuric acid did not show this effect. This paper includes a study of the effect of known plant hormones (indole acetic acid and gibberellic acid), cryoprotectants (glycerol and cysteine) and fungicides (Dithane Z-78 and Difolatan) in preventing freezing injury.

Seedlings of B. campestris L. var BSH-1 were raised in pots (6" diameter) containing a mixture of soil, sand and farmyard manure (2:1:1). Fertilizer, irrigation and plant protection practices were followed as described earlier (Dhawan et al. 1983). Twenty days after flowering, the plants had attained a stage that was comparable to the one in field plants in the middle of January, when frosts are very frequent in this region. Plants at this stage (30-40% siliquae formed) were sprayed with the test chemicals on an afternoon and freezing treatments following Dhawan et al (1983) given the next morning. Seed yield was recorded at maturity and reduction in yield due to freezing taken as an estimate of injury (Dhawan et al, 1983).

Data presented in the Table given below indicate that freezing reduces seed yield. Application of 50 mg/l gibberellic acid, 0.2% Difolatan (Cis-N-(1,2, 2,2-tetrachloroethythio)-4-cyclohexene-1, 2-dicarboximide; 80 w.p.; Cheuron Chem. Co., U.S.A.) or 0.3% Dithan Z-78 (zinc ethylenebis dithiocarbamate; 75 w.p.; Bayer India Ltd., Bombay) partly prevents the effect of freezing on seed yield. Other concentrations of these chemicals, as also, 50 and 100 mg/l indole acetic acid, 0.5 and 1.0 ml/l glycerol and 50 and 100 mg/l cysteine showed a little or no effect (data not included).

Gibberellic acid has no effect on cold tolerance in potato (Chen et al, 1979) and decreases it in tree plants (Levitt, 1980). A protective effect of Difolatan and Dithane Z-78 is in confirmity with a visual observation made in pathological trials at our farm, that plots sprayed with these two chemicals are less affected by natural frosts. This points out to a dual advantage of fungicide sprays in the areas where fungal diseases and frosts are coincident. The mechanism of fungicide action in preventing freezing injury however, remains to be explored.

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Table 1. Effect of chemical sprays on seed yield in frozen and unfrozen B. campestris L. plants

Chemical	Treatment	Seed yield/ 10 plants(g)
Control	U	20.8 ± 1.24
Control	F	12.6 ± 1.03
Gibberellic acid, 50 mg/l	U	21.8 ± 1.38
Gibberellic acid, 50 mg/l	F	18.8 ± 0.77
Dithane Z-78, 0.3%	U	23.2 ± 1.00
Dithane Z-78, 0.3%	F	17.8 ± 1.05
Difolatan, 0.2%	U	22.1 ± 2.71
Difolatan, 0.2%	F	19.5 ± 0.78

U = unfrozen; F = frozen; ± = standard error

VERNALIZATION OF SPRING AND WINTER RAPE CULTIVARS AS IMBIBED SEED

B. L. Bettis, D. L. Auld and G. A. Murray[1]

Winter cultivars of rape have an absolute requirement for vernalization which often cannot be met as imbibed seed (4). Unvernalized winter rape cultivars may fail to flower after 300 days of vegetative growth while most spring cultivars show only a quantitative response to vernalization (3, 4). Most winter rape cultivars are vernalized in the rosette stage for 6 to 8 weeks at 0 to 4 C (1, 2). However, spring rape responds to vernalization as imbibed seed at 2 to 4 C (3). The purpose of this experiment was to compare the effect of differential vernalization periods on imbibed seed of a spring cultivar (Tower) and three winter rape cultivars known to differ in their relative degree of winter hardiness.

Imbibed seed of the cultivars Tower, Indore, Sipal, and Norde were exposed to four vernalization periods. Seed of each cultivar were imbibed between germination pads in Petri dishes at room temperature for 24 hours and then transferred to a 4 C room with an 8-hour photoperiod provided by 40-watt cool-white fluorescent lights. Imbibition dates were staggered to allow removal of all treatments from the cold room on a single date. After vernalization the seedlings were transplanted into pots in the greenhouse and supplied with a 24-hour photoperiod by metal halide lamps. The experiment was conducted as a split-plot design with three replications in which vernalization treatments were main plots and cultivars were sub-plots. The plants were scored for the presence of floral buds on the primary raceme 40, 46 and 56 days after transplanting. Data were subjected to an analysis of variance and means were separated by LSD tests.

Forty days after planting non-vernalized, imbibed seed; none of the winter cultivars and only 16% of the Tower plants had flowered (Fig. 1). With 5 weeks of vernalization all of the Tower plants flowered but 9 and 13 weeks of vernalization tended to slightly delay flowering. Over 60% of the Indore plants flowered with 5 weeks of vernalization but longer periods of vernalization appeared to decrease flowering. Sipal did not flower until given a minimum of 9 weeks of vernalization. Norde did not flower within 40 days of any of the vernalization treatments.

Forty-six days after planting essentially all of the Tower plants flowered regardless of the vernalization treatments (Fig. 1). Five to 13 weeks of vernalization of the imbibed seed of Tower reduced the time to complete flower by only 6 days. The non-vernalized plants of Indore, Sipal and Norde had not flowered at 46 days. Both Indore and Sipal had over 60% flowering with 5 and 13 weeks of vernalization. Both cultivars showed a reduction in the rate of flowering at 9 weeks of vernalization. The flowering response of Norde increased from 7 to 44% as the percent of vernalization was increased from 5 to 13 weeks.

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[1] Former research associate, assoc. prof. of plant breeding genetics and professor of crop physiology, respectively.

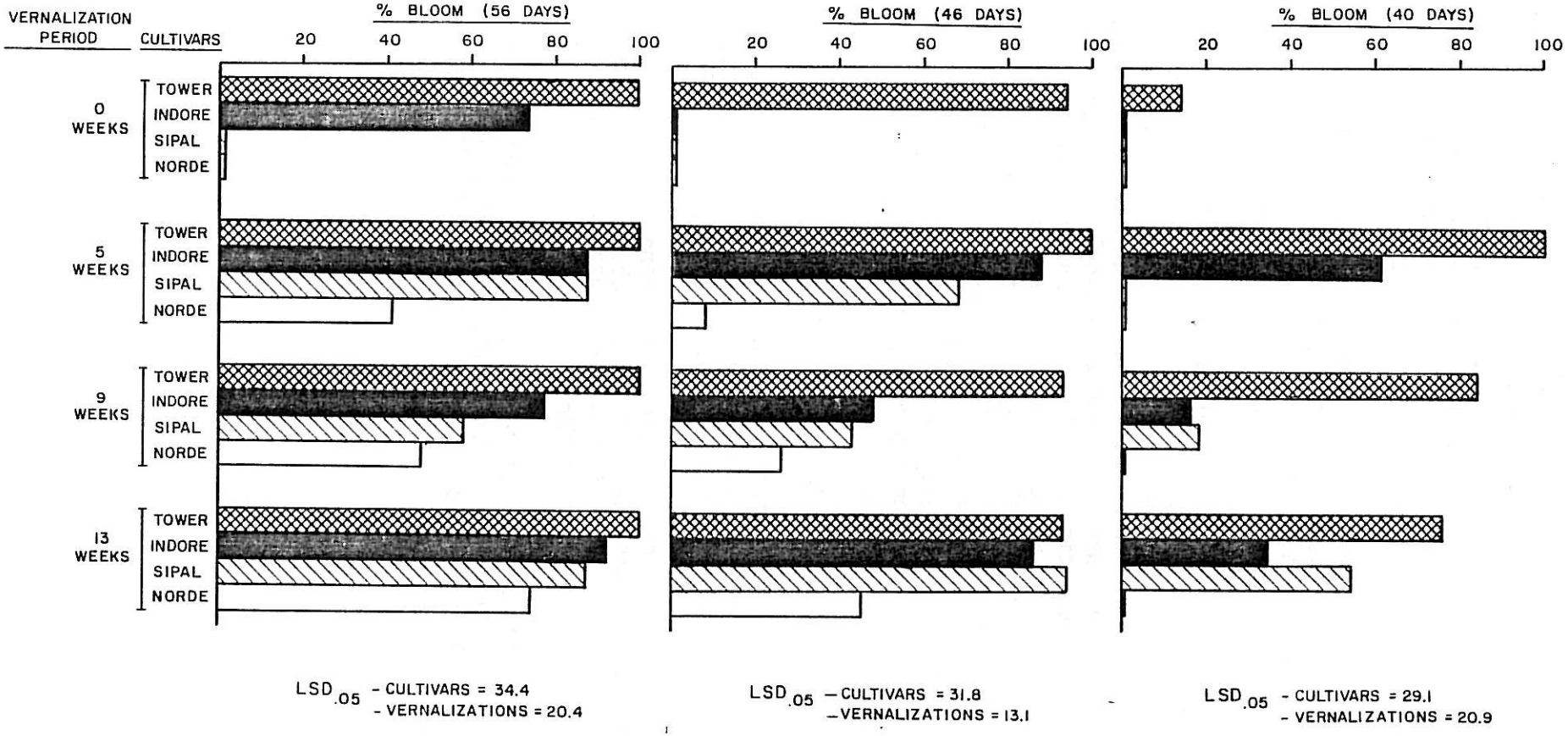

Fig. 1. Flowering response of Tower spring rape and three winter rape cultivars, 40, 46 and 56 days after vernalization as imbibed seed.

Fifty-six days after planting the non-vernalized seed, over 70% of the Indore plants had flowered, while both Sipal and Norde failed to flower (Fig. 1). The lack of an obligate requirement for vernalization of Indore indicates that this cultivar is intermediate between conventional spring and winter rape cultivars (5). With 5 and 13 weeks of vernalization over 80% of the Indore and Sipal plants had bloomed. As at 46 days, the seedlings of these two cultivars showed a decrease in flowering with 9 weeks of vernalization. This may indicate that the vernalization requirement of these two cultivars was met with 5 weeks of vernalization and that further exposure to cold temperatures for 9 weeks delayed floral development. At 56 days after planting more than 70% of the Norde plants given 13 weeks of vernalization had flowered.

The vernalization response of Indore, the least hardy of the winter cultivars, was intermediate to spring and winter cultivars. Indore, which was derived from a cross between the spring rape 'Bronowski' and the winter rape 'Gorczanski', did not have a qualitative vernalization requirement. Even a very hardy cultivar such as Norde showed a good flowering response with 13 weeks of vernalization. This level of flowering induction (70%) is adequate for most breeding programs and would prevent inadvertent selection of spring types. Throughout these studies Norde required longer cold treatments than Sipal to achieve a similar flowering response. Extreme care should be taken in the vernalization and selection of progeny from segregating populations which contain both spring and winter parents. Inadequate vernalization treatments could result in the inadvertent selection of genotypes which do not require vernalization to flower resulting in a serious reduction in winter hardiness (4). If only winter germplasm were involved it might be possible to select for early maturing genotypes and/or increased levels of winter hardiness on the basis of their response to vernalization as imbibed seed.

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cb/0272a

CURRENT ASPECTS OF THE TAXONOMY OF CULTIVATED *BRASSICA* SPECIES

The use of *Brassica rapa* L. versus *B. campestris* L. and a proposal for a new infraspecific classification of *B. rapa* L.

