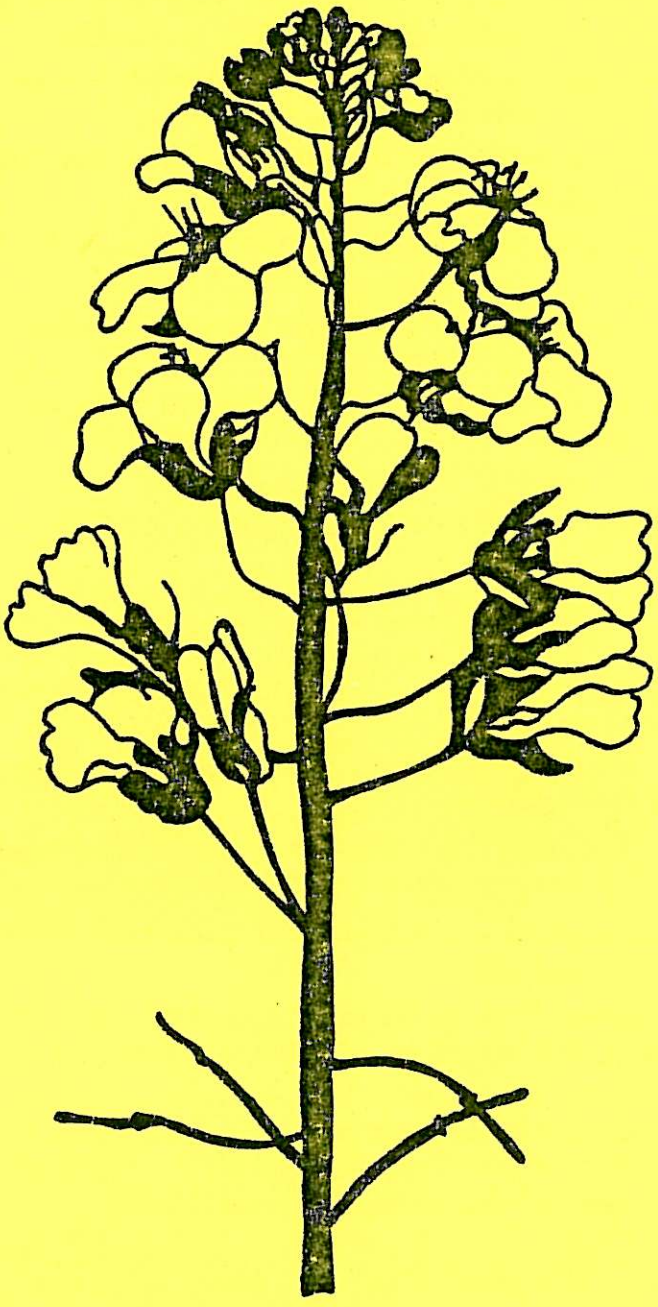
CRUCIFERAE NEWSLETTER No.5



November 1980

EUCARPIA

Editorial

It is encouraging to note the greater number of contributions submitted for the 5th Newsletter than for the previous one. All have been included. The Editors were pleased to see a widening of the range and scope of the papers but saddened to have to report that one of the new authors, Professor Li Chia Wen of the Peoples Republic of China died suddenly while studying at the University of Wisconsin-Madison, U.S.A.

Financial support for the publication of this issue has been received from the National Seed Development Organisation Ltd., Newton Hall, Newton, Cambridge and from the Eucarpia Conference 'Cruciferae 79'. The Editors gratefully acknowledge both of these sources and continue to subscribe to the view that making a charge for the Newsletter would be counter-productive. It will be necessary from time to time to revise the distribution list but Newsletter No. 6 will be sent, unless we are otherwise notified, to those on the current list and to those subsequently requesting to have their names added.

The policy of photocopying earlier numbers once the original stock of copies is exhausted, has had to be discontinued as it was becoming too time consuming. Readers requiring a back copy are advised to locate one from the distribution list and make their own arrangements to have it duplicated. At present the Editors hold a few copies of Nos. 3 & 4, but none of Nos. 1 & 2.

The Newsletter is produced by the staff of two Research Stations in Scotland which are to be amalgamated to form a new organisation named the Scottish Crop Research Institute, but for the present correspondence and contributions should continue to be addressed to The Editor, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian EH25 9RF, Scotland, U.K.

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REPORT OF THE SECOND ANNUAL MEETING OF BRITISH AGRICULTURAL RESEARCH SERVICE BRASSICA BREEDERS

Peter Crisp

Brassica breeders from British public research institutes met at SPBS, Pentlandfield, in 1979 to discuss their mutual interests. It was decided to hold such a meeting annually, and the second meeting took place at NVRS, Wellesbourne, on 4 and 5 September, 1980, with 26 brassica specialists in attendance.

Our discussions included collaborative trials between institutes, automatic data recording, and changes in the structure of brassica breeding in Britain. Of interest to a wider audience were three other topics.

1. Gene conservation

   A joint British/Dutch request has been made on behalf of European breeders to the EEC for funds to collect endangered land races and similar stocks in Europe. Further details can be obtained from Hille Toxopeus (SVP, Wageningen) or Peter Crisp (NVRS, Wellesbourne).

   A report has been prepared on the status of cruciferous crop conservation in Europe, at the request of the International Board of Plant Genetic Resources; this is reported separately in this Newsletter.

   Both developments owe a large measure of their success to the efforts of members of the Eucarpia Crucifer Gene Conservation Group.

2. Problems caused by oil seed rape

   Concern was expressed at the increase in horticultural and agricultural brassicas of *Alternaria brassicicola*, *A. brassicae*, *Phoma lingam* and *Gleosporium concentricum* arising by cross-infection from oil seed rape. This is due to the increase in oil seed rape in Britain, from 5000 to 74000 ha in eight years, and the establishment of rape populations as weeds.

   Problems are also caused by fertilisation of *B. napus* and *B. campestris* forage and fodder seed crops by oil seed rape pollen.

   In future, clubroot and the insect pests *Delia brassicae* and *Pieris* spp. may become more important because there is no resistance or agronomic measure used to limit them in oil seed rape crops.

3. Haploidy

   The meeting felt optimistic that haploid plants produced by culturing anthers would contribute to brassica breeding. It is likely that SPBS and NVRS will start research on this topic.

INTERNATIONAL CLUBROOT WORKING GROUP

Forthcoming events

(1) 6th Meeting of the International Clubroot Working Group to be held at Aas - Norway in August 1981

Gunnar Weisaeth has invited the I.C.W.G. to meet at the Department of Vegetable Crops of the Agricultural University of Norway on the 14 August 1981. He is hoping to get organized a meeting of the Vegetable Section of Eucarpia or perhaps Cruciferae 1981 as a so called natural occasion. Gunnar Weisaeth will give further informations in No. 11 of the Clubroot Newsletter.

(2) XXIst International Horticultural Congress at Hamburg, F.R. Germany from 29th August - 4th September 1982

The I.C.W.G. chairman has contacted the president of the XXIst International Horticultural Congress at Hamburg. He agreed with our proposal to have an informal meeting of all clubrooters together with others interested in this most important disease of cruciferous crops. A room will be available. Everybody who plans to attend this congress and who possibly is interested in Plasmodiophora brassicae please contact the undersigned.

(3) 4th International Congress of Plant Pathology at Melbourne from 17th - 24th August 1983

K.G. Tate, Levin, New Zealand has contacted Dr. Gretna Weste (organizer 4th ICPP) concerning the reservation of a room for 30 - 40 participants during the 4th ICPP at Melbourne. It would be of some help to the organizer if those clubrooters who intend to travel to Melbourne would keep the chairman informed so that we have an impression how many participants will be possibly there.

For further information please contact

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ANNOUNCEMENT - GLOBAL RAPESEED GERMPLASM COLLECTION

G. RÖBBELEN and E. CHONE

The Eucarpia Section Oil and Protein Crops together with the Groupe Consultatif International de Recherche sur le Colza (GCIRC), and the Eucarpia Cruciferae Genetic Conservation Group (CGCG) have recently taken a new initiative to build up a Global Rapeseed Germplasm Collection in order to preserve valuable breeding material from extinction.

During the last twenty years rapeseed breeding has gone through explosive developments. Most of the earlier cultivars have been replaced by types exhibiting entirely new characteristics, low content of erucic acid in the oil being the first remarkable example. In several instances such characteristics were found in a single genetic origin only and this source was introduced worldwide into all the existing cultivars sometimes using halfseed techniques and single plant descents for a new variety. Simultaneously, the crop has expanded its acreage and economic importance considerably and moved into areas, where its cultivation had so far been unknown.

The new GCIRC program will collect from the various countries adapted and important former cultivars of Cruciferae oilseeds which are going to be lost because they are no longer grown. The main recent cultivars from the various countries will also be included.

For this collection entries are requested of the following species:

Brassica napus	(n=19)	B. nigra	(n=8)
B. campestris	(n=10)	B. carinata	(n=17)
B. oleracea	(n=9)	Sinapis alba	
B. juncea	(n=18)	Other Cruciferae oil crops	

A minimum of 300 g of clean, highly germinative seeds of recent harvest is needed. The initial collection and preparation of the seed will be done at

Institut für Pflanzenbau und Pflanzenzüchtung, FAL, Dir. Prof.Dr. Dambroth, Bundesallee 50, D 3300 Braunschweig, Fed.Rep.Germany.

You are kindly requested to send your samples to this address. Here you may also ask for further details of the programme.

Storage will be at two places, one in Europe and one in Ottawa, Canada. Part of the seed will be placed in a long term storage for, e.g. 20 years. The rest will go into a working collection. It is intended to circulate information on the entries to all contributors yearly. Everyone is entitled to receive on request small seed samples from the working collection in Braunschweig.

Since the collection is expected in essence to contain well known and earlier widely distributed varieties only, at this stage no extensive informations on the samples are needed. It is only requested to note the following, when sending the seed:

	e.g.:	
Species		Brassica napus L.var.oleifera f. biennis
Cultivar		"Rapol"
Type		Winter rapeseed for kernel production
Origin		Middle Europe (Germany) - Norddeutsche Pflanzenzucht Lembke
Time of cultivation		1949 / 1974
Harvest date of sample		1979

and informations as far as known on:

Agronomical characteristics	winter hardy
Disease resistance against	Phoma lingam
Quality characteristics	normal content of erucic acid and glucosinolates

From present working collections some samples may be ready for delivery now. But we expect that preparation of other material will take more time. Multiplication may be required in order to send enough fresh seed and this multiplication may conveniently be spread over several years to avoid cross pollination within some of the species.

We roughly expect a total of 300 seed samples per year and an eventual collection of 1000 to 3000 samples of Brassica oilseed cultivars and relatives.

THE STATUS OF GENETIC CONSERVATION OF CRUCIFERS IN EUROPE

Hille Toxopeus and Peter Crisp

In May 1980 we were requested to prepare a report on genetic resources of European cruciferous crops as part of a world-wide assessment being prepared for the International Board of Plant Genetic Resources. Our report was submitted to the IBPGR in September, and the global report is likely to be ready before the end of 1980. We were assisted by Henny Roelofsen (IVT, Wageningen), members of the Crucifer Gene Conservation Group of Eucarpia and others who responded to the questionnaires sent out in early 1980. Useful contact was also established with the Rapeseed Breeding Subcommittee of the Group Consultatif International de Recherches du Colza.

We were asked by IBPGR to propose at the same time a minimal descriptor list - a request also made to us by the European Community Programme Committee on Resistance Breeding and the Use of Plant Genetic Resources. Again, our Eucarpia CGCG colleagues, notably Ian MacNaughton (SPBS, Pentlandfield), and Henny Roelofsen and Lothar Seidewitz (FAL, Braunschweig) were of considerable assistance.

Our report to IBPGR lists the numbers of seed stocks held by Eucarpia CGCG members, by the gene banks at Gatersleben and Braunschweig, and by other colleagues. The time-consuming job of identifying duplications has yet to be attempted. Nevertheless, the data were sufficient to allow us to state that most cruciferous crops in southern Europe were under-collected and that genetic erosion was already occurring. Deficiencies in other crops and areas could also be identified. We recommended that IBPGR should support the collection of endangered land races and local varieties, particularly in southern Europe.