Hille Toxopeus , Emiel Oost and Gerhard Reuling

A practical, yet scientifically sound and generally accepted system of naming the wide range of cultivated cruciferous plants is lacking. This is regrettable, especially because underlying this great variety of crops is a simple botanical principle, aptly summarized in the *Brassica* triangle (U, 1935).
The attempts to classify the wide variation within cultivated *Brassica* species have resulted in many infraspecific taxa of different ranks. In most cases these taxa were used to indicate crops. However, size, variation and consequently a description of a crop may vary considerably in the course of time. Crops are in fact groups of cultivars and cultivars may have a limited life span depending on the intensity and scope of the breeding work. Moreover, new crops are being developed as a result of infraspecific crosses and as efforts at collecting genetic resources continue,previously unknown crops are encountered!
Botanic classification is not designed to cope with such dynamic situations.
A flexible classification system is therefore urgently required. In the following the present taxonomic situation for cultivated *Brassica* species at generic, specific and infraspecific level is summarized, and a proposal for a new infraspecific classification system is being discussed and exemplified by the case of *B. rapa* L. (syn. *B. campestris* L.).

Classification and nomenclature at the genus level.

The relationship between the genera *Brassica* and *Sinapis* is still a point of discussion between taxonomists, but it is not expected that this will lead to any nomenclatural changes for the cultivated *Brassica* and *Sinapis* species.
The nomenclature of the intergeneric hybrid between *Brassica* and *Raphanus* will be dealt with in another paper in this Newsletter by the second author.

Classification and nomenclature at the species level.

Starting from the publication of the so-called *Brassica* triangle or triangle of U (1935), relationships between the important cultivated *Brassica* species have been much clarified. The scientific names of these species are generally accepted, with the exception of the long standing controversy about the use of the names *Brassica rapa* L. versus *Brassica campestris* L.
The third author studied the typification and nomenclature of *B. rapa* and *B. campestris* as a part of his Ir. study at the AU (Reuling, 1984).

Typification of *B. campestris* L. and *B. rapa* L.

The specimen no. 844.4 in the Linnaean Herbarium in London (LINN) has been designated as the lectotype of *Brassica campestris* L. (Jonsell, 1982).
The illustration of *Rapum* in Camerarius (1586) will be designated as the lectotype of *Brassica rapa* L. (Oost et al., in preparation for Taxon).

Brassica rapa versus *B. campestris*.

The first botanist to seriously discuss the conspecifity of the species *B. rapa* and *B. campestris* was Metzger* (1833). He studied the complete range of cruciferous crops existing in W.-Europe at the time by growing and studying plants over a number of years. Contrary to many contemporaries his species concept agrees perfectly with present day views. He combined turnips and turnip rape into one species with the name *B. rapa* L., because of their close relationship. As a consequence the name *B. campestris* L. has become a synonym of *B. rapa* L.
Many authors have followed the choice of Metzger (e.g. Mansfeld, 1959; Tutin et al, 1964; Wellington & Quartley, 1972; Terrel, 1977). The International Seed testing Association had the name *B. rapa* L. stabilized at the conference of 1974 (Quartley, 1974). Nevertheless, the synonym *B. campestris* is still often found in papers on genetics and breeding. Although the choice between *B. rapa* and *B. campestris* may seem to some rather an arbitrary one, the present authors hope that the above will help to put an end to this confusion.

Common names at the species level.

Examples of common English names that cover the whole genetic variation at the species level are: cole crops for *Brassica oleracea* L., black mustard for *B. nigra* (L.)Koch, Ethiopian mustard for *B. carinata* A. Braun and Indian mustard for *B. juncea* (L.)Czern. Unfortunately there are no common names for *B. napus* L. and *B. rapa* L. and the authors cannot think of any that might be acceptable.

Classification and nomenclature at the infraspecific level.

The cultivated *Brassica* species do not only cover a highly polymorphic series of crops, new forms are still being discovered or bred. Therefore, taxonomic problems are especially encountered at the infraspecific level.
There is a great need for a flexible classification system which will allow for new crops to be included and changes to be made without difficulty.
We would welcome discussion on a new infraspecific classification system, based on the classification of cultivars into cultivar groups. These cultivar groups are formed on the basis of certain shared characters (morphological, physiological, agricultural) between cultivars. In order to be functional the number of cultivars in a cultivar group should neither be too big nor too small. In accordance with Art. 26 of the ICNCP (1980)**, cultivar groups will preferably be named in a modern language. These names should be written with capital initial letters to distinguish them from common crop names, which are written in small letters.
Latin description and typification of a cultivar group is not necessary; a short description of the shared characters is sufficient, since the cultivar group is adequately characterized by the cultivars that constitute the group. An infra-specific classification system based on cultivar groups is in fact not a new phenomenon, but until now it has found little application, mainly for want of an

* A translation into English of Metzger's publication is being prepared at the SVP.

** International Code for the Nomenclature of Cultivated Plants (1980). This Code aims to promote uniformity, accuracy and fixity in the naming of agricultural, horticultural and silvicultural cultivars.

international agreement on the choice of cultivar group names.
In case of cruciferous species the Cruciferae Newsletter might prove to be an excellent medium to propose, discuss and officially accept cultivar groups and corresponding names. Further details have yet to be worked out, but as a start the authors have set up a provisional infraspecific classification for *B. rapa* L. using cultivar groups.

Proposal for a new infraspecific classifiction of *B. rapa* L.

Table 1 shows the classification and nomenclature we put forward for discussion and improvement to all readers fo the Cruciferae Newsletter.
As far as possible we have tried to use already existing common crop names as provisional cultivar group names. The definitive assignment of cultivars to cultivar groups is still being studied (lists of cultivar names, seed catalogues and cultivar descriptions are urgently needed).
We invite all readers to respond (pro or con). In particular we would welcome comments on the following:

- Spelling of names: Tori, Sarson, Pe-Tsai, Pak-Choi and Broccoletto.
- Common names for *Brassica japonica* Makino (note 3 in table) and *Brassica pervirides* Bailey (note 5 in table).
- Are there sufficient grounds for separating Tori (sometimes called brown sarson) from Spring Turnip Rape? Can the two cultivar groups be distinguished other than on the basis of origin?
- The status of the following taxa is uncertain and requires further study,also with regard to their position in *B. rapa* L.: *B. parachinensis* Bailey, *B. narinosa* Bailey, *B. dubiosa* Bailey.
- Can Turnip Greens function as a cultivar group?
- Should more cultivar groups be included; should any of the proposed be split up?

Authors would welcome lists of cultivars of *B. rapa* L. (preferably already assigned to cultivar groups), cultivar descriptions and seed catalogues for the further characterization of cultivar groups.

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Table 1. Proposed infraspecific classification of *B. rapa* L. based on cultivar groups.

Cultivar group	use	shared character(s)
Vegetable Turnip	vegetable	turnip (= swollen hypocotyl/root)
Fodder Turnip	fodder	rozette with many loose leaves (with or without turnip)
Winter Turnip Rape	oilseed	bienial
Spring Turnip Rape	"	annual
Tori[1]	"	annual
Sarson[2]	"	annual, yellow-seeds, siliques often with more than two valves
Pe-Tsai[3]	vegetable	heading, winged petioles
Pak-Choi[4]	vegetable	non-heading, conspicuous fleshy petioles
.....[5]	vegetable	heading, pinnate leaves
Brocoletto[6]	vegetable	annual, enlarged flowering head
.....[7]	vegetable	.....
Turnip Greens	vegetable	dense sowing, foliage of 6-8 weeks old seedlings harvested

Notes: 1. also known as subsp. *dichitoma*
2. also known as subsp. *trilocularis*
3. also known as subsp. *pekinensis*
4. also known as subsp. *chinensis*
5. also known as *Brassica japonica* Makino (no common name known to us)
6. parallel variation of broccoli (*B. oleracea* L.)
7. also known as *Brassica pervirides* Bailey (no common name known to us).

GENETIC VARIATION IN ETHIOPIAN *BRASSICA* spp.

Jan Engels

"Although of limited geographyical distribution, *Brassica carinata* is an important vegetable and source of edible oil in Ethiopia" (IBPGR,1981) The Plant Genetic Resources Centre/Ethiopia (PGRC/E) is being designated as the global base collection centre for *B.carinata* or Ethiopian mustard.

Since the start of PGRC/E a total of 569 *Brassica* accessions have been collected throughout the country (119 accessions in Welega, 94 in Shewa, 88 in Gonder, 68 in Gojam, 67 in Welo, and the remaining 133 accessions were collected in the rest of the 9 adminstrative regions). In addition, 297 *B.carinata* accessions were "repatriated", the majority through IBPGR from the Netherlands.

Beside the long term conservation of the germplasm in its headquarters at Addis Abeba, the PGRC/E is actively engaged in the utilization of its holdings. A strong cooperation exists with the highland oilcrop breeding programme of the Institute of Agricultural Research at Holetta, whose breeders are mainly concentrating on *B.carinata*. Yearly, some 300 accessions are planted for the multiplication of the individual accessions (isolation per accession is applied) and simultaneously these accessions are characterized for about 28 descriptors in close cooperation with the plant breeders. In Tables 1 and 2 the characterization results of the main characters are presented. Since only 287 accessions were classified so far (242 were *B.carinata*, 39 *B.nigra* and 6 *B.oleracea*) all the species are lumped in the presentation of the data. Since it was not always possible to identify accessions in an unambiguous way a side activity is under development to count chromosomes on a routine basis. Furthermore it is envisaged to start the determination of the oil content of the accessions by using the available Near-Infra-Red analyser.

The observed diversity is partly due to the inclusion of several species, but from the observations made it can be stated that *B.carinata* is highly diverse for many of the agriculturally relevant descriptors. Its use in Ethiopia varies from a pure oilcrop to a pure vegetable and, all combinations in between.

A detailed account on the cultural practices and uses of the different *Brassica* spp. is given by Astley *et al*. (1982).

ACKNOWLEDGEMENT. I am thankful to Mr. Solomon Zewdie who processed the data collected by PGRC/E's Multiplication Section.

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Table 1. Phenotypic variation (mean, coefficient of variation and range) for nine quantitative characters in 556 Brassica accessions.

Desciptor	Mean	C.V	Range Min	Range Max
1. Days to 50% flowering	93.3	18.0	44	153
2. Days to 50% maturity	176.6	12.7	109	223
3. Plant height in cm	175.2	22.4	75	317
4. Number of primary branches	9.1	29.1	1	18
5. Number of secondary branches	18.5	45.9	3	44
6. Number of siliquas/plant	130.6	37.9	23	373
7. Stem diameter in mm	11.1	23.1	5.6	24.0
8. Thousand seed weight in g	3.3	26.9	1.7	11.9
9: Number of seeds/siliqua	12.1	62.8	1	57

Table 2. Phenotypic variation expressed in frequencies for the qualitative characters in Brassica accessions.

Descriptor	Descriptor states	Freq.	Freq.[1]
1. Stem colour	light green	154	296
2. Leaf colour [1]	green	3	175
	dark green	8	62
	light purple	1	2
	purple	77	3
	dark purple	37	1
	light green and purple	267	10
3. Seed colour	yellow	20	
	yellow and brown	23	
	light brown	166	
	brown	196	
	dark brown	97	
4. Flower colour	cream	3	
	yellow and cream	60	
	yellow	293	
5. Leaf waxiness	1= shiny, no wax	263	
	2= little wax	128	
	3= intermediate	100	
	4= waxy	26	
	5= very waxy	24	

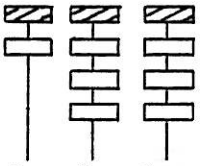
THE LEAF SURFACE OF BRASSICA OLERACEA AT DIFFERENT STAGES OF GROWTH

Aileen Quinlan and Ivor Simpkins

Observations were made on seedling leaves of two varieties of cauliflower (Brassica oleracea var. botrytis L. subvar. cauliflora DC.) in order to find the range of variation of wax morphology within the plant.

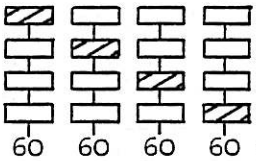
Seed of the two varieties (All The Year Round, Currawong) was germinated in compost consisting of peat, sand and fertilizer (50.85 kg peat- 50.85 kg sand- 3.12 kg Bio P Base). Plants were grown in a controlled environment room at a temperature of 18 + 1 C, a photoperiod of 16 hours and a light intensity of 24.5 W m^{-2}. Leaves of the same length on plants of different ages (figure 1.1) and leaves at different stages of expansion were sampled (figure 1.2) by stripping the epidermis (Grout, 1975). Specimens were taken from both the abaxial and adaxial surface and three replicates were prepared for observation in the scanning electron microscope using methods described by Wardle, Quinlan and Simpkins (1979).

1. leaf position



Samples from leaves of the same physiological age on plants of different chronological age.

2. leaf position



Samples from leaves of different physiological age on plants of the same chronological age. Sampling was repeated using plants which were 110 days old.

Figure 1 A diagramatic representation of plants showing the position of the leaf (marked ▨) from which samples were taken.

Observations on leaves of different sizes and therefore different physiological ages on a single plant showed a range in the density of wax structures. In All The Year Round, apart from differences in the density of the structures, wax morphology was similar in all leaves irrespective of the physiological age of the leaf or the chronological age of the plant. In Currawong however leaves of the same physiological age did not have a similar wax morphology on plants of different chronological ages.

The general appearance of epicuticular wax for the variety All The Year Round, observed as rods, tubes and plates on the leaf surface, was similar to that of other types of Brassica spp. reported (Martin and Juniper, 1970). The diameter and height of wax structures varied considerably and the distribution of the wax on the surface was uneven since areas in which the wax was dense were interspersed with those in which there was less wax production. There was consistantly less wax in the immediate area around the stomatal pore and a lower density of wax was observed on specimens at the first sampling time (20 days) compared with that observed on leaves after 60 and 110 days which was similar. Wax morphology on the first fully opened pair of leaves of All The Year Round was the same as that observed on the second and subsequent pairs of leaves.

The wax at 20 days on the first pair of leaves of the variety Currawong consisted of rods, tubes and plates similar to those of All The Year Round but after 60 days, unlike All The Year Round, there were

horizontal protuberances of wax on the vertical rods and tubes which developed further until after 110 days the rods, tubes and plates were almost covered by a lattice of fine filaments. The covering of wax on the first leaf was much greater at 110 days than at 60 days and unlike All The Year Round, Currawong also had a dense covering of wax around the stomata. After 110 days it was difficult to find stomata on the Currawong samples as they were often covered by the horizontal wax filaments. Therefore in Currawong, wax on the first pair of leaves was morphologically different on plants of different ages, older plants having a more intricate pattern of wax even though the leaves were physiologically the same age. Samples taken at 60 days from leaves successively down the Currawong plant (2nd, 3rd and 4th pair) showed that the position of the leaf on the plant did not affect wax morphology. All leaves sampled in seedlings grown for 60 days had wax in the form of rods and tubes with small horizontal protruberances similar to that of the first leaf. The protruberances were slightly more developed in samples taken from the third and fourth pair than from the first or second pair but even the oldest leaves sampled had not developed the lattice of horizontal filaments after 60 days whereas the filamentous mesh was clearly present in the youngest leaves of plants after 110 days growth even though it was not as well developed as in the older leaves. These findings suggested that it was the developmental stage of the whole plant and not the physiological age of individual leaves that determined wax morphology in Currawong although there was greater coverage of wax in older leaves. The abaxial epidermis was more readily stripped than the adaxial surface but wax morphology inboth was the same. There was in general a higher density of abaxial wax compared with the adaxial surface where structural damage was observed.

The increase in density of wax on leaves of All The Year Round from 20 to 60 days and the lack of increase between 60 and 110 days was in accordance with the view of Martin and Juniper (1970) that wax developed with increasing age and stopped increasing after leaf expansion ceased. Since wax morphology is a function of its chemical composition (Chambers, Ritchie and Booth, 1976) it is apparent that the biosynthetic pathways of older currawong plants are different from those operating in younger ones. Indeed Harwood, Wharf and Bolton (1978) found that the developing leaf contained rapidly changing enzyne systems for synthesising fatty acids the major precursors of wax.

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GLUCOSINOLATES CONTENT IN NEW GENOTYPES OBTAINED IN THE GENUS BRASSICA

Barbara Barcikowska, Piotr Mendelewski

Aiming at obtaining artificial Brassica napus with low glucosinolates content by crosses between B. campestris and B. oleracea new B. campestris genotypes have been found. They are characterized with high vitality, yellow seed coat and low glucosinolates content. These forms were the F_5 progeny of B. campestris ssp. pekinensis X B. campestris ssp. trilocularis Yellow Sarson. The glucosinolates content in the green matter of the hybrids oscillated between 8.1 and 13.5 µM/1g of air dried matter and, in the seeds, from 99.5 to 143.7 µm/1g defatted dry matter.

Determination of total glucosinolates content has been done according to the method of Brzezinski and Mendelewski (1984). The processing by using this rapid determination was as follows: meal of green matter (100 mg) was weighed in a test tube and heated in a boiling water bath for 3 min. Boiling water (9 ml) was added, and the mixture heated for a further 15 min. After cooling, 0.375 M lead acetate (1 ml) was added. Fifteen minutes after, the extract was centrifuged and the supernatant (1 ml) was poured in a micro-column with DEAE Sephadex A-25. The column was washed with 30% acetic acid (1 ml) and water (2 ml). Glucosinolates were eluted with 0.3 M potassium sulphate (4 ml). One ml sample from the eluate was taken into the test tube with 77% sulphuric acid (3.5 ml), then 1% thymol solution in ethanol (0.5 ml) was added to each tube. At the same time blank sample (1 ml of 0.3 M potassium sulphate) and standard samples (1 ml aliquots) of glucose solution in concentration ranging between 0.024 and 0.24 micromoles/ml were prepared. The tube content was mixed and placed in a boiling water bath for 35 min. and cooled under running water. Absorbance was measured at wavelength 505 nm against blank samples. Glucosinolates concentrations were calculated from the glucose standard curve.

The correlation coefficient between glucosinolates content in the seeds and in the green matter of the F_5 generation amounted +0.354 and was significant at $p = 0.05$.

Among the yellow seeded hybrids obtained, the most favourable seems to be the form 510 N because of the best recombination of low glucosinolates content in the seeds (99.5 µM/1g defatted dry matter) and in the green matter (8.1 µM/1g air dried matter).

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BRASSICA OLERACEA AND BRASSICA NAPUS ISOZYMES

Pere Arús

The number of isozymes of enzymes stained with natural substrates is highly conserved in diploid species (1). Moreover, polyploid species have more isozymes than the number usually found in diploids as a consequence of the addition of two or more genomes (1,2). In this study, the electrophoretic patterns at the regions of activity of six isozymes of B. oleracea (2n = 18) have been compared to those of its amphyploid derivative B. napus (2n = 38).

A sample of plants of each of four cultivars of B. napus were analyzed for the following enzymes: PGM-1 and PGM-2 of phosphoglucomutase, ADH-2 of alcohol dehydrogenase, PGI-2 of phosphoglucoisomerase, LAP-1 of leucine aminopeptidase and GOT-3 of glutamate-oxalacetate transaminase. Results have been summarized in Table 1. Starch gel electrophoresis and staining techniques used for B. oleracea (3) were also employed in this study. Information concerning the inheritance of the isozyme loci and allozyme terminology for B. oleracea can be found in (3) and (4).

Table 1. Electrophoretic phenotypes of B. napus. Phenotype notation: alleles previously described in B. oleracea have been labelled with numbers; the prefix C followed by a number (C1, C2, etc.) has been given to alleles present in B. napus but not in B. oleracea.
The number of plants of each phenotype in the variable regions has been indicated in parenthesis.

		Region of activity					
Cultivar	N	PGM-1	PGM-2	ADH-2	PGI-2	LAP-1	GOT-3
Dong Hae	20	2/4	C1/3 (5) C1/3/4 (7) C1/4 (8)	2	C1/3 (16) C1/2/3 (1) C1/2 (3)	C2/2	1/2
Midas	20	2/4	C1/3	2	C1/3 (10) C1/2/3 (6) C1/2 (4)	C2/2	1/2
Wesno	20	2/4	C1/3	2	C1/3 (4) C1/2/3 (1) 3 (10) 2/3 (3) 2 (2)	C2/2	1/2
Siberian Kale	100	2/4	C1/3	2	1/C1 (97) C1/3 (3)	C1/2 (2) C2/2 (98)	1/2

All plants had the heterozygous pattern at regions PGM-1, PGM-2, LAP-1 and GOT-3. All but 12 plants of cv. Wesno were also heterozygous at PGI-2. Individuals exhibiting three different allozymes were also observed in some regions (PGI-2 and PGM-2). These results suggest that two isozymes are expressed in B. napus at each of these five regions of activity. On the other hand, only one band was observed in all tested individuals for ADH-2. Thus, B. napus had at least 11 enzyme-coding loci where only 6 were expressed in B. oleracea. This observation is in agreement with the polyploid nature of B. napus. In this context, the absence of fixed heterozygosis in ADH-2 (and in some cases in PGI-2) can be explained if the electrophoretic mobility of the isozyme in the two diploid parentals is the same, or when null alleles occur in one of the duplicated loci.

At least one allozyme of each pair of duplicated isozymes of all plants examined had the same mobility that one of the allozymes of B. oleracea. This observation is consistent with the hypothesis that B. oleracea is one of the parental species of B. napus. Allozymes present in B. napus but not in B. oleracea are likely to belong to the other parental species (B. campestris). The fact that some regions of B. napus (PGM-1 and GOT-3) were fixed heterozygous for two alleles present in B. oleracea may be due to the existence of alleles common to both parental diploids for these enzymes.

Three different allozymes were visualized at regions PGM-2 and LAP-1 and four at PGI-2. This result implies that at least one of the two duplicated loci of these regions was polymorphic. Moreover, some individuals had three allozymes at one of these regions, indicating that they were heterozygous at one of the two loci. All plants analyzed were homozygotic at the polymorphic loci in "Siberian kale", whereas considerable proportions of heterozygotes were observed at the variable loci in the remaining three varieties, suggesting that important differences of outcrossing rates may occur among varieties of B. napus.