Our recommendation complements a request to the EC Programme Committee by Hille Toxopeus, Henny Roelofsen, Peter Crisp, and Bill MacFarlane-Smith (SPBS) for funds to collect such material in Europe. This proposal - which was approved by the EC Programme Committee but is still under consideration by the EC Agricultural Research Committee - also owes much to the efforts of Eucarpia CGCG members. If required, further details can be obtained from us.

While preparing the IBPGR report it became apparent that confusion existed over the nomenclature of crucifer crops, not only in their taxonomy, but in dialect names. This will be an impediment to identifying duplications and deficiencies in collections, and to the effectiveness of new collecting programmes. We therefore recommended to IBPGR that a taxonomist with pragmatic agricultural sympathies should be employed to produce a definitive taxonomy and list of local names.

The minimal descriptor list largely follows IBPGR recommendations by dividing descriptors into categories. 'Passport' descriptors are for characteristics recorded at the time that the seed stock was

collected:- site, date, collector, and so on. However, we have greatly extended this category to include abnormal environmental stresses and prevalent pests and diseases in the area where the crop developed. We believe that this could give useful information to the breeder by inferring, in the absence of positive evaluation, where he may find resistance to these factors. We have also listed descriptors observed during seed multiplication - ie. highly heritable characteristics, and those obtained during evaluation.

We have departed from the normal IBPGR practice by constructing the minimal descriptor list to cover the general characteristics of all cruciferous crops, and there will be separate lists for each type of crop. In this way we hope to prevent the descriptor list being too cumbersome, which would surely be the case if all characteristics of all cruciferous crops were included in a single list.

We stress that this is a proposed descriptor list, and that in due course our colleagues will be asked for their comments. We will also be eliciting their help in producing a more comprehensive survey of cruciferous genetic resources in Europe.

EFFECTS OF STORAGE HUMIDITY AND GERMINATION TEMPERATURE ON GERMINATION PERCENTAGE OF *BRASSICA* SEEDS

S. Tokumasu and M. Kato

In Cruciferae Newsletter No.4, we mentioned that seed dormancy is being studied in combination with different humidities and different temperatures at Ehime University. The present paper reports the result of this study. Three species, i.e., *Brassica japonica*, *B. cernua* and *B. napus* were used. Freshly harvested seeds were stored in five different humidities, 0%, 20%, 40%, 60% and 80% RH. Germination tests were carried out at five different temperatures, 5°, 15°, 25°, 35° and 45°C, at one or two week intervals during more than 8 months. Therefore, 25 combinations of storage humidity and germination temperature were made in each species.

(1) The optimum storage humidities for the removal of seed dormancy corresponded to 40-60% RH in *B. japonica*, 20-60% RH in *B. cernua* and 0-40% RH in *B. napus*. In these humidities dormancy could be completely removed in 4, 2 and one weeks, respectively, at their own optimum temperatures. In other humidities than the optimum ones, the removal of dormancy was more or less prevented. The preventing effects of desiccation or moistening were semipermanent.

(2) It is sometimes said that freshly harvested seeds can germinate only at a limited temperature. However, as the removal of dormancy proceeds, they soon widen the temperature range within which germination can occur. In the present study, seeds began to germinate at 15°C, which was followed by 25°, 35° and 5°C, and finally at 45°C in every species. When dormancy was removed, seeds could germinate at all the temperatures, 5°-45°C.

(3) The effect of storage humidity on seed viability was not ascertained because of a short term of the experiment. All seeds were viable at least until the end of the experiment. As an only exception, the seeds of *B. cernua* in 80% RH lost their viability in 14 months after harvest. In *Brassica* species, it is surmised from the literature that high storage humidity as well as extremely low humidity causes the deterioration of seeds and the optimum humidity for seed viability might lie around 20-30% RH.

(4) With respect to temperatures for germination of after-ripened (non-dormant) seeds, cardinal temperatures — minimum, optimum and maximum — are considered. The optimum temperature was 15-35°C in *B. japonica* and *B. napus*, and 15-25°C in *B. cernua*. The minimum temperature was below 5°C in every species. The maximum was around 45°C in *B. japonica*, but above 45°C in *B. cernua* and *B. napus*. There was a tendency that the range of temperatures for germination narrowed as the activity of seeds weakened with the lapse of time.

(5) As to the combinations of storage humidities and germination temperatures, germination percentages decreased in the following order: (i) optimum humidity and optimum temperature, (ii) optimum humidity and non-optimum temperature, and *vice versa*, and (iii) non-optimum humidity and non-optimum temperature. This demonstrates that storage humidity and germination temperature work on germination percentages of seeds independently of each other.

PRE-GERMINATION AND ITS GENETIC BEHAVIOUR IN RAPESEED

F. Bini, A.M. Olivieri and P. Parrini

During the spring 1977 from diallel crosses there was some indication that germination at a very early stage (when the seed is still inside the pod) occurs in some combinations. Pre-germination was observed in descents involving winter varieties such as Lesira, Girita, Quinta, Gira, Eragi, Sano, Status, Ramses O and a summer variety, Torch. On the contrary, progenies of Tower, Midas, Esora, Kosa, Dolora, Oro, Zepher, Vanda and Cressor (summer varieties) and of Primor, Eurora, Kara I, Brink, Sinera (winter varieties) did not show any pre-germinated seed.

In order to assess the genetic behaviour, a set of diallel crosses was made. In March 1980 two plants of each variety, as indicated in Table 1, were transplanted from the field to an insect proof glasshouse and pollinations were made by hand from April 16th throughto 21st. Since crossing and until the end of June, temperature and humidity reached values as high as 27°C and 80% r.h., respectively.

At maturity single pods were taken and pre-germination was observed.

Table 1 shows the percentages of seed germinated within the pod for each variety in itself and when used in combination as female and male. All the varieties gave pre-germinated progenies, but a large variability amongst them was observed. No relationship with growth habit (winter and summer) was found. Hayman's analysis (Hayman, 1954) applied on transformed data, gives (a) and (c) components significant ($P < 0.01$), suggesting that additive and maternal effects control pre-germination, and in relation with the previous data, genotype x environmental interactions have a large role to play in the variation of this trait.

Hayman B.I. (1954). The analysis of variance of diallel tables Biometrics, 10, 235-244.

Table 1 - Percentages of seed germinated within the pod (data reconverted after arcsin transformation).

Variety	*	Parental	Female			Male			Mean
				Range			Range		
		Mean	Mean	From	To	Mean	From	To	
OLIMPIADE P.	W	0.0	0.0	0.0	0.0	7.0	0.0	100.0	2.0
LEONESSA	W	11.7	13.0	0.0	100.0	11.0	0.0	100.0	12.2
TORRAZZO	W	2.8	13.0	0.0	100.0	7.0	0.0	70.0	9.6
MATADOR	W	39.8	9.0	0.0	100.0	15.0	0.0	100.0	11.9
GIRITA	W	8.6	8.0	0.0	80.0	17.0	0.0	100.0	12.3
LESIRA	W	100.0	89.0	25.0	100.0	22.0	0.0	100.0	57.8
JET NEUF	W	0.0	1.6	0.0	20.0	9.0	0.0	100.0	4.5
ELVIRA	W	29.5	5.2	0.0	70.0	10.0	0.0	100.0	7.4
CRESOR	S	12.4	10.0	0.0	70.0	6.0	0.0	50.0	8.0
QUINTA	W	0.0	0.1	0.0	15.0	16.0	0.0	100.0	4.8
PRIMOR	W	0.0	12.6	0.0	100.0	5.0	0.0	100.0	8.4
Mean		18.6	14.7			11.4			12.6

* W = Winter; S = Summer.

The cytoplasm of Japanese local varieties of turnip rape, *B. campestris*

Yasunobu OHKAWA

The cytoplasmic male sterility in *B. napus* which was discovered by Shiga and Baba (1971), is controlled by a male sterile cytoplasm (S cytoplasm) and fertility restorer gene(Rf)s in the nucleus. Most of all the Japanese cultivars in *B. napus* were previously reported to have S cytoplasm and the remaining ones N cytoplasm (non male sterile cytoplasm).

Before the 1870s, in Japan, the main oil crop had been turnip rape, *B. campestris*. After the 1870s, oil-seed rape was introduced via Korea and cultivated in Japan. Many Japanese varieties of rapeseed plant had been bred by an interspecific cross, *B. napus* × *B. campestris* or *B. campestris* × *B. napus* (NC cross). Some of the cultivars, which were reported to have N cytoplasm, were bred from special NC crosses, that is Miezairai(*B. campestris*) × Wasechosen(*B. napus*), Shiromizuzairai(*B. campestris*) × Wasechosen (*B. napus*).

In this paper, I report some results on the cytoplasmic type(S or N) of four Japanese varieties of turnip rape. As shown in Table 1, the F_1 plants (the male sterile line in *B. napus* × varieties in *B. camp.*) were male sterile or partially male sterile, while the F_1 plants (varieties in *B. camp.* × the maintainer in *B. napus*) were as fertile as the F_1 hybrids (a maintainer in *B. napus* × varieties in *B. campestris*). From these results, the four cultivars tested here were assumed to have N cytoplasm. The cultivar of *B. napus*, Wasechosen, used as a parent of the NC cross, however, has not been tested for its cytoplasmic type, yet. The experiment is now in progress.

Table 1. Degree of male sterility in hybrids between local turnip rape cultivars, *B. campestris*, and *B. napus* cultivars, Isuzu-natane and (*chi*) Isuzu which is a cytoplasmic male sterile line.

Cultivars or Hybrids	Relative position of Anther/Stigma						
	ms 1	2	3	4	5	6 f	Mean
(Chi) Isuzu	2	**43**	6	1	1		2.17
Isuzu-natane					1	**56**	5.98
Isobezairai					1	**10**	5.91
(Chi) Isuzu x Isobezairai	1	**14**		1			2.06
Isobezairai x Isuzu					3	**7**	5.70
Isuzu x Isobezairai					**8**	6	5.43
Inakashu				1	4	**5**	5.40
(Chi) Isuzu x Inakashu		5	**8**	1			2.71
Inakashu x Isuzu						1	6.00
Isuzu x Inakashu					2		5.00
Miezairai					**7**	5	5.42
(Chi) Isuzu x Miezairai		3	5	2	1	1	3.33
Miezairai x Isuzu				1	2		4.67
Isuzu x Miezairai					**7**	6	5.46
Shiromizuzairai				2	8	**7**	5.29
(Chi) Isuzu x Shiromizuzairai		5	**7**				2.58
Shiromizuzairai x Isuzu					8	**11**	5.58
Isuzu x Shiromizuzairai					**11**	8	5.42

Note: Figures in "gothic" characters refer to the mode.
ms means male sterile, f means fertile

CYTOPLASMIC MALE STERILITY IN RAPE - (BRASSICA NAPUS L.)

P. ROUSSELLE

At the Plant Breeding Station at Le Rheu, we study four systems of cytoplasmic male sterility in rape.

- Using interspecific crosses, male-sterility of radish discovered by OGURA (1968), has been transferred in kale by BANNEROT et al (1974) and then in rape. Two major problems are not yet solved using either kale or rape : strong chlorophyllous deficiency and absence of restorer genes. As HEYN (1978) has already described, we use Raphano-Brassica as genitor to solve these two problems. In F1 of crosses between male-sterile plants and Raphano-Brassica we found all plants without chlorophyllous deficiency and enough fertility to have progeny. Our project is to breed this material to obtain male-sterile and male fertile plants without deficiency, male fertile ones having restorer genes.

- As THOMPSON (1972), we use "Bronowski" cultivar to induce male sterility. We have already studied the genetic system (ROUSSELLE et al 1978). We have found that this sterility is unstable with high temperature and at the end of flowering stage. But in this case, we find easily restorer and maintenor plants. Our project is to screen with winter rape (and not spring rape) male sterile plants which can be used to produce F1 hybrids.

- SHIGA gave us his system. For the time being, this material is not adapted to our climatic conditions and we cannot study the quality of this sterility.

- DICKSON (1975) has transferred nucleus of some Brocoli on B. nigra cytoplasm. We began to transfer B. napus on this sterile cytoplasm. It seems, a priori, that most of the lines of rape are restorer and few one are maintenor.

First, I have made interspecific crosses between B. nigra, B. carinata, B. campestris, B. juncea with rape in order to induce cytoplasmic male sterility. In F2 of these crosses, we found either male-sterile plants or male-fertile ones. Our project is to screen for male-sterility, maintenor and restorer in this material after several backcrosses with rape. This year, we begin crosses involving other Brassicae, even other genera (Diplotaxis, Eruca, Sinapis, Moricandia) to induce other system of cytoplasmic male sterility.