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OBSERVATIONS ON THE LARGE SCALE ISOLATION OF GLUCOSINOLATES

A.B. Hanley, C.L. Curl, G.R. Fenwick and R.K. Heaney

The toxicity of glucosinolates (mustard oil glycosides) and their breakdown products has led to concern over the levels of the intact thioglycosides in oilseed and fodder crops (1). The physiological effects of feeding glucosinolate - containing material to animals and poultry have been documented and recent studies have suggested that as many as 2 million people in the U.K. may consume in excess of 300 mg of glucosinolates per day in the winter months through consumption of commercially produced brassica vegetables (2).

The study of the clinical and physiological effects of glucosinolates has been limited by the difficulty in isolating sufficient quantities to enable definitive feeding trials to be carried out. Much of the information concerning the metabolic fate and physiological effects of glucosinolates has been gleaned as a result of feeding crude glucosinolate mixtures, high glucosinolate rapeseed meals or certain glucosinolate breakdown products.

We have recently reported a method for the isolation of gram quantities of certain glucosinolates from brassica seed and leaf material (3). The discovery and characterisation of two novel ring oxygenated indole glucosinolates and the need for the rapid production of glucosinolates for biological evaluation has led to improvements and modifications to the original techniques which we report here.

The initial stages of glucosinolate extraction using boiling 80% methanol (to inactivate endogenous myrosinase enzyme) and preliminary purification on an acidic alumina column were carried out as reported earlier (3). Elution from the alumina column using potassium sulphate solution (1%) was followed by evaporation of the salt solution to dryness, dissolution of the glucosinolates by agitation of the residue with methanol and evaporation of the methanol extract to afford a partially desalted glucosinolate mixture. This sample was dissolved in the minimum volume of water and applied to a Sephadex G-10 column which was then eluted with water, thus fractionating the glucosinolate from the residual potassium sulphate. The former can then be freeze dried and, where possible, recrystallised. In those instances where the plant material contains essentially a single glucosinolate then 150-300g of seed or leaf material can be dealt with at one time producing 0.8-4.0g of glucosinolate (purity > 95% as judged by glucose release, gc and hplc). From "single" glucosinolate sources we have isolated 2-propenyl - (I), 3-butenyl- (II), (-)-2-hydroxy-3-butenyl- (III), (+)-2-hydroxy-3-butenyl- (IV), benzyl- (V), 4-hydroxybenzyl- (VI) and indolyl-3-methyl- (VII) glucosinolate.

Using this method 5-10g amounts of glucosinolates I-VII have been prepared. Smaller amounts of two of the substituted indole glucosinolates, 1-methoxyindolyl-3-methyl-(VIII) and 4-hydroxy indolyl-3-methyl glucosinolate (IX) and 2-phenylethylglucosinolate (X) have also been produced for use as chromatographic standards and for limited structural and degradative studies from "multiple" glucosinolate sources. 4-Hydroxyindolyl-3-methyl glucosinolate (IX) is unstable and we have observed an increased yield if the seedmeal is treated with methanol to inactivate myrosinase and then extracted with cold water.

While a number of potential sources exist for the isolation of substituted indole glucosinolates (VIII and IX) they do not occur in single-glucosinolate material; therefore separation from other glucosinolates is required. We have found that passage of complex

mixtures of glucosinolates through Sephadex G-10 allows some separation of the different classes of glucosinolates (Fig 1).

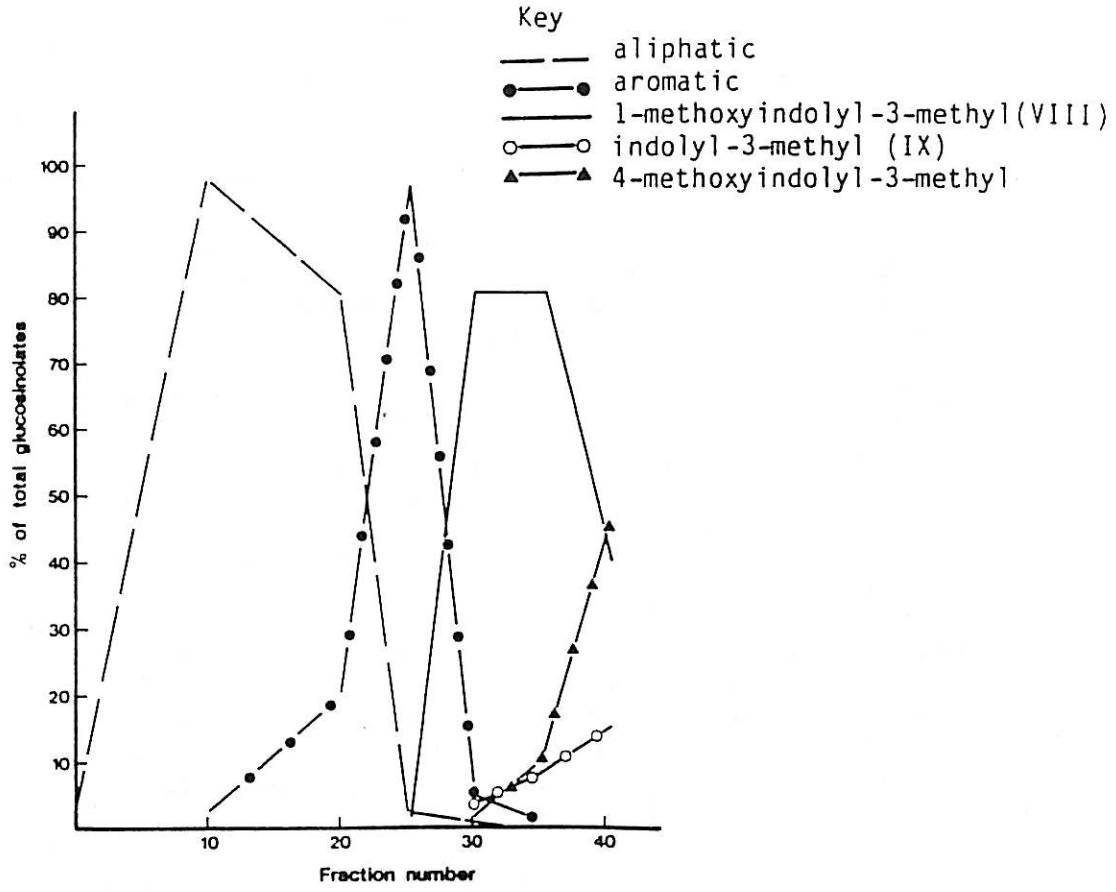

Fig. 1. Composition of fractions from a Sephadex G-10 column

Careful selection of fractions or recrystallisation produces glucosinolates of high purity. The method has general applicability for the isolation of 4-hydroxyindolyl-3-methyl, 1-methoxyindolyl-3-methyl and 2-phenylethyl glucosinolates from swede; however it has been found that 4-methoxyindolyl-3-methyl glucosinolate is not easily separated from indolyl-3-methylglucosinolate by these means and therefore the use of DEAE Sephadex A-25 as described previously is advocated (3).

Attempts to isolate pure 4-pentenyl and 2-hydroxy-4-pentenyl glucosinolates are underway since these compounds are important chromatographic standards, particularly in the analysis of rapeseed meal. Further investigation of the chromatographic (gc and hplc), chemical and biological properties of indole glucosinolates (VII-IX) are underway in order to present a more complete picture of this important group of glucosinolates.

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REPEATABILITY ESTIMATES OF GLUCOSINOLATE CONTENTS IN MOTHER PLANTS OF *BRASSICA OLERACEA* AND THEIR CLONES.

Curtis B. Hill, Paul H. Williams and Diana G. Carlson.

In studies of the inheritance of GS's in a rapid-cycling population of *B. oleracea* derived from CRGC-3, using shoots at first flower, we found GS analyses from cloned plants often did not agree with the initial analyses from the mother plants.Therefore, the following experiments were designed to estimate the repeatability in measurements of GS contents to provide us with information on the variation. Plants were cloned to provide adequate fresh tissue for analyses.

Repeatability as used in this paper is the correlation of GS analyses and the proportion of phenotypic variance due to genetic and general or permanent environmental factors (3,4). Temporal repeatability is the correlation of analyses of individual plants made at different times. Clonal repeatabilty is the correlation of analyses among clones of an individual plant.

Eleven rapid-cycling *B. oleracea* plants (mother plants), belonging to families having GS contents which represent the the full range found in the experimental population, were grown at 24C under continuous $250 \mu Em^{-2}s^{-1}$ irradiation and were irrigated daily with 50% Hoagland's nutrient solution. Shoots at first flower were sampled from each plant and analyzed for GS's (1,2). The mother plants were allowed to grow new shoots and three cuttings from each plant, were rooted and grown in the same environment as the mother plants. Approximately 1 mo. after initially sampling the mother plants, clonal shoots at first flower and shoots from the mother plants were sampled individually and analyzed for GS's.

Variation between plants and between sampling times was calculated by analysis of variance and the interclass correlation coefficients were calculated for individual and total GS's to give the temporal repeatabilities (3,4) (Table 1). To estimate clonal repeatabilities (3), variation between and within mother plants (clones) was calculated by analysis of variance and the intraclass correlation coefficients were calculated (3,4) (Table 1).

Differences among the mother plants were significant in several cases (Table 1). Measurements of some GS's in shoots of the mother plants taken at different times varied considerably as did measurements from clones of individual mother plants. For individual GS's the average variation among clones of individual mother plants was always higher than the variation among sampling times. Clonal repeatabilities (X=.33) averaged less than temporal repeatabilities (X=.60). The fact that each clone derived from a single mother plant was genetically identical suggests that rooting of cuttings may have introduced

variation. Therefore, cloning to increase fresh weight for GS analysis might increase variation. The relatively high temporal repeatabilities suggested that repeated GS measurements over time are fairly consistent.

Table 1.
Temporal and clonal repeatabilties of glucosinolate content in eleven *Brassica oleracea* plants.

Aglucon	Clonal Repeatability	Temporal Repeatability
Allyl	.36*	.59
Butenyl	.38*	.79*
4-Methylthiobutyl	.33*	.82*
Vinyl-OZT	.23*	.80*
4-Methylsulfinylpentyl	.47*	.62*
2-Phenylethyl	.44*	.47
Indolyl as SCN ion	.10	.51
Total GS	.35*	.23

*indicates that the variation between plants was significantly higher than the variation within plants at P=.05.

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A COMPARISON OF GLUCOSINOLATES IN OPEN POLLINATED AND F_1 CABBAGES

Calvin Chong, M.S. Chiang and R. Crête

Research conducted 50 years ago indicated that the goitrogenic potency of fall or late maturing cabbage cultivars was as much as twice that of summer or early maturing cultivars (Webster *et al.* 1931). Later studies indicated that environment influenced glucosinolate contents in cruciferous crops (Joseffson 1970). Bible and Chong (1975) reported correlations between various temperature rainfall parameters and yield of the glucosinolate hydrolysis product, thiocyanate, in roots of radish. Bible *et al.* (1980) analysed 14 cabbage cultivars and indicated a positive correlation between thiocyanate content in cabbages and days to maturity. In a study of 50 cabbage cultivars, Tookey *et al.* (1980) reported significant correlations between individual glucosinolates in head and seed.

In this study, we compared differences in glucosinolate contents between 22 open pollinated and 37 F_1 cultivars and related the glucosinolate contents of head and seed with selected variables.

As shown in Table 1, total glucosinolates and hydrolytic products were notably higher in seeds than in head tissue of the cabbage cultivars. The content of volatile isothiocyanates, which was particularly high in seeds, was the only product showing a significant difference between open pollinated and F_1 cultivars.

In open pollinated cultivars, volatile isothiocyanates in head was correlated with both volatile isothiocyanates and total glucosinolates in the seed (Table 2). In F_1 cultivars, a correlation was found only between head and seed goitrin. While days to maturity was correlated with head weight in open pollinated cultivars, interestingly days to maturity also was found to be correlated with contents of volatile isothiocyanates, thiocyanate ion, and also total glucosinolates in the F_1 cultivars.

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Table 1. Comparative contents of glucosinolate products (µg/g dry weight) in head and seed of open pollinated and F_1 cabbage cultivars.

Glucosinolate product	Open pollinated	F_1	t-value
	Head		
Goitrin	43	31	1.22
Volatile isothiocyanate	597	546	0.66
Thiocyanate ion	354	332	0.48
Total glucosinolate	994	909	1.25
	Seed		
Goitrin	518	466	0.49
Volatile isothiocyanate	2428	3222	2.48*
Thiocyanate ion	566	558	0.24
Total glucosinolate	3615	4245	1.78

NS Not significantly different

* Significant at 5%

Table 2. Correlation of glucosinolate constituents between head and seed and with other variables.

Head	Seed				
	Goitrin	Volatile isothiocyanate	Thiocyanate ion	Total glucosinolates	Days to maturity
			Open Pollinated Cultivars		
Goitrin	NS	NS	NS	NS	NS
Volatile isothiocyanate		0.483*	NS	0.443*	NS
Thiocyanate ion			NS	NS	NS
Total glucosinolates				NS	NS
Head weight					0.458*
			F_1 Cultivars		
Goitrin	0.459**	NS	NS	NS	NS
Volatile isothiocyanate		NS	NS	NS	0.547**
Thiocyanate ion			NS	NS	0.330*
Total glucosinolates				NS	0.607**
Head weight					NS

NS Not significantly different

*, ** Significant at 5% and 1%

IDENTIFICATION OF ZERO-OR LOW-GLUCOSINOLATE CULTIVARS AND CLUBROOT RESISTANT SELECTIONS OF CABBAGE

M.S. Chiang, Calvin Chong and R. Crête

Our research project has been examining the composition, variation, and physiology of glucosinolate-derived products in vegetable crops of the Cruciferae. Similar to rapeseed, these vegetables contain glucosinolates which form a number of breakdown products, the most prominent being isothiocyanates, thiocyanates, and 5-vinyl-oxazolidine-2-thione goitrin. These breakdown products are important because they contribute significantly to the flavor of cole crops. Furthermore, some of them are also toxic and may suppress growth when fed to animals. There is concern that levels of natural toxicants such as glucosinolates might be inadvertely increased during plant breeding. In a joint program with researchers at Agriculture Canada St-Jean Research Station, Quebec, we are determining the mode of inheritance of selected glucosinolates in commercial cabbage cultivars, and improving the quality of clubroot resistant cabbage selections by selecting those that are also low or lacking in glucosinolates.

In a recent communication (Chong *et al.* 1984), we studied the glucosinolate composition of commercial cabbage cultivars and of clubroot resistant cabbages from the breeding program at St-Jean Research Station. The mean thiocyanate ion content was significantly lower in the breeding selections (199 µg/g dry weight) than in the commercial cultivars (340 µg/g). In contrast, the mean goitrin was significantly higher in the breeding selections (193 µg/g) than in the commercial cultivars (35 µg/g). Similar to goitrin, the range of volatile isothiocyanates and total glucosinolate were higher in the breeding selections, but the mean contents of each were not statistically different between selections and cultivars. Fourteen cultivars and four selections were found to be free of goitrin; three cultivars, but no breeding selection were free of volatile isothiocyanates. The breeding selections will provide germplasm for breeding new clubroot resistant and low glucosinolate cultivars.

Similar studies are underway with other types of cole crops.

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TOXICITY OF THIOCYANTE ION TO SEEDLING GROWTH OF WEED AND CROP SPECIES

Bernard B. Bible

Thiocyante ion (SCN), which commonly forms in tissues of cruciferous plants, may be partially responsible for allelopathic effects of residues of cruciferous plants.

Twenty seeds each of tobacco (Nicotiana tabacum L. cv. Nicki Red), cabbage (Brassica oleracea var. capitata L. cv. Early Greenball), portulaca (Portulaca grandiflora), pigweed (Amaranthus retroflexus), and lambsquarter (Chenopodium album) were placed in 9-cm Petri dishes containing 30 ml of pasteurized sand and wetted with 10 ml of solutions with four concentrations of SCN supplied as KSCN (0, 0.09, 0.44, and 2.2mM). Fifteen seeds of green bean (Phaseolus vulgaris L. cv. Contender) were placed in 10.4-cm dishes containing 60 ml of sand and wetted with 20 ml of the various test solutions. After 7 days incubation at 20+1° C for the cabbage, portulaca, and tobacco, and at 26+1° C for pigweed, lambsquarter, and green bean, the shoot and root length and percent germination were recorded. The treatments were replicated three times and regression analysis was run on all data.

Thiocyante ion was not inhibitory to the germination of any of the test species, and it did not inhibit the seedling growth of bean plants. Tobacco and portulaca seedling growth appeared to be the most sensitive to SCN, particularly for root elongation. Cabbage and lambsquarter shoot growth was less sensitive to SCN, but their roots were almost as sensitive as those of portulaca. Pigweed shoot elongation was sensitive to SCN, but its roots were much less so. These results suggest that species differ widely in their response to SCN, and that some species could be adversely affected by modest concentrations of SCN in the environment.

TABLE 1. Estimated SCN concentration required for fifty percent reduction in seedling growth.

Test species	Millimoles of SCN required for 50 percent reduction in elongation	
	Shoot	Root
Tobacco	2.6	1.7
Portulaca	2.7	2.1
Pigweed	2.9	4.0
Lambsquarter	3.2	2.6
Cabbage	4.5	2.2
Bean	-a	-a

a Showed no inhibition of elongation

HERITABILITY FOR TOTAL GLUCOSINOLATE IN A RAPID-CYCLING *B. OLERACEA* POPULATION.

CURTIS B. HILL, PAUL H. WILLIAMS AND DIANA G. CARLSON.

Heritability of total GS content was estimated by regressing offspring on eight parents (four with high GS, 275-418 ppm and four with low GS, 39-49 ppm) which were reciprocally crossed in all possible combinations. Five offspring from each cross were grown in a standard greenhouse soil in a 20C greenhouse with supplemental fluorecsent light and were fertilized weekly with a 10:10:10 fertilizer. Young stems at first flower of the eight parents and the 320 total offspring were analyzed for total GS content (1,2). Regression of offspring on parents provided an estimate of the heritability in the narrow sense or the additive genetic variance (3) and the estimate was .32.

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AMINO ACID CONTENT IN INDIAN RADISHES AND THEIR HYBRIDS

N. Dayal and B.B. Lal

Chromatographic analysis of biochemical constituents in plant tissue has been successfully used in solving taxonomic problems, in tracing phyllogenetic affinities and in understanding the genetics of several important chemical compounds. In the present investigation an attempt has been made to analyse and identify amino acids in leaves of some varietal populations of Indian radishes and their F_1 hybrids in order to understand the genetics of amino acids and their distribution pattern.

Four varietal populations of Indian radish (Raphanus sativus), 'Japanese White' (JW), 'Long White Green Top'(GT), 'Kalamikati Red' (KR) and 'Rainy Season Red' (RR) and their two F_1 hybrids, JW x GT and KR x RR have been used in the present investigation. They differ in several morpho-physiological characteristics. Plants were raised in identical field condition at Ranchi during winter. Young leaves from 8-10 plants in each population were analysed chromatographically (1).

The population varied markedly in the number and amount of amino acids as indicated by the size and number of spots on the chromatograms. Significant between population variation was noted in amino acid content. Among the populations, JW had 8 and KR 9 amino acids in all, while RR and GT each had 12 amino acids. All of them had 6 amino acids in common, namely valine, dl-alanine, threonine, glycine, glutamine and glutamic acid. JW had leucine/isoleucine and B-phenylalanine in addition to these basic amino acids. RR contained proline, y-aminobutyric acid, methionine, homoserine, phenylalanine and glutamic acid in addition to basic amino acids. Thus, it had 5 more amino acids than JW, but lacked leucine/isoleucine. KR had one more amino acid than JW; it had tryptophan and glutamic acid but lacked B-phenylalanine. RR and GT had 11 amino acids in common and differed in one amino acid only (proline in RR and leucine/isoleucine in GT). There was no noticeable variation within population in amino acid content.

In contrast to populations, their F_1 hybrids showed noticeable variation in the number, amount and distribution pattern of amino acids. In general they had a summation of amino acids of their parents. In JW x GT, there were 14 amino acids against 8 in JW and 12 in GT. KR x RR averaged midway between their parents. Some new amino acids appeared and disappeared in the hybrids. The vulnerable amino acids were: tyrosine, methionine, tryptophan, L-cysteine, homoserine, serine, valine and y-aminobutyric acid which either appeared or disappeared in the hybrids.

Thus, variation in the amino acid content in the Indian radishes has a genotypic basis and that there is an element of genetic control for this parameter.

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POLYSACCHARIDE-DEGRADING ENZYMES PRODUCED *IN VITRO* BY *LEPTOSPHAERIA MACULANS*

P. Hanacziwskyj and R.B. Drysdale

Leptosphaeria maculans is known to produce polygalacturonase (PG) *in vitro* with pectin or polypectate as the sole carbon source (Szajer and Bousquet, 1975; Nathanials, 1981; Easton, 1982) and to have a relatively low pectin methyl esterase (PME) activity (Szajer and Bousquet, 1975). Szajer and Bousquet (1975) and Nathanials (1981) each used one isolate of *L. maculans* while Eston (1982) used two isolates varying in virulence and recorded higher PG activity with the isolate of lower virulence.

When grown on cell walls of rape cv. Jet Neuf an isolate of *L. maculans* was shown to produce a range of extracellular cell wall degrading enzymes (Nathanials, 1981). Using a 'highly virulent' isolate Easton (1982) found no difference in PG or Cx activity in filtrates of cultures grown on Primor or Jet Neuf cell walls, but PL activity was significantly higher in the former filtrates.

However, *L. maculans* is now known to exist in Britain as three pathotypes: highly virulent, low virulence and non-pathogenic (Hanacziwskyj and Drysdale, 1984*a* and *b*) on the basis of disease severity on winter oilseed rape cvs. Jet Neuf or Primor or cabbage cv. January King.

The nature and amount of extracellular cell wall degrading enzymes of isolates of *L. maculans* grown in liquid medium (Boudart, 1978), with glucose replaced by 1% w/v citrus pectin or 0.5% w/v oilseed rape cell walls, was determined.