In every case of male-sterility, we study histology, cytology of sterile plants and cytogenetics when it is necessary. I am interested in exchanging papers, seeds... concerning cytoplasmic male sterility and interspecific crosses in rape or other Brassicae.

HETEROSIS AND GENETIC PARAMETERS IN WINTER RAPE (BRASSICA NAPUS)

M. BUSON

An incomplet diallel experiment has been realized involving 25 winter rape lines in order - firstly to give an indication of the relative differences between inbred lines and their F1 progenies, and - secondly to assess the relative importance of general and specific combining ability (respectively G.C.A. and S.C.A.) in the population of selected lines.

130 F1 hybrids were obtained by crossing these lines ; parents were chosen according to their consanguinity. Hybrids and parental lines were grown in field plots (four row plots, 3 m long with rows spaced 30 cm a part, with three replications each.

Meanly, hybrids showed 23% superiority over inbreds for plot yield, but some of them reached 50% more than the mean parent. Hybrid vigor is expressed also on characters connected with vegetative development (leaf area, plant height) and yield components (numbers of siliqua and seeds per siliqua).

These results corroborate the studies of SHELKOUDENKO (1968) SCHUSTER & MICHAEL (1976) and CAMPBELL & KONDRA (1978).

The estimates of G.C.A. variances were much higher than those of S.C.A. variances for all the characters except the height of plant. Reciprocal effects were significant for yield, leaf area and date of flowering, but their variances were not important compared to those of G.C.A. effects.

In this trial, it is essentially G.C.A. effects wich have created variability ; it means that the part of additive effects is important within the considered population of inbred lines ; it is not very surprising, because we used lines selected for their own value.

These results have to be corroborated before used in selection; so, we have taken an other sample of the considered population (selected inbred lines) and we have made 60 crosses between selfed lines ; we shall get results in July 1981.

If the variances of G.C.A. effects are again more important than those of S.C.A. effects, the selection of F1 hybrids will have to take into account the G.C.A. values of inbreds.

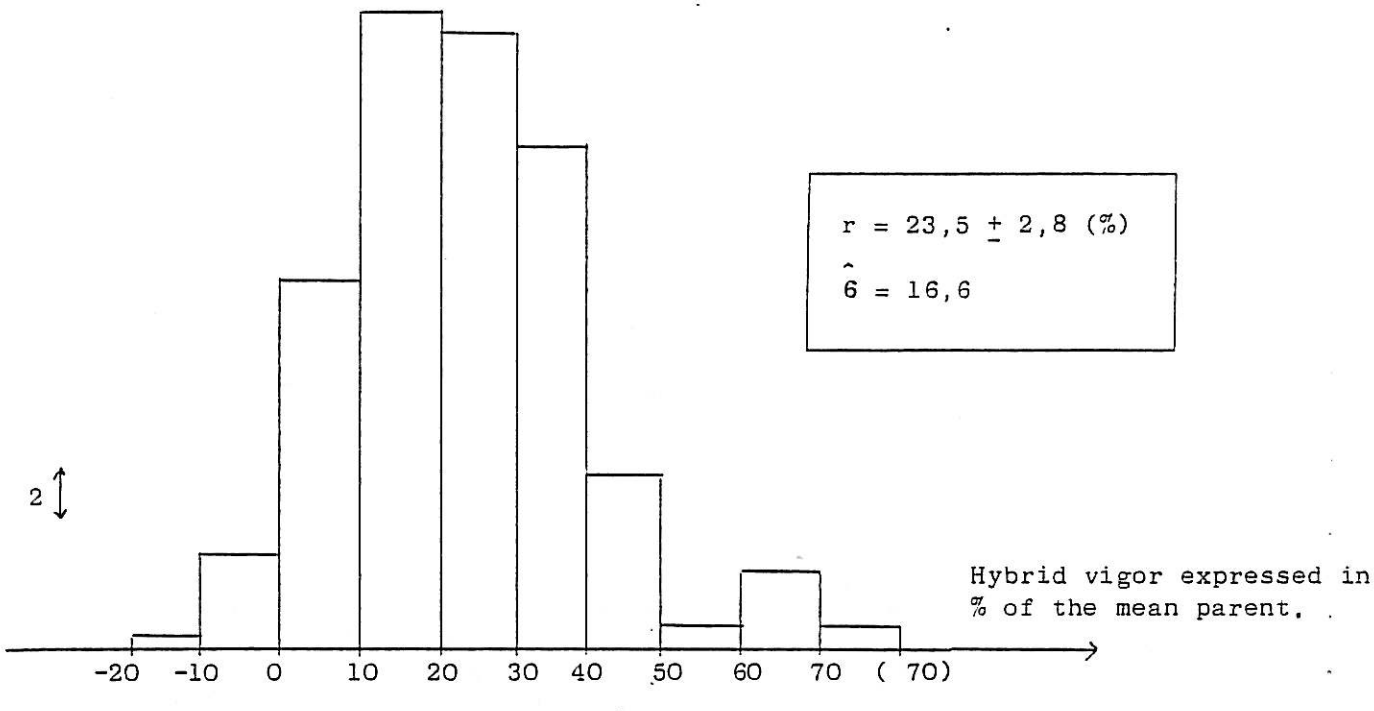

FIGURE 1 : Histogram of values of hybrid vigor for the 130 F1 hybrids

Effect	Estimates of variances : $\hat{\delta}$		
	lenght of plant	Date of flowering	Seed yield
- General combining ability	3,9	3,48	5,39
- General reciprocal effect	/	/	0,49
- Specific combining ability	3,7	0,23	/
- Specific reciprocal effect	5,6	0,15	/
- Error	26,0	1,41	25,53

TABLE 1 : Estimates of variances for three of the characters studied

The Production of Hybrid Oil-Seed Rape using Self-Incompatibility

Stuart Gowers

The possible use of self-incompatibility for producing hybrid oil-seed rape is complicated in swede-rape by the presence of two loci controlling incompatibility. Assuming a double-cross is necessary to allow multiplication on a commercial scale, the use of four S-gene lines could lead to high levels of cross-incompatibility unless the S-genes in the single-crosses were in the right combinations.

Using four S-alleles at a single locus would give 25 per cent cross-compatibility, which, under the pollen load of a field crop, should give a full seed set. This result is also obtained when the single-crosses are at the same locus, but with the double-cross being between different loci. Problems could arise, however, if the single-crosses involved both loci, with the possibility of some plants only being cross-compatible with one sixteenth of the population. These problems could be overcome by the use of a modified double-cross using self-incompatible and self-compatible lines. Using isogenic lines for the single-crosses to give an 'F_1' double-cross would give, with a single locus:

$$A_1A_1 \times A_0A_0 \qquad B_2B_2 \times B_0B_0$$

$$A_1A_0 \qquad \times \qquad B_2B_0$$

$$1\ A_1B_2 : 1\ A_1B_0 : 1\ A_0B_2 : 1\ A_0B_0$$

Even if the two S-genes used are at different loci, the same degree of cross-compatibility is obtained, namely:

$$1\ A_1A_0B_2B_0 : 1\ A_1A_0B_0B_0 : 1\ A_0A_0B_2B_0 : 1\ A_0A_0B_0B_0$$

The overall effect in both cases, therefore, gives a quarter of the plants cross-compatible with a quarter of the population, half the plants cross-compatible with half of the population, and the remaining quarter should be fully self- and cross-compatible.

Although work at S.P.B.S. has been concerned with the possibility of producing hybrid swedes, some of the self-incompatibility genes being used come from Panter and Matador oil-seed rape. Two of the lines have been used in a small experiment to test the possible use of self-incompatibility in oil-seed rape. Single-crosses were made in isolation cages between the self-incompatible Panter line (P_I) and the parent variety (P_C), between the self-incompatible Matador line (M_I) and the parent variety (M_C) and between P_I and M_I. Twenty-four plants of each progeny, plus twelve plants of each parent cultivar, were randomised in an isolation plot to establish a population with the expected proportions of a modified double-cross, namely 1 P_IM_I: 1 M_IM_C: 1 $M_C + P_C$. Five capsules from ten plants of each class were sampled to estimate the number of ovules per capsule and the proportion of seed set, and the plants were later harvested to obtain total seed yields per plant.

	$P_I M_I$	$P_I P_C$	$M_I M_C$	$P_C + M_C$	S.E.D.
% seed set	81.5	77.8	76.3	83.7	6.1
seed yield g/plant	21.2	27.7	22.8	21.4	2.4

There were no significant differences in the percentage of seed set between the four groups. The seed set of $P_I M_I$ was, in actual fact, higher than that of Matador (79.1%), and it appears that there should not be any reduction in the seed set with a normal double-cross. The degree of outcrossing in the field could not be assessed because there was no suitable marker gene available, but the S-genes used usually score well on pollen-tube screening.

There was no sign of heterosis in the Panter x Matador hybrid, whose yield was only equal to that of the mid-parent value. The only possible sign of heterosis would appear to be in the intra-varietal Panter hybrid, but a comparison of $P_I P_C$ with P_C alone (mean yield 23.7 g/plant) does not show significance (S.E.D. = 2.7). A result which did show intra-varietal heterosis but no inter-varietal heterosis would not have been too surprising, as the latter depends upon the combining ability of the parents (which was unknown in this case) whereas, with the breeding system of rape leading to quite a high inbreeding coefficient, an intra-varietal cross could be expected to give increases in yield.

No definite conclusions can be reached from this experiment in the absence of any significant heterosis, but it would appear if there were any slight problem with seed set when using self-incompatibility, then this could be more than overcome by yield compensation and heterosis. If sufficiently strong S-genes could be found to work in the backgrounds needed to produce a specific hybrid, then this method would appear promising. However, it is possible that a normal double-cross would be needed, and in this case there should not be any problem due to low cross-compatibility if the appropriate combination of crosses is made.

STUDIES ON THE F_1 HYBRID SEED PRODUCTION IN INDIAN CAULIFLOWER (BRASSICA OLERACEA L. VAR. BOTRYTIS L)

S.S. Chatterjee & Vishnu Swarup

In the past improvement of Indian cauliflowers has been achieved to a certain degree by inbreeding and selection, by inter-varietal hybridisation and also by developing synthetics. However, the greater heterotic effects expressed in the form of higher yield, early and uniform maturity could not be exploited due to the lack of a cheap and convenient method for commercial production of F_1 hybrid seeds. In advanced countries self-incompatible lines are being utilized more freely in recent years for the commercial F_1 hybrid seed production in spite of certain limitations. Following the earlier observation on Indian cauliflowers that early and mid-season cauliflowers are predominantly self-incompatible, intensive investigations were initiated to develop homozygous S-allele lines in agronomically suitable materials that could be utilized for F_1 hybrid production and to relate the identity of S-alleles present in the Indian materials to the S-alleles that have already been isolated in the kale, Brussels sprouts, other coles and allied species.

Studies over the past few years have indicated that the early maturing types of Indian cauliflowers are highly self-incompatible while the mid- and late-maturing varieties are somewhat self-compatible. A few homozygous S-alleles have been isolated, of which one has been found to have the S-allele that corresponds to the S-allele detected by Julia Hoser-Krauze of Poland in the c.v. Pusa Katki introduced from India. Incidentally this variety also belongs to the early type.

In general, the results could be explained on the basis of sporophytic self-incompatibility system governed by multiple alleles at the S-locus, as have been reported by other research workers. However these studies were entirely based on the seed set data, obtained under field conditions, resulting at times some indifferent seed set, possibly due to environmental and physiological factors that are known to influence the strength of incompatibility.

Dominance relationships between all possible combinations of the S-alleles, already isolated, are being worked out. Besides, multiplication of these lines are being standardised with the help of tissue culture technique. Some heterotic combinations involving self-incompatible lines are currently being tested.

BORON INCREASES POLLEN TUBE NUMBERS IN SELF-POLLINATED *BRASSICA OLERACEA* STIGMAS

T. Hodgkin

Boron is known to improve pollen germination and tube growth *in vitro* in many plant species (Visser, 1955) and it has recently been suggested that it plays a role in the production of anti-fungal substances (phytoalexins) and in the self-incompatibility reaction (Lewis, 1980). The effect of supplying additional boron to self-pollinated *Brassica oleracea* flowers was tested by Gates and Ockendon (1976) who reported that solutions containing 610 ppm (10 mM) boric acid had no effect on the numbers of self pollen tubes present. However, some recent experiments carried out at SCRI suggest that this is not always the case.

Materials and Methods

For each of the three experiments, 75 mature buds were removed from plants of two families. The families were heterozygous S_2S_{46} and S_5S_{13} and had shown some partial self-compatibility. The buds were placed in the wells of microtitre plates which contained boric acid solutions (1, 10, 20 or 40 mM) or distilled water. These were kept under high RH in plastic boxes at room temperature. The buds opened the next day and were self-pollinated, cross-pollinated or left unpollinated. Pollen tube numbers and callose deposition were subsequently examined using standard procedures (Hodgkin, 1977). Five flowers were tested for each boric acid pollination combination in each experiment.

Results and Discussion

In both families the number of pollen tubes penetrating self-pollinated stigmas supplied with 1 mM boric acid was twice that of stigmas supplied with distilled water (Table 1). High concentrations of boric acid, up to 20 mM, increased self tube numbers in only one of the families. Pollen tube numbers decreased at boric acid concentrations of 40 mM.

Table 1. Pollen tube numbers found in self- and cross-pollinated *B. oleracea* stigmas at different boric acid concentrations.

Pollination	Family	mM boric acid				
		0	1	10	20	40
self	1	33	71	75	54	41
	2	10	19	24	51	28
cross	1	146	143	169	145	71
	2	111	137	159	153	113

Pollen tube numbers in cross-pollinated stigmas showed a more variable response. Higher numbers of pollen tubes were found in one family, in flowers supplied with 1, 10 or 20 mM boric acid, but not in the other. In both families fewer pollen tubes penetrated stigmas supplied with 40 mM boric acid.

The clarity of the pollen tubes in the stained preparations was improved by the addition of boric acid, as a result of an intensified fluorescence of the tube walls. At 40 mM boric acid the tubes contained noticeably more fluorescent material (presumably callose) and some growth abnormalities were observed. There was also an increase in the fluorescence of the stigmatic pappillae in both self- and cross-pollinated stigmas at high boric acid concentrations. This also occurred in some of the unpollinated controls.

It is clear that boric acid can increase the number of pollen tubes found in self-pollinated styles although whether this is a specific feature of the self-incompatibility reaction is not certain. It is probable that the optimum concentration of boric acid will depend on the level of boron present in the plants used and the chemical may not have an effect on plants with high endogenous levels. At high concentrations boric acid appeared to inhibit both self and cross pollen tube penetration and growth.

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TESTING FOR SIBS BY ACID PHOSPHATASE ISOENZYME ANALYSIS: NEW GENETIC EVIDENCE

A.B. Wills and Eveline M. Wiseman

Previously we have demonstrated three types of interaction between alleles of acp-3, the locus controlling those acid phosphatase isoenzymes that are found as bands in zone 1 on polyacrylamide gels following electrophoresis of seedling extracts. Each of the four homozygous genotypes that have been found, acp-$3^a/3^a$ to $3^d/3^d$, has a single band with characteristic mobility. The heterozygotes $3^a/3^c$, $3^a/3^d$, $3^b/3^c$ and $3^b/3^d$ have three bands, one corresponding to each allele and one intermediate (Wills and Wiseman, 1980). By contrast, heterozygous $3^a/3^b$ plants show only band b; while $3^a/3^{b'}$ seedlings do not show any clear bands, instead they have an area of general staining spreading between the positions that the a and b bands would be expected to take (Wiseman and Wills, 1979).

More recently we have found that most $3^c/3^d$ seedlings also give an area of staining, rather than separated bands, but with more intense staining in the region of band c than elsewhere. However a family in which the heterozygous plants expressed two clear bands was also found. It is not yet certain whether this exceptional result has a genetical or a technical cause. A more detailed account of these results and some anomalies is in preparation.

In view of these different interactions, it seems possible to us that acp-3 heterozygosity in an inbred line might not be recognised in some circumstances. This could lead either to difficulty in interpreting results of sib assessment tests or, exceptionally, to erroneously high assessments of sib proportions in some hybrids. In the simplest breeding situation the remedy would be to evaluate progenies produced by hand crossing between inbreds at an early stage of inbreeding when segregation of a and b bands, or c and d, would be readily observed.

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BREEDING WORK ON COLE CROPS AT THE IVT, WAGENINGEN, THE NETHERLANDS

Q.P. van der Meer

Work is going on in respect of introducing male sterility into autumn cauliflower. The most promising results were archieved by using the Bannerot material. All varieties investigated proved to be complete maintainers for this type of male sterility. Eliminating the chlorosis phenomenon by maintainer line selection delivered promising results.

The Raphanus type of male sterility incorporated into Brassica campestris (received from F. Heyn, Germany, via H. Toxopeus, SVP, The Netherlands) has been introduced into Chinese cabbage giving results simular to the cauliflower results. In Chinese cabbage also a genic type of male sterility has been found.

Brassica campestris chinensis and stubble turnips are very tipburn resistant.

In a collection of 63 Chinese cabbage entries all of them bolted when sown as early as in the middle of March or the middle of April. Most entries did bolt after sowing in early May, but some of them did not, stubble turnips and a variety from Taiwan (JS 84) being most bolting resistant. Bolting in early July resulted in the longest flower stalks (120-150 cm). After sowing in early August not any bolting did occur at all. Most plants of this sowing date showed internal flower stalk formation in the end of November. Stubble turnips did not.

Phytotron experiments in respect of bolting in Chinese cabbage resulted in the following preliminary results:

- Prenatal vernalization could not be realized.
- Lower light intensities resulted in slower vernalization at supra-optimal temperatures.
- Clear differences were found between different Chinese cabbage populations in respect of cold requirement and in level of vernalizing temperatures.

NEW CULTIVARS OF OIL CROPS FROM SVALÖF, SWEDEN.

Gösta Olsson, Roland Jönsson, Lena Bengtsson and
Nils Johansson

As results of the breeding work with oil crops carried out at Svalöf 5 new cultivars have been included in the official Swedish list of cultivars during the last year. Here will be given very short descriptions of these cultivars.

The winter rape cultivar HERKULES (Sv 749379) has been selected from the cross combination (Panter x Sv 581119) x Sinus. Herkules is of the low erucic acid type. The new cultivar, which has been tested in official Swedish trials since 1976, has as a mean of 71 trials surpassed Brink with 7 % both in seed and oil yield. Herkules is more vigorous and has a better winter hardiness than Brink. The protein content in the meal is about 1.5 per cent units higher than in Brink. Herkules was marketed in the autumn 1980.

KASPER (Sv U 761300) is the first cultivar of winter turnip rape with a low erucic acid content marketed in Sweden. The genes for low erucic acid has been transferred from summer turnip rape. Therefore Kasper is inferior to the old high erucic cultivar Rapido III in winter hardiness and as a consequence in yield too. Kasper was marketed for the southern part of Sweden in the autumn 1980.

The summer rape cultivar NIKLAS (Sv U 74507) is selected from crosses between Sv 691256 and Sv 69615. Niklas has a low erucic acid content but a normal glucosinolate content. In official trials during 4 years Niklas has surpassed the now cultivated low erucic acid cultivars Gulliver and Olga with 6 % in oil yield. Niklas has a higher protein content in the meal and a better stalk stiffness than Olga. The chlorofyll content is lower than in Gulliver and Olga. Niklas will be marketed for sowing in the spring 1981.

The summer rape cultivar LERGO (Sv 751516) is a pedigree selection from the cross combination Hermes x (Bronowski x Gulle). Lergo is a double low variety. It is early ripening and is of particular interest in districts, where both the low glucosinolate content and the earliness are valued. Lergo is included in the Swedish list of cultivars as an export cultivar. In 1980 Lergo was cultivated in Finland.

TORKEL (Sv 7510223) is a low erucic acid cultivar of summer turnip rape originating from the cross combination (From Canadian material x Torpe) x Sv 67389. Compared with Span, which has been grown in Sweden during the last years, Torkel is much more vigorous and has a better competition against weeds. As a mean of 91 trials Torkel gave 1.6 per cent units higher oil content and 11 % higher yield of oil than Span. Torkel is about 4 days later in ripening and has a better stalk stiffness than Span. Torkel was marketed in the spring 1980.

THE ASSOCIATION OF YELLOW SEED COAT WITH OTHER CHARACTERS IN MUSTARD *B. juncea*.

D. L. Woods

There is considerable emphasis in rapeseed (*B. napus* & *B. campestris*) breeding programs in Canada on producing cultivars with yellow seed coats. The *B. campestris* cultivar Candle which has a rather yellow-brown seed coat has been licensed. The value of this lighter coloured seed coat is the associated reduced fibre content, and higher oil and protein percentages.

Some observations on the association of seed size, oil %, and protein % with seed colour in the closely related mustard *B. juncea* may give some indication of the effect *per se* of seed coat colour on these characters, something which cannot be readily measured yet in the rapeseeds due to the relatively poor agronomic performance of the pure yellow seeded strains so far developed.

The data reported here were obtained from two sources. For the parental material samples from the Canadian co-operative mustard test were used - 66 station years for oil data, and 4 station years for seed size and protein data, with two replicates for each station year. The progeny material was field grown back-cross-one plants from a cross of brown and yellow seeded cultivars with the yellow seeded cultivar as the recurrent parent. This material had been grown primarily as part of a study on seed colour inheritance (1), and the residue from each group of crosses was harvested as a bulk. The bulked seed lots were screened through a series of round hole seives with holes 2.2, 2.0, 1.8, & 1.6 mm in diameter. The proportion by number of yellow and brown seeds in each fraction was determined. For each colour within each size class oil content, protein content, and seed weight was obtained. (Oil by wide line NMR, Newport Analyser Mk IIIA, Protein by Kjeldhal digestion and colorimetry, protein = N x 6.25)

CHARACTERISTICS OF THE PARENTS

Cultivar	Seed coat colour	Seed wt. (mg)	Oil %	Protein %
Domo	yellow	3.31	38.4	21.5
Blaze	brown	2.88	34.8	22.1
Comm brown	brown	2.92	35.1	21.8
Ekla	brown	2.86	33.1	22.1

Larger seed size was clearly associated with yellow seed coat, and within any size class yellow seed coat was associated with higher oil content than was brown seed coat. The largest size class of yellow seeds were heavier than the largest class of brown seeds, possibly suggesting a higher proportion of irregular shapes in the largest brown class. Similarly with the smallest size class, the brown seeds were lighter, presumably because the size distribution for browns extended into smaller sizes than did the distribution of yellow seeds. As for protein content, no particularly distinct trend was noticed.

CHARACTERISTICS OF THE PROGENIES

Cross, quantity, sieve	% of total	Seed coat colour %Y	%B	Seed wt. (mg) Y	B	Oil % Y	B	Protein % Y	B
(DxB)xD 41.8 kg									
> 2.2	0.02	67.0	33.0	5.07	4.51	39.3	36.5	27.2	25.8
2.2-2.0	0.30	82.0	18.0	4.62	4.65	39.8	37.0	27.9	28.1
2.0-1.8	31.28	45.5	54.5	3.62	3.48	42.2	39.2	25.3	27.2
1.8-1.6	35.13	39.5	60.5	3.02	3.04	42.3	39.4	24.1	25.6
< 1.6	33.27	19.2	80.8	2.46	2.42	42.8	40.1	25.1	26.3
(CxD)xD 74.9 kg									
> 2.2	0.02	75.5	24.5	4.55	4.05	34.8	33.8	29.8	26.5
2.2-2.0	0.20	85.8	14.2	4.55	4.54	37.9	34.6	29.1	29.8
2.0-1.8	22.85	47.0	53.0	3.62	3.50	40.5	36.6	28.6	29.1
1.8-1.6	36.36	32.0	68.0	3.13	3.10	40.1	37.0	27.0	27.9
< 1.6	40.57	11.8	88.2	2.53	2.46	40.0	37.8	27.7	27.7
(ExD)xD 31.8 kg									
> 2.2	0.02	88.5	11.5	5.19	4.92	35.5	33.0	28.1	26.7
2.2-2.0	0.32	71.0	29.0	4.57	4.51	39.7	36.8	27.0	26.5
2.0-1.8	18.59	50.0	50.0	3.67	3.65	41.8	38.7	24.1	27.2
1.8-1.6	40.73	32.0	68.0	3.07	3.14	43.1	40.0	24.8	26.7
< 1.6	40.34	17.0	83.0	2.50	2.37	43.1	40.6	24.4	25.3

D=Domo - yellow seeded
B=Blaze, C=Commercial brown, E=Ekla - all brown seeded

Conclusions

The yellow seeded parent was both higher in oil content and larger in seed size than any of the brown seeded parents, so the fact that the yellow seeded progenies were also larger and contained higher oil levels could be either a pliotropic effect of the yellow seed character, or a linkage effect The optimistic belief is that the effect is pliotropic, in which case an advantage of about 2.8% in oil content and an increase in seed size of 10-15% may be hoped for associated with change to yellow seed colour.