A. Citrus pectin as carbon source

Total PG of culture filtrates was determined by the release of reducing sugars from sodium polypectate. All isolates demonstrated PG activity and this amount increased with age of the cultures. Low virulence isolates had higher PG activity (76 to 167 units[1]) than highly virulent isolates (17 to 50 units[1], with the exception of one isolate, 112 units[1], that produced several additional isoenzymes compared to the other highly virulent isolates)(Hanacziwskyj and Drysdale 1984*a* and *b*).

B. Oilseed rape cells walls as carbon source

Isolates grown for 6 days on cv. Primor cells walls produced a range of extracellular cell wall degrading enzymes. Similar, though slightly higher activities, were obtained with cv. Jet Neuf cell walls.

All isolats produced PG, β-D-glucosidase, α-D-galactosidase and α-L-arabinosidase and activities were not correlated with pathotype. However, isolates belonging to the highly virulent pathotype were generally the only isolates to produce PME and protease activity (detected by cup plate methods) whereas generally only low virulence isolates possessed relatively high cellulase activities (measured by release of reducing sugars from CMC, and to a lesser extent by reduction in viscosity of CMC solutions). Phosphatidase activity was not detected and levels of β-D-xylosidase were insignificant.

[1]Pectolytic activity was expressed as:
μmol equivalent galacturonic acid released/h/ml of culture filtrate.

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BLACK ROT - resistant cauliflower

S. Honma

Hybridization between Self-Blanche cauliflower and Early Fuji cabbage made 10 years ago has resulted in two cauliflower lines that are resistant to Black Rot as Early Fuji.

These lines were observed at several locations for resistance to the disease and have performed well horticulturally. One of the lines are being considered for release in 1985.

CORRELATIONS OF VIRULENCE, GROWTH RATE, PIGMENT PRODUCTION AND ALLOZYME BANDING PATTERNS WHICH DIFFERENTIATE VIRULENT AND AVIRULENT ISOLATES OF *LEPTOSPHAERIA MACULANS*.

Curtis B.Hill, Xu Xiao hua and Paul H. Williams.

Isolates of *Leptosphaeria maculans* from Australia, New Zealand, France, South Africa and the United States from several crucifer hosts were tested for virulence on a set of six crucifer species, for radial growth rate on three agar media, for the production of brown water-soluble pigment and for allozyme banding patterns. Virulence tests were conducted by inoculating wounded 5-day-old cotyledons of *Brassica campestris, B. nigra, B.juncea, B. oleracea, B.napus* and *Raphanus sativus* with 10^5 conidia in a 10μl drop of water. Plants were grown at 20C under continuous fluorescent light in the greenhouse and symptoms were rated 10 days after inoculation with a 0-9 scale, where 0=immunity and 9=full susceptibility. Radial colony growth was measured after 1 wk on potato dextrose agar, V8 juice agar and malt extract agar. Pigment production was positive if a brown, water soluble pigment was present in cultures grown for 3 wk in the dark at 20C in Czapek's Dox broth (1). Allozyme patterns were assessed using starch gel electrophoresis (2).

Of 51 isolates tested, 10 were avirulent (AV), producing no symptoms on any host and 41 produced symptoms of varying degrees on four or more host species. None of the isolates strongly attacked *B. nigra* or *B. juncea*. Two isolates from *B. juncea* were AV. Isolates from *R. raphanistrum* and *B. hirta* were moderately V. Isolates from *B. campestris, B. oleracea* and *B. napus* ranged from weakly V to highly V and five from *B. campestris* were AV.

Of the media used, V8 juice agar was the best for differentiating V and AV isolates based on colony growth rate. AV isolates generally grew faster than V isolates. Colony growth rate on non-acidified media was not as effective in differentiating the isolates as with acidified agar media (1). All AV isolates produced pigment whereas no V isolates did.

Eleven enzyme-buffer systems were examined using starch gel electrophoresis. For malate dehydrogenase (E.C. 1.1.1.37) in tris-citrate buffer (pH=6.7), AV isolates produced a characteristic faster moving band in addition to the slower moving band produced by all isolates.

These correlations suggest that AV and V isolates may be from reproductively isolated populations. Many matings of V x V isolates have been fertile while V x AV and AV x AV matings have been infertile. We are working on methods for improved induction of the sexual stage and exploring other ways for determining the relationship among AV and V isolates, such as making forced matings and heterokaryon analyses. More isolates from the United States, Europe and elsewhere are being tested for the correlations. We are also examining the possible coexistence of AV and V isolates on the same plant or in the same lesion. The possibility of differential susceptibility of various plant organs is also under study.

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RESISTANCE TO BLACKLEG, *LEPTOSPHAERIA MACULANS*, IN RAPE AND SWEDES, *BRASSICA NAPUS*

J. Lammerink

Leptosphaeria maculans is a fungal pathogen of brassicas causing blackleg or basal stem canker in seed crops and dry rot in swedes grown for winter feeding of sheep in the southern part of New Zealand.

An autumn-sown trial was carried out at Lincoln to compare 12 cultivars and breeding lines of *Brassica napus* for resistance to blackleg: 3 breeding lines and 2 cultivars of oilseed rape, including Tower, which is normally sown in spring; 3 cultivars of forage rape and 4 cultivars of swedes including Tina, a new cultivar with a high level of resistance to dry rot, bred in New Zealand by Crop Research Division, DSIR. Tina was selected from a cross between Doon Spartan//Debra turnip *(C. campestris)* /Sensation, from which Kiri swede was derived, and Wilhelmsburger/Wye//Gartons Parkside with Gartons Parkside being a major source of dry rot resistance (Smith 1960). The trial was sown on 3 April adjacent to infected rapeseed stubble from the previous season. It consisted of three-row plots with 0.5 m row spacings and 4 replications. There was adequate natural infection and by mid-winter all plants showed a large number of leaf lesions. The first stem lesions were observed when the plants started to bolt in early spring. After flowering on 24 November the trial was scored for blackleg symptoms. 50 plants in the middle row of each plot were graded for stem infection in five classes:

0	=	no symtoms
1	=	superficial lesions < 10 mm long
2	=	superficial lesions > 10 mm long
3	=	deep lesion or crack
4	=	stem broken off

A disease index (DI) was calculated for each plot using the equation:

$$DI = \frac{25 \sum (n \times c)}{N}$$

in which n = number of plants in each class; c = class number and N = total number of plants scored per plot. Analysis of variance was carried out on percentages of severely infected plants (classes 3 + 4) and of broken plants (class 4) after arc sin transformation and on disease index. Waller and Duncan's multiple range test was applied.

Table 1: Infection of *B. napus* cultivars and breeding lines with *L. maculans*

Type and cultivar or breeding line	Severely infected incl. broken plants (%)	Broken plants (%)	Disease Index
Oilseed rape			
Primor	19 ab A †	7 b A †	27.7 a A †
Line A	28 b A	8 b A	39.2 b A
Line B	78 d C	40 de BC	75.4 de C
Line C	82 d CD	45 e CD	78.5 e CD
Tower	94 e DE	75 g E	92.1 f E
Forage rape			
Rangi	17 a A	3 ab A	26.8 a A
Giant	29 b A	5 ab A	34.8 ab A
Wairangi	60 c B	26 c B	60.8 c B
Swedes			
Tina	29 b A	0 a A	34.0 ab A
Kiri	73 d BC	26 c B	67.4 cd BC
Doon Major	74 d BC	28 cd B	67.6 cd BC
Calder	98 f E	64 f DE	89.4 f DE
CV%	12.4	19.6	12.8

†Within each column values without a letter in common are significantly different at P = 0.05 (small letters) or P = 0.01 (capital letters).

There were large differences in disease reaction within each type of crop with Rangi and Primor, followed by Tina, Giant and Line A, showing resistance (Table 1). Results confirm that despite the occasional high incidence of leaf infection in seed crops of Rangi rape, little stem infection occurs in this cultivar in contrast to some other cultivars of forage rape, such as Wairangi.

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PRELIMINARY INVESTIGATIONS OF RELATIONSHIP BETWEEN LEAF GLUCOSINOLATES AND SUSCEPTIBILITY TO *LEPTOSPHAERIA MACULANS* IN WILD AND CULTIVATED BRASSICAS

R.F. Mithen, B.G. Lewis, R.K. Heaney and G.R. Fenwick

Stem canker disease of oilseed rape (*B. napus*) caused by the fungus *Leptosphaeria maculans* remains an important problem in the U.K. The most destructive cankers arise from systemic growth of the pathogen from leaf lesions to the stem (1). Resistance to the leaf spot phase would therefore reduce the frequency of cankers at harvest.

Glucosinolates and their breakdown products, mainly the isothiocyanates have a wide range of biological activity and have been implicated in resistance to fungal diseases (2). A collaborative project between the University of East Anglia and the Food Research Institute, Norwich, has been initiated to investigate the relationship between leaf glucosinolates and susceptibility to leaf infection by *L. maculans* in a range of wild and cultivated plants of *B. campestris* and the *B. oleracea* complex.

Infection of newly expanded leaves of these plants with a pycniospore suspension results in a range of symptoms which can be grouped into three reaction classes.

Class 1	Hypersensitive	Very small areas of necrosis.
Class 2	Localised Lesions	Grey lesions 0.5-2 cm in diameter which may or may not result in a systemic reaction as the leaf ages.
Class 3	Systemic	Systemic fungal growth usually in vascular tissue with little or no necrosis around inoculation site but often extensive chlorosis.

Individual plants from each of these classes were selected and the leaf glucosinolates analysed as previously reported (3). Plants from classes 1 and 2 had the infected leaf removed and subsequent analysis was undertaken on the oldest leaf prior to senescence and the youngest newly expanded leaf from each plant. Analyses of glucosinolates from class 3 were done on uninoculated plants from cultivars which had been shown to have little variation in susceptibility between plants.

Total glucosinolates are shown in Figure 1. The mean levels of glucosinolates in class 3 are significantly lower (95%) than those in classes 1 or 2, between which there are no significant differences. The relatively large levels of glucosinolates in Plant no 9 which exhibits a systemic reaction may imply that either glucosinolates are unimportant or that the interaction is more complex. It may be significant that 95% of the glucosinolates in Plant no 9 is gluconapin.