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INTERGENERIC HYBRIDIZATION BETWEEN *BRASSICA OLERACEA*(♀) AND *RAPHANUS SATIVUS*(♂) BY EMBRYO CULTURE

M.SARASHIMA, Y.MATSUZAWA and T.KIMURA

Raphanus sativus(2n=18,RR) has been successfully crossed by *Brassica oleracea*(2n=18,CC) when former species was used as female parent and derivative amphidiploid and its offspring are well known as artificially synthesized new crop "Raphano-*brassica*"(2n=36,RRCC). Reciprocal hybridization,*B.oleracea* x *R.sativus*,however, has been burdened with severe cross-incompatibility and only three have succeeded in the hybridization (Fukushima 1929, U *et al.* 1937, Makarova 1963).

Nowadays,embryo culture is one of the most practical technique to overcome cross-incompatibility in plants. Therefore,by using embryo culture technique we tried to obtain so many F_1 hybrids as to give sequrity for the intergeneric hybridization breeding.

We had accomplished diploid and tetraploid level pollinations from *R.sativus* onto *B.oleracea* and 35 - 40 days after pollination removed juvenile hybrid embryos from siliques onto culture media (Whithe 1963, added 30g/l sucrose,500mg/l CH and 8g/l agar powder).

We obtained 12 true hybrids from 92 cultured embryos derived from 9,121 flower pollinated in diploid level mating and six from 83 embryos out of 4,514 flowers in terapioid level. In the former 12 hybrids three were amphihaploids(2n=18,CR) and nine were sesquidiploids(2n=27,CCR and CCR), and in the latter six ones five amphidipoids(2n=36,CCRR) and and hypo-amphidiploid (2n=34).

Their morphological characteristics were generally intermediate between the parents with the exception of sesquidiploids and pure- and hypo-amphidiploids closely resembled previous *Raphano-brassica*. Out of nine sesquidiploids seven and two plants more strongly resembled *oleracea* and *Raphanus*,respectively than amphihaploids and amphidiploids. Therefore,we considered that genome constitution of the former seven sesquidiploids were CCR and that of the latter being CRR.

All of three amphihaploids had no viable pollen but yielded 48 seeds from 9,325 flowers in total pollinated by diploid *R.sativus*. Sesquidiploids had also no viable pollen and did not yield any seed,even though we had pollinated more than thousand flowers by diploid and tetraploid *R.sativus*,respectively. Although hypo-amphidiploid produced neither viable pollen nor seed,three true amphidiploids were 60 - 85% in pollen fertility and yielded 63 seeds from 812 selfed flowers in total and three seeds from 424 back-crossed flowers by terapioid *R.sativus*.

The *in vitro* embryo culture technique proved best for obtaining intergeneric hybrid,*B.oleracea*(♀) x *R.sativus*(♂). Our intergeneric F_1 hybrids and their progenies might be very useful to study nucreus-cytoplasm interaction,to make nucleus substitution and to transfer useful characters in both directions between *B. oleracea* and *R.sativus*.

EFFECT OF FLOWER BUD AGE AT BUD POLLINATION ON CROSS FERTILITY IN RECIPROCAL INTERGENERIC CROSSES, RAPHANUS SATIVUS X BRASSICA OLERACEA AND B.CAMPESTRIS X R.SATIVUS

H. NAMAI

Crosses between R.sativus(RR) and B.oleracea(CC) and between B. campestris(AA) and R.sativus(RR) are successful in some degree, while their reciprocal crossings are not so easy. Therefore, basic and practical studies to overcome cross-incompatibility are very important for obtaining many intergeneric F1 hybrids so as to enable to give promising intergeneric hybridization breeding in Cruciferous crops.

Intergeneric cross-pollinations are more easily effected at bud stage than at open flower stage and we usually give the bud pollinations. Recently, in vitro embryo culture has applied very well in intergeneric and interspecific crosses of Criciferous crops. In these cases the embryo culture technique is very useful, but juvenile embryo extraction rate from siliquae is fairly low in general.

This paper presents the study on effect of flower bud age at bud pollination on cross fertility in reciprocal intergeneric crosses between R.sativus and B.oleracea and between B.campestris and R.sativus to improve the juvenile embryo extaction rate.

Parents included seven cultivars of R.sativus(vegetable and fodder radish), six of B.oleracea(kohlrabi, kale and cabbage) and six of B.campestris(turnip and chinese cabbage). All open flowers and young buds were removed from each selected inflorescence of maternal plants and large and medium sized buds opened and emasculated, then fresh pollen of father plant was applied to the stigma in pot culture under greenhouse. Pollinated inflorescences were protected by paraffin paper bags for at least ten days.

In 1967 - 1971 a little less than 8,000 buds were pollinated in all in the reciprocal crosses, R.sativus x B.oleracea and B.campestris x R. sativus and 61 and 13 hybrids were obtained from R.sativus pollinated by B.oleracea and from B.campestric pollinated by R.sativus, respectively. Whereas, no hybrid was obtained from the reciprocal crossing in both cross combinations(Table 1).

Silique setting percentage, number of seeds including abortive ones and number of hybrids obtained were calculated one by one of the pollinated buds in some cross combinations. In cross, R.sativus x B.oleracea hybrids were obtained in No.1 to No.8 buds, with a range from 1.0 to 6.3 on an average per 100 pollinated buds and No.7 and 8 buds yielded most number of hybrids. From 6.3 to 10.1 abortive seeds per 100 pollinated buds were also counted in No.1 to No.8 buds. On the other hand reciprocal crossing yielded 4.2 to 9.9 abortive seeds in No.3 to No.8. Hence it follows that in the reciprocal crosses young buds of No.9 and upward must be removed in order to avoid waste of the pollinations.

In cross, B.campestris x R.sativus hybrids were obtained in No.1 to 10 buds, with a range from 0.6 to 3.9 and No.3 and 4 buds yielded most number of hybrids. From 38.3 to 87.6 abortive seeds were counted in No.1 to No.12. Reciprocal crossing yielded no seed and only 2.8 abortive

seeds in No.7 and 8 buds.

The summary is as follow:

The intergeneric cross-incompatibility probably decrease in buds being two to three days before flowering in R.sativus as female and being one to three days in B.oleracea and B.campestris especially in two to three days before flowering.

Table 1. Comparative results of reciprocal intergeneric crosses between R.sativus and B.oleracea,and B.campestris and R.sativus at the diploid level in 1967 - 1971

Cross combination*	Number of pollinations	Number of siliquae obtained	Total number of seeds obtained**	Number of seeds germinated	Number of hybrids obtained
RR x CC(19)	3,594	268	426	106	61
CC x RR(9)	1,024	576	106	61	0
AA x RR(16)	2,111	1,106	889	35	13
RR x AA(9)	1,111	20	11	0	0

*Figure in parentheses means number of cross combination.
**They includes false hybrid seeds and abortive ones.

Table 2. Effect of flower bud age at bud-pollination on cross fertility in reciprocal intergeneric crosses,R.sativus x B.oleracea,B.campestris x R.sativus

Cross combination*		Flower bud No. at bud-pollination**					
		No.1, 2	3, 4	5, 6	7, 8	9,10	11,12
RR x CC (7)	Silique setting percentage(A)	7.9	10.3	10.7	10.5	1.4	0.0
	Number of hybrids obtained per 100 flowers(B)	1.0	1.8	3.3	6.3	0.0	0.0
	Number of abortive seeds per 100 flowers(C)	7.8	10.1	9.9	6.3	0.0	0.0
CC x RR (3)	(A)	44.1	55.1	65.3	35.7	40.3	20.6
	(B)	0.0	0.0	0.0	0.0	0.0	0.0
	(C)	0.0	4.2	9.9	8.4	0.0	0.0
AA x RR (9)	(A)	83.7	86.7	87.3	84.4	79.2	65.1
	(B)	1.1	3.9	0.6	1.1	1.3	0.0
	(C)	62.6	87.6	77.1	59.7	51.6	38.3
RR x AA (2)	(A)	7.5	10.6	15.9	15.9	3.2	0.0
	(B)	0.0	0.0	0.0	0.0	0.0	0.0
	(C)	0.0	0.0	0.0	2.8	0.0	0.0

*Figure in parenthesis means number of cross combinations.
**Flower buds in each inflorescence are given consecutive numbers from basal to upper bud.

SYNTHESIS OF NEW GENOTYPES WITHIN THE GENUS BRASSICA

M.Balicka, B.Barcikowska, W.Mlyniec, E.Zwierzykowska

From the 607 F_1 hybrids between B.campestris ssp. pekinensis cv. Granaat and B.oleracea var. acephala cv. Normal one allohaploid plant of 2n=19 chromosomes was identified. It was a plant of great vitality, of well developed, relatively large leaf surface and relatively high growth. The flowering time lasted about six months, but almost all flowers were completely sterile. After colchicine treatment, conducted at the early stage of flower development, restoration of the fertility of pollen grains was observed. By way of self pollination about 70 seeds were obtained. A cytological test revealed 2n=38 chromosomes, which indicated that we are dealing with a synthetic B.napus form. Owing to the low thioglucosinolates content in the initial forms of this hybrid it may be possible to reproduce this feature in the progeny, this may also occur because in haploid state it was cross-pollinated with some B.napus varieties, which were characterised not only by low eruca acid, but also by low thioglucosinolates content.

In the production of interspecific hybrids / already announced in the Cruciferae Newsletter No. 3 p. 10 / besides conventional methods, in vitro of excised ovaries and embryo culture techniques were recently applied. From 1464 excised ovaries 67 embryos were obtained, which gave 5 plantlets, now cytologically tested.

Hybrids, mainly semisynthetic Napocampestris leafy-fodder forms, obtained from the beginning of our project / see also: Cruciferae Newsletter No. 3 p. 10 / up to the present, are tested under field conditions. According to their destination in the crop rotation and utilization as forage, they are divided into three groups: late summer, late autumn and winter intercrops. Besides their quantitative features their physiological and qualitative properties are also evaluated.

ON THE TAXONOMIC IMPORTANCE OF POD SEPTUM ANATOMY

Walter TITZ

Pod septum anatomy is of differing taxonomic importance for the Cruciferae: thickness and patterns of epidermal cell walls are either constant or variable within the species that have been investigated thoroughly till now. This becomes evident from our recent studies (TITZ, 198o[1]) on Arabis and some other Cruciferae. The features of pod septa can be used for characterizing e.g. the following species: Arabis hirsuta (L.)Scop.s.str., A.allionii DC.s.str., A.sudetica Tausch, A.ciliata Clairv., A.collina Ten.s.l., A.pumila (Jacq.) (always with thin epidermal cell walls), and A.hornungiana Schur or Sisymbrium officinale (L.)Scop. (regularly thick epidermal cell walls occurring in the septa of each plant). On the other hand different types of epidermal cell walls occur in the pod septa within species as Arabis sagittata (Bertol.)DC., A.planisiliqua (Pers.)Reichenb.s.str., A.nemorensis (Hoffm.)Koch, A.juressi Rothm.s.l., A.soyeri Reuter & Huet and A.brassica (Leers)Rauschert (=A.pauciflora (Grimm)Garcke): some individuals display only thin epidermal cell walls, others at least partially ± thick ones.

The inheritance of the tendency towards thickened cell walls in the septum epidermis proved to be dominant in the progenies of artificial crossings of different types of Arabis sagittata, A.planisiliqua and A.nemorensis.

Thus the taxonomic value of pod septum characters is obviously limited to the level of species and inferior to other characters as radicle position or trichome types, though even these features are only rarely uniform within the tribes as discriminated by SCHULZ (1936[2]) or others (cfr. TITZ 198o[1], TITZ & SCHNATTINGER 198o[2]).