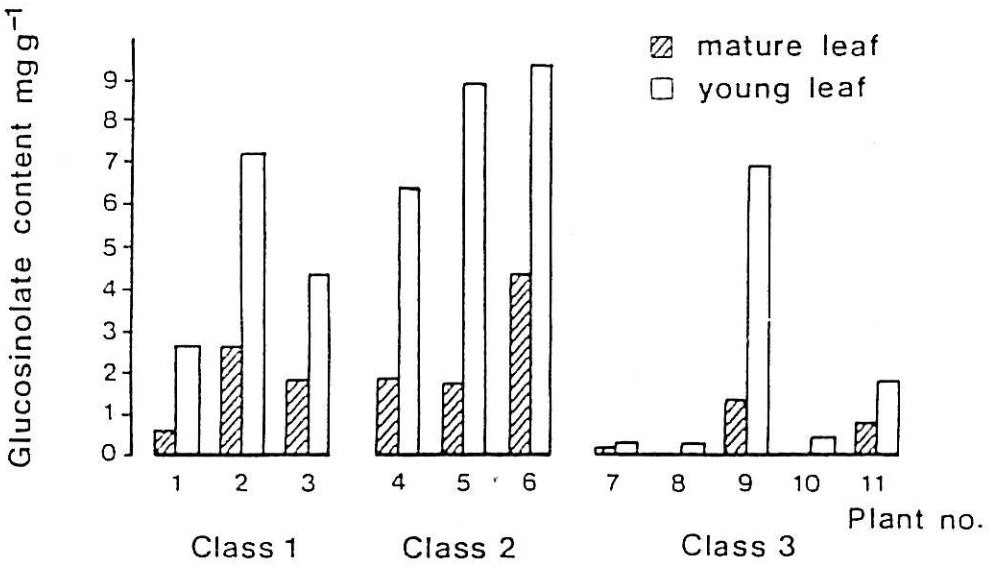

Figure 1. Glucosinolate content of 11 Brassica plants showing different reactions to leaf infection by L. maculans. Class 1 - Hypersensitivity Class 2 - Localised lesions Class 3 - Systemic. Plant no.

1. B. oleracea
2. B. insularis
3. B. cretica
4. B. oleracea
5. B. incana
6. B. rupestris
7. B. campestris ssp. campestris
8. B. campestris ssp. oleifera
9. B. campestris ssp. campestris
10. B. campestris ssp. pekenensis
11. B. campestris ssp. japonica

High levels of glucosinolates may be expected to occur in wild brassicas and may be unrelated to disease resistance. Assessment of the glucosinolates in a wider range of material, particularly plants from resistant and susceptible wild B. campestris populations and susceptible B. oleracea varieties is in progress.

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EPIDEMIOLOGY OF ALTERNARIA BLIGHT OF RAPESEED AND MUSTARD

G.S. Saharan and A.K. Kadian

The epidemic development of Alternaria blight of rapeseed and mustard caused by Alternaria brassicae (Berk.) Sacc. is influenced by a number of factors under field conditions. In the present study the prevailing environmental conditions and cultivars grown were analysed to measure the progress of disease.

Materials and Methods

To measure the progress of disease under field conditions in relation to environment, 20 leaves of each cultivar were tagged on 9.1.81. The observations on increase in number and size of lesions were recorded at an interval of 5 days till the death of leaves.

In another experiment the progress of disease was measured on the basis of five plants of each cultivar viz, RC-781, Tower, Prakash and YRT-3 tagged before appearance of the disease in nature. The number of infected leaves, number and size of lesions per infected leaf were recorded at intervals of seven days.

The data on weather variables viz maximum temperature (MXT), minimum temperature (MNT), mean temperature (MT), mean relative humidity (MRH), rainfall (MM) and wind velocity (km/hr) prevailing during the period of study were obtained from meteorological department, HAU, Hisar located 800 metres away from the experimental area.

Results and Discussion

The progress of disease under nature is influenced by many factors viz prevailing environmental conditions, resistance status of the cultivars sown and cultural practices followed, even if an abundant amount of inoculum is available. Fig 1 indicates periodical and cumulative increase in number of lesions/leaflet on cultivars Prakash and Tower, respectively susceptible and resistant. The number of lesions per leaflet were less on Tower and RC-781 (resistant cultivars) than on Prakash and YRT-3 (susceptible cultivars). The maximum periodical increase of lesions per leaflet on all the cultivars was recorded from 24.1.81 to 3.2.81 where weather variables influencing the disease were found at MXT from 18.78-23.14°C, MNT from 6.80-10.21°C, MT from 14.21-15.42°C, MRH from 53.80-80.80% and rainfall from 0.2-2.12 mm with 4.50-8.20 km/hr wind velocity. Substantial increase in disease was also observed from 25.2.81 to 2.3.81 because of favourable weather conditions. The disease progressed at a slow rate before 24.1.81 since weather variables were quite low.

Increase in size of lesions on different cultivars showed similar trend as far as their influence by environmental factors was concerned. However, the size of lesions on resistant cultivars like Tower and RC-781 was less but on susceptible cultivar YRT-3 lesions up to 33.20 mm were observed. On Tower, the infection was delayed by up to 15 days

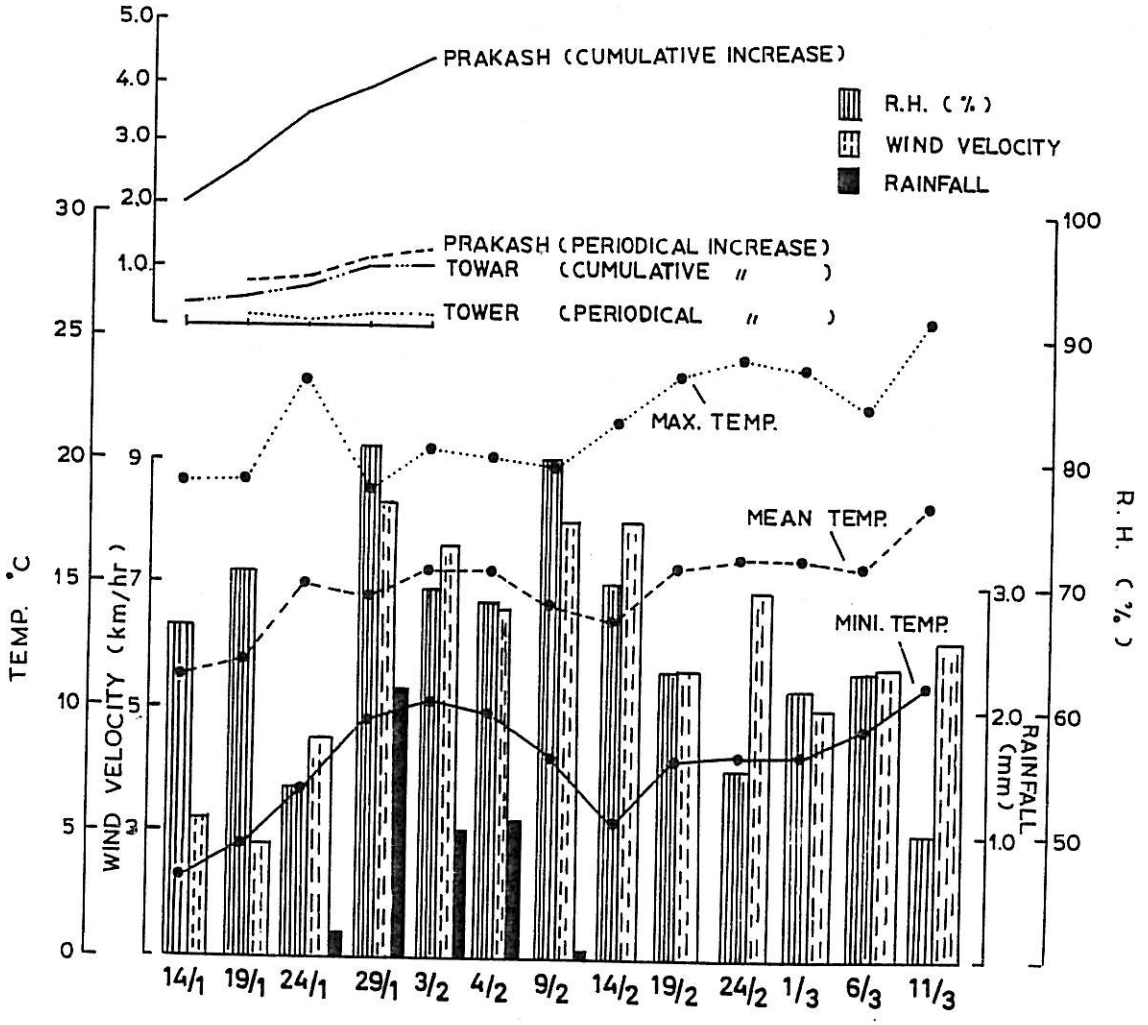

Fig. 1. INFLUENCE OF WEATHER VARIABLES ON DEVELOPMENT OF ALTERNARIA BLIGHT.

and there were only a few lesions per leaflet (0.95) up to the third week of March. In RC-781, although infection took place along with other susceptible varieties, the number of lesions was restricted to 2.98 per leaflet. In Prakash and YRT-3 lesion numbers were more than 3.33 and 3.22 respectively right from the initiation of infection. The percentage of infected leaves per plant was only 15.62% in Tower but it was 36.10% in Prakash up to the third week of March.

Saharan et al (1981) analysed the environmental factors involved in the progress of disease under field conditions on different cultivars. According to their study, disease increased with a slow speed with less number of spots per leaflet on cultivar RC-781 (2.2) followed by Tower (2.4). On cultivar Prakash the progress was rapid with maximum spots/leaflet. The results obtained in the present study corroborated these findings.

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INCIDENCE OF *ALTERNARIA* INFECTION IN OILSEED RAPE (*BRASSICA NAPUS L*) CROPS IN EAST SCOTLAND, 1984

Kothanur P R Prasanna and J H Lennard

From 1982 there has been a considerable expansion in the area of production of oilseed rape in Scotland. During this period of intensification of oilseed rape growing, surveys of *Alternaria* infection have been carried out in east Scotland (Prasanna and Lennard, 1982; 1983). The present paper reports the results of the survey carried out in 1984: 51 crops were assessed and the results are summarised in Table 1.

Table 1 Incidence of *Alternaria brassicae* and *Alternaria brassicicola* in oilseed rape crops in South-east Scotland, 1984

District	Number of fields inspected	Number of fields infected with *Alternaria*	
		A brassicae	*A brassicicola*
Borders	14	10	0
Lothians	11	4	1
Fife	14	3	0
Perth, Kinross and Angus	12	2	0

As in previous years, the most prevalent pathogen was *Alternaria brassicae* (Berk) Sacc, while *Alternaria brassicicola* (Schw) Wiltshire was found in only one crop. The incidence of both species was much less than in 1982 and 1983: at the flowering stage only 19 of the 51 fields showed infection by *A brassicae* along with the one occurrence of *A brassicicola*, and no infection was subsequently recorded in seed samples from any of the crops. Spotting symptoms in the growing crop were generally confined to the lower leaves. The low incidence in 1984 may be related to the prolonged period of dry weather during most of the flowering and seed development stage (Table 2).

Table 2 Monthly temperature and rainfall in South-east Scotland (1984)

District	Daily mean temperature °C				Monthly rainfall mm			
	May	June	July	August	May	June	July	August
Borders	9	13	16	16	11	69	47	40
Lothians	9	13	16	16	55	51	25	23
Fife	10	14	16	16	33	51	34	13
Perth	9	13	15	15	31	37	30	12

In the previous year there was a decline in disease incidence during the growing season from flowering to seed maturation, which might again be attributed to reduced rainfall over the period. Humpherson-Jones and Hocart (1983) have indicated that *Alternaria* infection requires free water. A reduction in disease incidence at seed maturation in east Scotland in 1983 may also be attributed partly to the use of aerial sprays of iprodione (Prasanna and Lennard, 1983). However, in 1984 few crops were sprayed.

The pattern of incidence of *A brassicae* in relation to different regions was similar to that in previous years, infection occurring more frequently in the Borders region and declining towards the north. As discussed before (Prasanna and Lennard, 1982), this may be related to regional differences in intensity of cropping.