[1] TITZ W., 198o, Ber.Deutsch.Bot.Ges. (in press)

[2] SCHULZ O.E., 1936, in: ENGLER A.& HARMS H., Die natürlichen Pflanzenfamilien 17b: 227-658. Engelmann, Leipzig

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TAXONOMY, CYTOGENETICS AND ORIGIN OF CROP BRASSICAS, A REVIEW BY SHYAM PRAKASH AND KOKICHI HINATA

A review of taxonomy, cytogenetics and origin of crop Brassicas will be published this year (1980) in a separate issue of Opera Botanica.

The authers have summarized the content of the review in the following way:

From the points of view of taxonomical nomenclature, cytogenetical relationships and the origin of cultivated types, information was assembled concerning the diversification of Brassica crops: B. nigra (n = 8, BB genome), B. oleracea (n = 9, CC), B. campestris (n = 10, AA), B. carinata (n = 17, BBCC), B. juncea (n = 18, AABB) and B. napus (n = 19, AACC). Variable pairing of chromosomes has been observed in the hybrids of these species and wild allies, which suggests that close genetic relations exist between different genomes. These observations substantiate the idea that the monogenomic Brassica species are aneuploid in the ascending order evolving from a common archetype. Alterations in level of polyploidy and the artificial reconstruction of naturally occurring amphidiploids have been successfully utilized as a means of breeding. Oleracea, campestris and juncea are highly polymorphic. The wide variations, particularly regarding their foliar organs, have been brought about in Europe where oleracea is concerned and in Asia where campestris and juncea are concerned, quite independent of each other in the long course of domestication. In general, the taxonomic nomenclature is rather confusing and it is therefore necessary to compile a simplified classification of the crop Brassicas at the same time bearing in mind the morphological and cytogenetical information.

This publication presents an exhaustive review of the relations between the different Brassica species both from a taxonomical and a cytogenetical point of view and discusses in addition the possibilities to utilize interspecific and intergeneric hybridization in practical plant breeding. This publication will, thus, be most valuable for all interested in crop Brassicas.

This separate issue of Opera Botanica (Opera Botanica 1980, number 55, p. 1-57) can be ordered from:

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Gösta Olsson

The Origin, Evolution, Taxonomy and Hybridization of Chinese Cabbage (*Brassica campestris*, ssp. *pekinensis*)

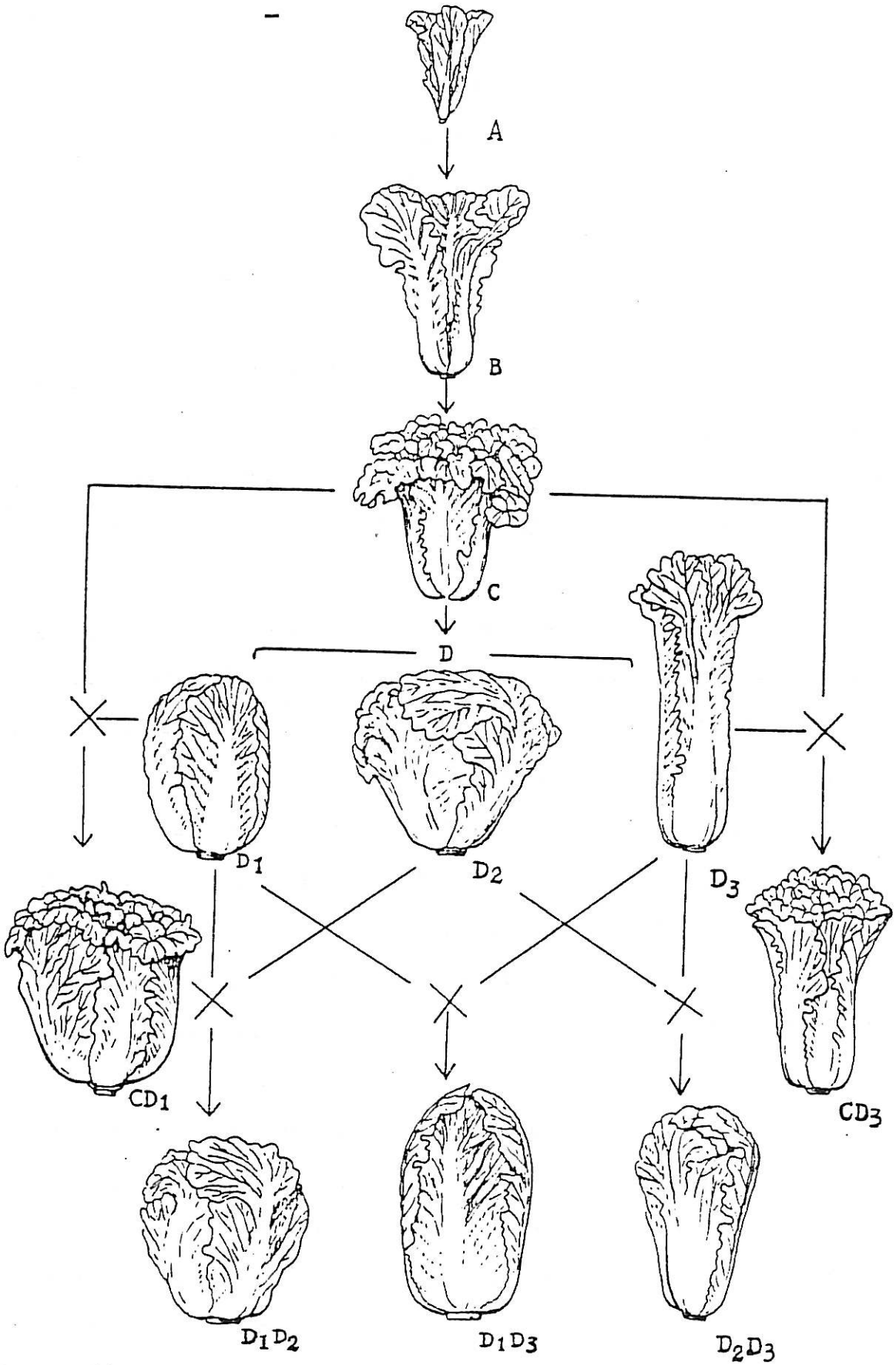
Li Chia Wen

Chinese Cabbage (*Brassica campestris* L. ssp. *pekinensis* [Lour.] Olsson) is known as a vegetable crop which originated in China. However, its wild form has not been found there. Its primary form, loose leaved var. *infacta* Li (Fig. A) was first mentioned in Chinese literature in the 5th century A.D., which was probably a hybrid produced by a natural cross between Pak-choi, ssp. *chinensis* (L.) Makino, and turnip ssp. *rapifera* Metzg. By development of the head-forming habit, it evolved successively to semi-heading var. *infacta* Li (Fig. B), fluffy-topped heading var. *laxa* Tsen et Lee, and then heading var. *cephalata* Tsen et Lee. The last one has developed into three morphotypes in different climatic areas, each having a different head shape and adapting to rather different ecological conditions. They are oval-headed f. *ovata* Li (Fig. D-1), flat-topped f. *depressa* Li (Fig. D-2), and cylindrical-headed f. *cylindrica* Li (Fig. D-3).

By natural crossing between the previous varieties and forms, five hybrid forms have developed. They are fluffy-topped oval form (CXD-1), fluffy-topped cylindrical form (CXD-3), flat-topped oval form (D-1XD-2), stout-cylindrical form (D-1XD-3), and flat-topped cylindrical form (D-2XD-3).

Based on the results of 198 cross combinations which have been conducted in China during the last 30 years, it was found that the average heterosis percentages on a yield basis were significantly different between combinations of different parentages: those crosses between cultivars and inbred lines within the same variety or form, 10.16 $\pm$ 2.47%; those between different varieties and forms, 20.44 $\pm$ 2.19%; those between a variety or form with a hybrid form, 26.24 $\pm$ 3.22%; and those between hybrid forms, 44.2 $\pm$ 8.42%. Therefore, crosses between different varieties or forms, and especially between different hybrid forms are preferable for obtaining heterosis for yield.

THE CLASSIFICATION AND EVOLUTION OF CHINESE CABBAGE (BRASSICA CAMPESTRIS L. SSP. PEKINENSIS (LOUR.) OLSSON)



A: var. dissoluta Li, B: var. infarcta Li, C: var. laxa Tsen et Lee, D: var. cephalata Tsen et Lee, D_1: f. ovata Li, D_2: f. depressa Li, D_3: f. cylindrica Li, CD_1, CD_3, D_1D_2, D_1D_3, D_2D_3: Hybrid forms

Classification and Evolution of Mustard Crops (*Brassica juncea*) in China

Li Chia Wen

Mustard (*Brassica juncea* Coss.) was mentioned early in the Chinese literature during the Chou Dynasty (1122-247 B.C.). Over the centuries, this species has become highly variable in morphology and presently there are more varieties than in *B. oleracea* L.

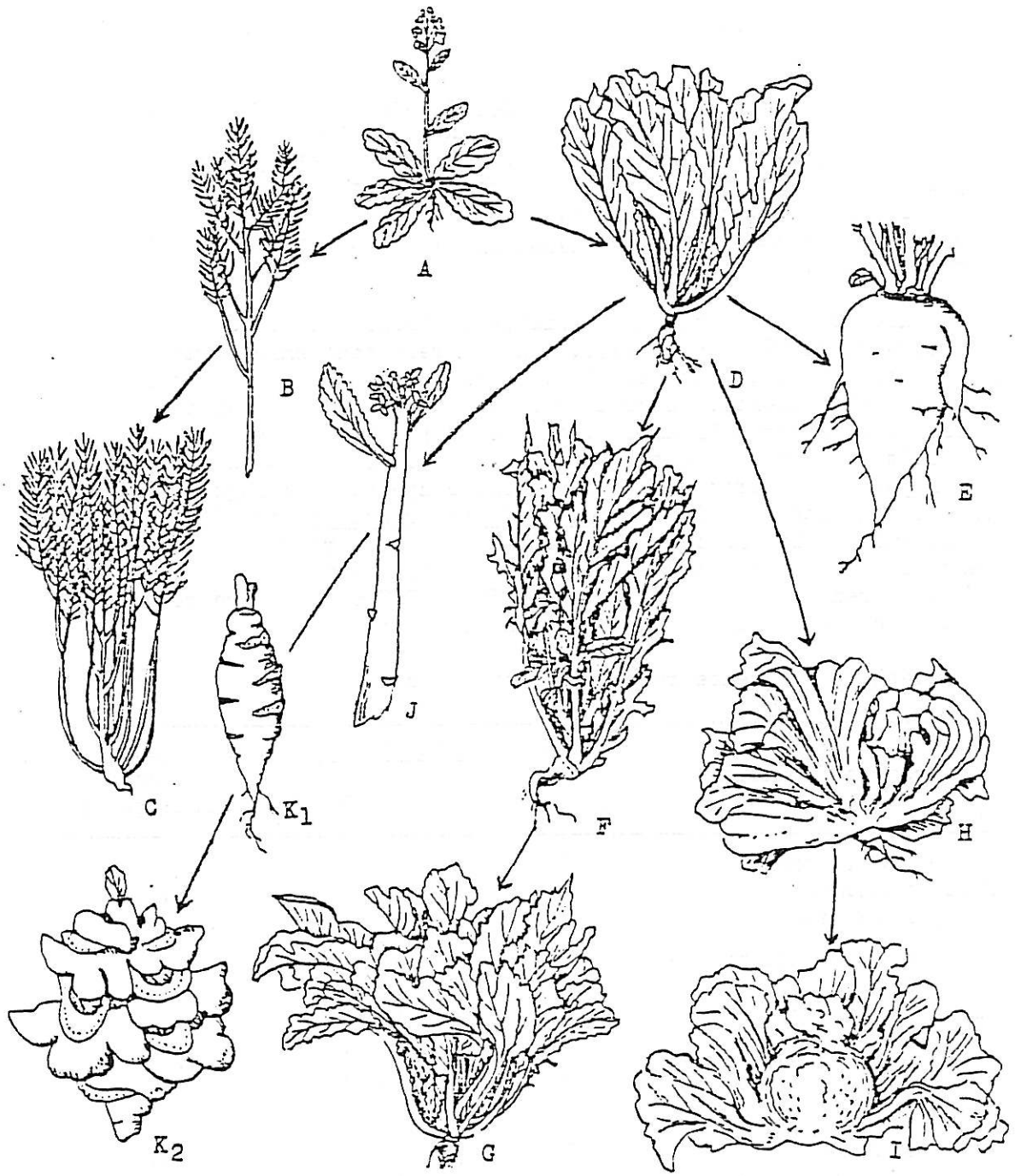
As described in the literature through the 5th century A.D., its ancient form was a small annual plant as shown in Fig. A (Copied from a book written 1400 years ago). Since then, by improving the seed yield, it developed to var. *spicea* Li (Fig. B), a crop with seed used as a condiment, and then later an oil crop, var. *oleifera* Li (Fig. C), which is now grown in the northwestern area of China.

In the Tang Dynasty (618-907 A.D.), mustard with well-developed broad leaves, var. *rugosa* Bailey (Fig. D), was developed and used as greens in the temperate and humid area of southern China. From this variety, a form with a fleshy tap root developed because of the need for the plant to store the excess photosynthates produced by the broad-leaved form.

Later, a form with deeply dissected leaves, var. *crispifolia* Bailey (Fig. F), was developed in northern China which is adaptable to the arid climate there. From this, a tillering form, var. *multiceps* Tsen et Lee (Fig. G), was developed which is more productive than the former and good for pickling.

During the Chin Dynasty (1644-1911 A.D.), a mustard form with broad and thick midribs and petioles as storage organs, var. lapita Li (Fig. H), was developed. Subsequently, a form which produces a head with its fleshy midribs and petioles, var. capitata Li (Fig. I), was developed on the southeastern coast of China where the mild and humid climate in late autumn and winter is especially favourable for the growth of mustard.

During the same dynasty, an early flowering mustard form that produces fleshy flower shoots, var. faciliflora Li (Fig. J), and another form that is late flowering and produces a fleshy stem weighing 0.5-1.0 kg, var. Tai-tsai Mao (Figs. K-1 and K-2), were developed. The former is used in the same way as B. oleracea L. var. italica Plenck., while the latter is used for making a famous pickle named "tsa-tsai" in Chinese.



THE CLASSIFICATION AND EVOLUTION OF CHINESE MUSTARD CROPS
(BRASSICA JUNCEA COSS.)

A: ancient form, B: var. spicea Li, C: var. oleifera Li,
D: var. rugosa Bailey, E: var. napiformis Pall et Bois.
F: var. crispifolia Bailey, G: var. multiceps Tsen et Lee,
H: var. lapita Li, I: var. capitata Li, J: var. faciliflora Li,
K_1 and K_2: var. tsa-tsai Mao

INTRODUCTION OF CLUBROOT RESISTANCE OF BRASSICA OLERACEA INTO 'HAKURAN', B.NAPUS, A NEW HEAD FORMING VEGETABLE

H. Yamagishi, T. Mochizuki and H. Yoshikawa

An artificially synthesized B.napus, named 'Hakuran' (Nishi, 1968), has been in commercial production since 1972, in some restricted area of Japan, mainly because of low seed productivity and no resistant cultivar to clubroot. For the establishment of this new vegetable crop, we have tried to introduce clubroot resistant genes from B.oleracea, through interspecific hybridization.

Three resistant cabbage varieties, Bindsachsener 72754, Böhmerwaldkohl 72755 and 72756, and one resistant kale variety K 269 were crossed respectively with three Chinese cabbage cultivars as male parents, Shimoyamachitose, Chosen and Shoseiko. Thirty days after pollination, embryos were excised and grown on the culture media in test tubes. About 50% of cultured embryos developed into hybrid seedlings. These hybrid seedlings were inoculated with race 2 of Plasmodiophora brassicae Wor. (10^7 spores/ml). It was found that there were differences in resistance of the hybrids tested (Table 1). The hybrids whose female parent was Böhmerwaldkohl 72755 or 72756 were more resistant than the hybrids of other cross combinations.

Table 1. Disease reaction of hybrid seedlings to clubroot.

Cross combination*	Number of plants	Disease score** 0	1	2	3	Average, F_1s of common female parent
K 269 x Shimoyamachitose	6	1	1	1	3	
K 269 x Chosen	3	0	1	1	1	1.75
K 269 x Shoseiko	7	1	3	2	1	
72754 x Shimoyamachitose	13	0	1	5	7	
72754 x Chosen	11	1	3	4	3	2.18
72754 x Shoseiko	4	0	1	1	2	
72755 x Shimoyamachitose	1	0	0	1	0	
72755 x Chosen	3	2	0	1	0	1.00
72756 x Shimoyamachitose	14	3	7	4	0	
72756 x Chosen	13	3	3	4	3	1.39
72756 x Shoseiko	4	0	1	2	1	

Hybrid seedlings acclimatised well were planted to pots inoculated by pouring method.

* 72754; Bindsachsener 72754, 72755 and 72756; Böhmerwaldkohl 72755 and 72756

** 0 (healthy)-3 (severely infected)

After treating hybrids with colchicine, we obtained amphidiploids and tested them for clubroot resistance again (Table 2). The frequency of resistant plants was very low, probably because of the doubling of chromosomes or the dilution of resistant genes. However, we found differences between lines. Among the six lines whose parents were Böhmerwaldkohl 72756 and Shimoyamachitose, two lines (1-24 and 1-35) showed relatively high numbers of resistant plants (disease score 0 or 1).

These lines, artificially synthesized B.napus, n=19, are expected to serve as a genetic basis of resistance to clubroot in the breeding program of 'Hakuran'.

Table 2. Disease reaction of amphidiploids derived from Böhnerwaldkohl 72756 x Shimoyamachitose.

Lines	Number of plants	Disease score 0	1	2	3	Average
1- 2	59	1	7	24	27	2.31
1-12	87	0	12	26	49	2.43
1-21	47	0	5	15	27	2.47
1-24	33	2	6	16	9	1.97
1-33	86	0	2	38	46	2.51
1-34	71	0	0	17	54	2.76
1-35	85	1	20	49	15	1.92
Shimoyama*	87	0	0	5	82	2.94

Tested plants were grown in soil inoculated by inserting method.

* Shimoyama; Shimoyamachitose

SOME INVESTIGATIONS ON LIGHT LEAF SPOT DISEASE OF SWEDES

T.D. JOHNSTON

The fungal disease light leaf spot (Pyrenopeziza brassicae) occurs on leaves of swedes in the glasshouse at the Welsh Plant Breeding Station during late winter and spring, and in recent years has been found on oilseed rape in several parts of Britain.

Various studies, including a formal description of the perfect stage, are described by Rawlinson et al (1), with a list of a number of references to this relatively neglected disease. A few brief comments are given below on my preliminary investigations into the disease on swedes.

1. Inoculum preparation, application and storage: Sporulating pustules on leaves brought into the laboratory from the field or on laboratory-maintained swede leaf segments produce large numbers of small conidiospores which can be used to make inoculum for experimental applications. Aqueous suspensions with around 10^5 - 10^6 spores/ml are then applied by light brush application to the leaf to be tested. The addition of a trace of non-ionic wetting agent to the suspension facilitates applications but may affect the relative contribution of the epicuticular wax of the leaf to the relative degree of resistance to penetration (2).

Long term storage, eg. from season to season, has been successfully achieved by maintenance of fresh, freely sporulating segments of leaf in sealed petri dishes kept at around -20°C in a deep freeze.

2. Leaf segment infection and pustule development: The use of cleanly-cut detached leaf segments, about 20 mm x 20 mm in dimensions, 'floated' on 0.3% agar in petri dishes has been found convenient for assessment of susceptibility of plants. This flotation method is a useful improvement on the use of moist filter paper as described in Cruciferae Newsletter No.4, p.28 for similar mildew tests.

The dishes, each containing about six segments, are then maintained under cool, moderately illuminated conditions. After an incubation period of about 12 - 15 days pustule development commences; sporulation may then continue abundantly for 2 weeks or more.

3. Some test findings: A number of observational tests have been carried out at Aberystwyth. Some of the more interesting findings are as follows:

(a) Spore germination tests in aqueous suspensions showed little germination and slow germ tube growth of spores in distilled water. The number germinating and rate of germ tube growth were increased considerably by addition of a little juice expressed from Brassica plants or the limited number of non-cruciferous weeds also tested.

(b) Marked variations in reaction to inoculation existed in the swede varieties and breeding lines tested. Differences of up to about 20% in the duration of the latent period from inoculation to sporulation were observed together with considerable variations in degree of apparent susceptibility and in some genotypes the appearance of a necrotic reaction with no sporulation. This reaction tended to appear 2-3 days before sporulation on identically treated susceptible leaf segments. Intermediate lines with some spotting and moderate sporulation were also found.

References

1. RAWLINSON, C.J., SUTTON, B.C. and MUTHYALU, G. 1978. Trans. Br. mycol. Soc. 71: 425 - 439.

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Resistance of *Raphanobrassica* seedlings to downy mildew, *Peronospora parasitica*.

S.M. Kluczewski, J.A. Lucas, and I.H. McNaughton.

As part of a study on the host range of different isolates of the downy mildew fungus, *Peronospora parasitica*, the reaction of *Raphanobrassica* seedlings to this pathogen was tested under controlled conditions. Two isolates of *P. parasitica* were used; isolate C1 was obtained from *Brassica oleracea* cauliflower CV. Barrier Reef, and isolate R1 from *Brassica napus* winter oilseed rape (cultivar not known). Thirteen 1 g samples of *Raphanobrassica* 'breeding material' seed were tested. The pathogen was applied as a sporangial suspension to pots of 10-14 day old seedlings using a nasal atomizer at an inoculum rate of 10^5 sporangia/ml. After inoculation the seedlings were maintained inside propagators at high humidity in a growth room at 19 $\pm$ 1 ^{o}C under fluorescent lights (16h day, 5000 lux). Seedlings were assessed visually after 5 days for sporulation of the fungus, and necrotic flecking on the cotyledons. At this time susceptible control seedlings of cauliflower or oilseed rape showed heavy infection. Results are presented in the table overleaf:

Scores represent the mean of all seedlings tested.

	Isolate C1		Isolate R1	
Seed batch code	Sporulation	Necrosis	Sporulation	Necrosis
RB 25/8	0	1	0	0
RB 25/4/12	0	1	0	1-2
RB 25/4/13	0	1	0	1
RB 26/12/B	1-2	2	0	1
RB 27/8/B	2	1	0	1
RB 27/8/19	0	1	0	1
RB 27/8/20	0-1	1	1	2-3
RB 30/6/B	1-2	1-2	0	1
RB 30/8/B	0	2	0	1
RB 30/9/B	0	1-2	0	1
RB 30/12/B	1-2	2-3	0	1
RB 31/6/B	1	1	0	1
RB 31/10/10	2	2-3	0	1

Assessment scale 0 = no sporulation/necrosis

4 = heavy sporulation/extensive necrosis

There was quite wide variation in the reaction of individual seedlings within each seed batch, and many seedlings showed no symptoms. In view of the high inoculum dose used the scores in general indicate good resistance to P. parasitica. All the seed batches showed high resistance to the rape isolate R1, with some necrosis but scarcely any sporulation. The cauliflower isolate C1 was able to make limited growth on six of the thirteen batches, but in no case was sporulation more than slight.

In an additional test, detached Raphanobrassica cotyledons were inoculated with droplets containing a mixture of sporangia of both fungal isolates. Using susceptible hosts, R1 and C1 are sexually compatible and produce oospores in cotyledon tissues. When cleared and stained in lactophenol-ethanol and trypan blue 5 days after inoculation and examined microscopically, no oospores were seen in any of the samples. Extensive necrosis of host epidermal and mesophyll cells was noted, apparently restricting development of the pathogen. Small amounts of mycelium were seen in three samples, RB 27/8/20, RB 30/12/B and RB 31/10/10. These all supported slight sporulation of the pathogen when inoculated with isolate C1 alone.

The results of these tests confirm a previous field observation of the resistance of Raphanobrassica to downy mildew. As in Brassica spp, seedlings are more susceptible to P. parasitica than adult plants, it seems likely that the resistance noted here will also be effective in older plants. It is not known, however, whether Raphanus isolates of the pathogen will prove to be more virulent than the Brassica isolates used in this study.

PROGRESS IN DEVELOPMENT OF CLUBROOT RESISTANT CABBAGE

M.S. CHIANG and R. CRETE

A number of plants derived from our two backcross progenies are resistant to clubroot races 2 and 6 in field test. The pedigrees of the two backcross progenies are as follows.

B_1-A-1: *B. napus* x 2x-cabbage → triploid F_1 hybrid (2n = 28) x 2x-cabbage → B_1-A-1 (2n = 18) x 2x-cabbage → clubroot resistant cabbages with attractive head.