The results over the 3 years indicate, in general, the importance of rainfall during the flowering and seed maturation phase in its effect upon disease incidence and severity; a further contributory factor is the intensity of cropping of susceptible crops. Within individual fields, dense crops and sheltered situations which encourage a high atmospheric humidity were observed to favour infection, while farms with a history of oilseed rape cropping were more likely to be associated with severe infection.

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OCCURRENCE AND RELATIVE ABUNDANCE OF FUNGI ANTAGONISTIC TO *ALTERNARIA BRASSICICOLA* ON THE PHYLLOPLANE OF MUSTARD

S.K.Sarkar and K.R.Samaddar

Mustard (*Brassica juncea* (L.) Coss) cultivars B-85 and T-59 are extensively cultivated in West Bengal. Leaf spot caused by *Alternaria brassicicola* is the most destructive disease of the crops in West Bengal[2]. The disease usually appears on 45-to 60-day-old mature plants in the field. Occurrence of mycoparasites of *A. brassicae* on the phylloplane of rapeseed has been reported[4]. The occurrence and relative abundance of fungi antagonistic to *A. brassicicola* on the phylloplane of the cultivars B-85 and T-59 of mustard were investigated.

The phylloplane mycoflora of the two cultivars were isolated by leaf washing method[1] and screened for their antagonistic activity against *A. brassicicola* in dual culture[3]. Of the 20 species of fungi isolated, only 3 organisms viz. *Cladosporium cladosporioides*, *Penicillium variable* and *Penicillium* sp. isolate B-2 were inhibitory to *A. brassicicola* on PDA and the inhibition zones ranged from 4 to 8 mm.

The relative abundance of the phylloplane organisms especially the antagonists and the pathogen as a function of age of the cultivars was estimated. The result (Table 1) indicates that the total number of surface organisms including the fungi increased with age. The population density of *A. brassicicola* gradually increased and was maximum on 60-day-old plants. In contrast, the antagonistic fungi gradually declined on the mature plants. The data suggest influence of non-pathogenic surface flora on the population build-up of the foliar pathogen *A. brassicicola* on the phylloplane of mustard plants. Mycoparasites of *A. brassicae* isolated from the phylloplane of rapeseed[4] could not be isolated from the leaf surfaces of test cultivars of mustard.

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Table 1. Population dynamics of leaf surface microorganisms in relation to the age of mustard cultivars B-85 and T-59

Plant age : Days after sowing (Sampling date)[d]	Number[a]/cm^2 of surface (X10^4)		Total No.[b] of fungal colonies		Percentage abundance[c] of							
					A. brassicicola		C. Cladosporioides		Penicillium variable		P. Sp. isolate B-2	
	B-85	T-59	B-85	T-59	B-85	T-59	B-85	T-59	B-85	T-59	B-85	T-59
30 (December 5)	0•22	0•18	120	106	16•7	16•9	15•0	9•4	1•67	1•89	0	0
45 (December 20)	1•16	0•63	262	153	17•6	13•7	7•6	10•4	0	0	4.6	2.6
60 (January 4)	2•52	1•24	178	109	21•4	21•1	5•6	8•2	0	0	1•7	2•8

[a]Total number of bacteria, actinomycetes and fungi were considered.

[b]Total number of fungal colonies on PDA were counted.

[c]Percentage abundance was calculated based on total number of fungal colonies.

[d]Seeds were sown on November 5 and leaf samples were collected on indicated days.

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OCCURRENCE AND RELATIVE FREQUENCY OF DIFFERENT SPECIES OF *ALTERNARIA* ON CRUCIFEROUS CROPS OF WEST BENGAL

S. Kundu and K.R. Samaddar

Many cruciferous vegetables and oil seed crops are cultivated in West Bengal. Black spot caused by *Alternaria* spp. is the most destructive disease of these crops. Although most of the literature claims *A. brassicae* as the causal agent of black spot disease in India, information regarding the occurrence and relative frequency of *A. brassicae*, *A. brassicicola* and *A. raphani* on cruciferous crops in West Bengal is lacking.

In our survey of black spot disease of cabbage (*Brassica oleracea* var *capitata*), cauliflower (*B. oleracea* var *botrytis*), kohlrabi (*B. oleracea* var *gongylodes*), turnip (*B. campestris*), radish (*Raphanus sativus*), Brown Sarson (*B. campestris* var *Brown Sarson*), Yellow Sarson (*B. campestris* var *Yellow sarson*) and Toria (*B. campestris* var *toria*) in West Bengal, we isolated the pathogen from diseased leaves of the standing crops and identified them following Neergard (1977). Out of 82 samples 89.02% were *A. brassicicola*, 6.09% were *A. raphani* and 4.87% were of mixed infection with both *A. brassicicola* and *A. raphani*. None of the samples yielded *A. brassicae* (Table 1).

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Table 1. Occurrence and relative frequency of different species of *Alternaria* on cruciferous crops of West Bengal

Host	No. of samples	No. of samples yielding			
		Brassicicola	*Brassicae*	*Raphani*	Mixed
Cabbage	13	13	-	-	-
Cauliflower	21	21	-	-	-
Kohlrabi	10	7	-	3	-
Turnip	3	2	-	-	1
Radish	15	10	-	2	3
Brown Sarson	3	3	-	-	-
Yellow Sarson	13	13	-	-	-
Toria	4	4	-	-	-
Total number of of samples	82	73	-	5	4
Relative frequency (%)		89.02	0	6.09	4.87

RESISTANCE OF CAULIFLOWER AND BROCCOLI /B. OLERACEA L. BOTRYTIS L./ SEEDLINGS TO DOWNY MILDEW, PERONOSPORA PARASITICA

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

The reaction of cotyledons to this pathogen was tested under controlled conditions agree with the method described by P.H. Williams /3/ and introduced to Poland by J. Robak /4/. The pathogen was applied as a sporangial suspension instead of micropipette using handsprayer at an inoculum rate of 10^5 sporangia/ml to 4 days old seedlings. The seedlings at this time had fully expanded cotyledons. Isolate of P. parasitica was obtained from broccoli cv. Cotyledons were assessed visually agree with Williams scale. As resistant were assessed seedlings with no sporulation or hypersensitive necrosis /classes 0+1/; seedlings with different degrees of sporulation were designed as susceptible /classes 3+5+7+9/.

Resistance to downy mildew originated from broccoli line. It was used in crosses with Pp susceptible broccoli line. Both lines obtained from R.L. Gabrielson /1/. Parental lines, F_1 and F_2 progeny of these crosses were investigated. The second source of resistance - cauliflower cv. P.I. 208474 obtained from Plant Introduction Station Genewa N.Y. USA. This cultivar was described as having some resistance /5/. S_2 and S_3 progeny of it was tested to investigate the segregation of resistance to Pp.

The inheritance of resistance to Peronospora parasitica Polish isolate of broccoli cv. derived from the broccoli line of R.L. Gabrielson /1/ and cauliflower introduction PI 208474 was found to be governed by one recessive gene /tab. 1/. It was on the contrary to the results of Natti at al. /2/. They have found resistance to the race 1 and 2 was inherited independently by one dominant gene to each race. The recessive type of resistance in many cases was overcome by extending the exposure period of inoculated plants in the greenhouse for 3-4 days. It was agreed with observations of Natti at al. /2/.

Table 1 - Segregation of seedlings tested

Generation and pedigree	Downy mildew reaction Res. no of	Susc. plants	Genetic relations Expected	Obtained	Ch^2	P
Broccoli check /susc./	12	126	100% susc.	90,5 %		
P_1 broccoli	76	8	100% res.	90,5 %		
P_2 broccoli	1	42	100% susc.	97,6 %		
F_1 /P_1 x P_2/	0	33	100% susc.	100 %		
F_2 /F_1/	46	142	1 : 3		0,0106	>0,80
S_2 PI 208474 cauliflower	48	104	1 : 3		1,3158	>0,20
S_3 PI 208474 cauliflower	31	98	1 : 3		0,0171	>0,80

P_1; P_2 parental lines of broccoli

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FIVE STRAINS OF TURNIP MOSAIC VIRUS IN CRUCIFERS IN TAIWAN

Green, S. K. and T. C. Deng

An islandwide survey of Chinese cabbage, radish, and smooth-leaf mustard was conducted in Taiwan to determine the presence, distribution, and prevalence of turnip mosaic virus (TuMV). TuMV was isolated by sap inoculation to Nicotiana tabacum L. 'White Burley', a non-host for other viruses known to occur on the surveyed crops. Strain differentiation was done on four of the nine Chinese cabbage differentials proposed by Provvidenti for TuMV strain classification. Host range studies were conducted using 5 TuMV strains and 88 plant species. TuMV was found in each of the major vegetable production areas surveyed and was isolated from 52 of the 102 leaf samples collected. The isolates represented all four TuMV strains described by Provvidenti and a fifth strain that infected B. campestris subsp. pekinensis PI418957 systemically. This new strain, tentatively named TuMV C-5 has only been found at the Asian Vegetable Research and Development Center (AVRDC) on Chinese cabbage and mustard. The most commonly found strain was C-4; it was present in eight of the nine locations surveyed. TuMV strain C-1 was found only in 2 samples from different locations in southern Taiwan. The following plants were identified in host range studies as immune to all five virus strains: Brassica oleracea subsp. capitata 'Cia-Chio' and 'Hitoma' and B. caulorapa 'Grand Duke', and B. campestris subsp. pekinensis, AVRDC Acc. 730. This line, however, included a small percentage of individual plants susceptible to TuMV C-5, indicating that it is not genetically pure. Host range studies and recent screenings of AVRDC Chinese cabbage accessions and breeding lines indicate that susceptibility to all strains of TuMV, except C-1 is common.

A NEW PATHOTYPE OF THE CRUCIFER YELLOWS ORGANISM CAPABLE OF CAUSING DISEASE IN COOL SOILS

Paul W. Bosland and Paul H. Williams

Pathotypes of *Fusarium oxysporum f. sp. conglutinans* cause yellows and wilt disease of various crucifers; race 1 is found predominately on *Brassica*, race 2 on *Raphanus* and races 3 and 4 on garden stock, *Matthiola incana*. Recently, a pathotype designated race 5 has been found to be pathogenic against the monogenic dominant "type A" resistance gene introduced by Walker over 50 years ago (2). Race 5 appears to be restricted to one locality on a coastal plain of California. Unlike the common race 1 which causes yellows at soil temperatures normally above 18C, race 5 is able to cause disease at soil temperatures as low as 12C. This low temperature pathotype may be of concern to crucifer breeders in countries whose soil temperatures are normally too low to favor the expression of yellows incited by race 1. A survey of cabbage, broccoli, and radish cultivars from Japan, China, Northern Europe, North America, and Brazil for sources of resistance to race 5 is currently underway. There appears to be resistance in cultivars adapted to each country.

As part of a comprehensive study of pathotypic and ecotypic variation in *Fusarium oxysporum f. sp. conglutinans*, we are assembling a collection of isolates from as many different hosts and geographic regions as possible. We would appreciate receiving any isolates that you might be able to send us. Please write us for USDA permits prior to sending your cultures.

References

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