B_1-20 : *B. napus* x 4x-cabbage → tetraploid F_1 hybrid (2n = 37) x 2x-cabbage → B_1-20 (2n = 26) x 2x-cabbage → I-1 (2n = ?) x 2x-cabbage → clubroot resistant cabbages with attractive head.

Since both B_1-A-1 and B_1-20 are male sterile, we expect that most of the selected plants will be male sterile too. We hope, however, that some of them will produce some pollen grains so that homozygous resistant plants can be selected in later generations by self or sib pollination.

INFLUENCE OF BORON NUTRITION ON CONTENTS OF GLUCOSINOLATES IN CRUCIFEROUS CROPS

H.-Y. Ju, B.B. Bible and C. Chong

Boron deficiency is more damaging to cruciferous crops than any other micronutrient deficiencies. Recently we noted that boron deficiency significantly altered the yield of thiocyanate ion and reducing sugars in radishes (*Raphanus sativus* L.) grown in hydroponic culture. Excessive amounts of thiocyanate ion and reducing sugars were found in foliage of boron-deficient plants. This evidence suggested that boron nutrition may be an important factor influencing glucosinolate content in cruciferous plants.

In a subsequent investigation we studied the influence of six boron levels (0, 0.1, 0.5, 2.5, 5.0 and 10.0 ppm) on content of glucosinolates and reducing sugars in hydroponically grown 'snowball' turnip (*Brassica rapa* L.). The glucosinolates were determined by quantifying the hydrolysis products, goitrin (L-5-vinyl-2-thio-oxazolidinethione), volatile isothiocyanates, and thiocyanate ion.

While plants not receiving boron (0.0 ppm, control treatment) were very poor in growth, those receiving 0.1 to 5.0 ppm boron were equally vigorous in growth; growth of plants receiving 10.0 ppm boron were slightly inhibited.

Compared with the other treatments, relatively high or maximum quantities of goitrin, volatile isothiocyanates, and thiocyanate ion were found in both top and root tissues of turnips grown in the 0.1 ppm boron treatment. Although the foliage and external root appearance of plants grown in the 0.1 ppm boron treatment appeared healthy, the roots showed internal brown discoloration, characteristic of boron deficiency.

CONTENT AND PATTERN OF GLUCOSINOLATES IN SEEDS OF RESYNTHESIZED *BRASSICA NAPUS* AND ITS DIPLOID PROGENITORS, *B. OLERACEA* AND *B. CAMPESTRIS*

Astrid GLAND

The use of rapeseed meal for animal feeding is limited by its glucosinolate content (ASTWOOD et al. 1949, VIRTANEN 1961, PAPAS et al. 1979). But extensive selection for low glucosinolate content within *B.napus* was only successful in one case: In seeds of the Polish variety 'Bronowski' the content of total glucosinolates was reduced to one tenth (JOSEFSSON and APPELQVIST 1968, KRZYMANSKI 1970, LEIN 1970). However, the diploid ancestors of *B.napus*, i.e. *B.oleracea* and *B.campestris* exhibit many differences not only in content of total glucosinolates but also in the proportion (pattern) of individual glucosinolates. Therefore, it seemed possible to select genotypes of these two species and to construct new rapeseed cultivars by interspecific crossing and polyploidization of the amphihaploid hybrids. This report presents some of our experiments in this direction (for details see GLAND 1980).

Glucosinolate content and pattern were determined by gaschromatography according to THIES (1977, 1978). More than 700 origins from the worldwide Brassica collection of the Institute of Plant Breeding in Göttingen were analyzed and 102 different genotypes of *B.oleracea* and *B.campestris* were selected for hybridization. Because seed development was rather poor, especially in combinations with *B.campestris* as the female, embryos could be raised by in-vitro culture (for medium see VELEMÍNSKÝ and GICHNER 1964). Hybrid plants were most effectively diploidized by soaking seedlings at the six leaf stage for 8 hrs into 0.05% colchicine solution. Selfed seeds were analyzed for their glucosinolate content and pattern. Some of the data are exemplified in Table 1.

Large differences were found between the 367 varieties of *B.oleracea*: The glucosinolate content ranged from 1 µmol/g defatted meal (e.g. broccoli and turnip kale) to about 200 µmol/g (e.g. fodder borecole cultivars) and the pattern varied widely, too. This result is most plausible, since this species has long been used as a vegetable and the contents of the leaves and of the seeds are highly correlated (JÜRGES and RÖBBELEN 1980).

Between the 256 *B.campestris* forms the variability was similarly complex: Total glucosinolates ranged from 8 µmol/g defatted meal (e.g. turnip rape) to 200 µmol/g (e.g. yellow sarson). Members of this species are widely used as a salad and vegetable crop in South East Asia. One important difference between *B.campestris* and *B.oleracea* is the absence of sinigrin (Table 1). Generally, gluconapin is the major compound in *B.campestris* seeds.

Table 1: Glucosinolate pattern (content of a single glucosinolate in % of the total content of analyzed glucosinolates) and total glucosinolates (in µmol/g defatted meal).
SIN = Sinigrin, GNA = Gluconapin, GBN = Glucobrassicanapin, PRO = Progoitrin, NAP = Gluconapoleiferin

Collection No.	Cultivar	Single glucosinolates in %					Total glucosinolates
		SIN	GNA	GBN	PRO	NAP	
	1. Cabbage, Brassica oleracea						
525	White cabbage	92.5	2.9	0.7	3.7	0.2	45.6
575	Red cabbage	18.5	4.6	0.1	75.8	0.1	76.6
2184	Savoy cabbage	93.9	1.6	0.1	2.5	2.0	76.5
182	Brussels sprouts	36.0	8.6	0.2	54.9	0.2	45.5
2902	Cauliflower	95.8	1.5	0.2	2.4	0.2	61.8
2345	Broccoli	6.7	6.7	6.7	73.3	6.7	1.5
513	Broccoli	0.5	18.0	1.3	76.4	3.7	129.3
450	Kale turnip	71.1	13.3	2.2	11.1	2.2	4.5
2155	Curly kale	64.6	7.4	0.4	26.0	1.5	91.4
2243	Fodder borecole	60.0	9.3	0.7	27.7	2.3	150.8
2150	B.alboglabra	24.1	14.8	0.6	58.2	2.3	154.8
	2. Turnip, Brassica campestris						
2473	Chinese cabbage	-	85.4	12.2	2.0	0.3	29.5
35	Chinese cabbage	-	90.2	8.5	1.3	0.1	151.1
849	Yellow sarson	-	97.8	2.1	0.1	0.1	205.9
2016	Turnip rape	-	34.1	25.0	37.5	2.3	8.8
76	Turnip rape	-	34.9	29.0	28.4	7.7	88.6
2130	Common turnip	-	65.5	6.2	26.5	1.8	98.5
	3. Rapeseed, Brassica napus						
109	Bronowski	-	26.5	6.2	65.5	1.8	16.0
2499	0559, Spain	-	26.1	4.5	65.7	3.8	73.4
2534	Diamant	-	21.3	5.3	70.1	3.3	156.1
2682	Rutabaga York	-	0.4	0.4	99.1	0.1	167.9
	4. Resynthesized rapeseed						
Hybrid No.	Total glucosinolate ♀	♂					
H 474	129.3	151.1	-	29.1	3.4	66.1	1.5	143.0
H 7	45.6	111.7	10.1	13.1	7.0	66.8	3.0	195.8
H 485	89.8	91.5	14.6	13.1	6.5	63.0	2.7	66.3
H 523	25.1	86.1	7.4	18.1	3.8	68.2	2.2	142.0
H 865	31.4	62.9	10.5	33.4	7.8	46.7	1.8	73.4
H 829	70.5	151.1	14.0	14.2	6.0	63.3	2.5	176.2
H 878	130.8	86.1	9.7	21.3	2.9	63.9	2.2	120.4
H 65	158.7	119.7	11.5	29.7	9.2	46.2	3.4	121.5

The glucosinolate content of natural B.napus ranged from 10 to 250 µmol/g defatted meal. It is composed of approximately two thirds of progoitrin and a quarter of gluconapin. Only some cultivars of rutabaga showed an extremely high content of progoitrin, which could amount up to 95% (Table 1). Sinigrin was not found in any of the analyzed rapeseed samples.

Seeds of our first 35 combinations of resynthesized rapeseed have been analyzed and the data of eight different combinations are given in Table 1. In all of these combinations the glucosinolate content ranged from 66 µmol/g defatted meal (e.g. H 485) to 196 µmol/g (e.g. H 7). It thus was as high as in any natural rapeseed cultivar. Similarly, the single glucosinolates were present in the known amounts. However, seeds of all hybrid plants, except 'H 474', exhibited 7-15% of sinigrin.

Two hybrids from the pollination of two B.oleracea by one B.campestris cultivar are shown in Figure 1. The female cultivars were extremely different, No. '513' (broccoli) representing the progoitrin-type and No. '2179' (savoy cabbage) the sinigrin-type. Nevertheless, seeds of both hybrids showed a rather similar glucosinolate pattern.

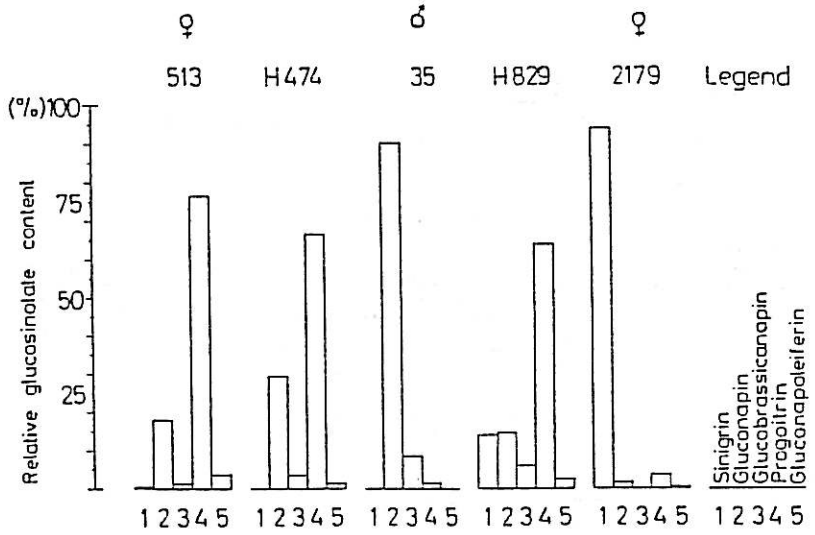

Fig. 1: Glucosinolate pattern of resynthesized rapeseed forms as compared to their progenitors. The cross includes two different B.oleracea females and one B.campestris cultivar as pollen donor.

Some resynthesized rapeseed forms had a glucosinolate pattern, which resembled that of B.juncea. These hybrids were shown to possess only 2n = 36 chromosomes. In root-tips of hybrid plants with the rapeseed glucosinolate pattern 2n = 38 chromosomes were determined.

The fertility of hybrid plants was different according to the combination; but seed weight was similar to that of natural rapeseed.

Although the variation of glucosinolate content and pattern in the seeds of diploid Brassica species, which we were able to combine is beyond everything known from the literature, most of our resynthesized rapeseed forms so far showed a glucosinolate pattern similar to any natural rapeseed cultivars. The parents were of the pure sinigrin or the gluconapin-type or they contained progoitrin in addition. But no similarity with these progenitors was detected in the new rapeseed hybrids, which indicates strong interactions between the two parental genomes. This situation evidently contributes to the uniformity of the glucosinolate pattern, which is typical throughout the present B.napus cultivars.

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FACTORS INFLUENCING THE GLUCOSINOLATE CONTENT OF BRASSICAS

R.K. Heaney, C.L. Curl, E.A. Spinks and G.R. Fenwick

Glucosinolates occur in common vegetables, e.g. cabbage and swede, salad crops, e.g. radish, cress and animal feedingstuffs, e.g. rapeseed, forage rape and kale. Their effects may be beneficial, e.g. by contributing significantly to the aroma and taste of the product, or harmful, e.g. causing goitrogenic effects, rendering rapeseed meal unpalatable. Consequently methods are needed for the analysis of both total and individual glucosinolates in products intended for animal and human consumption. Such methods have been developed at the Food Research Institute.

Individual glucosinolates, including for the first time all of the goitrogenic precursors, may be quantified by gas chromatography. In an investigation of new varieties of Brussels sprouts we have found levels of total glucosinolates varying between 100 and 400 mg/100 g (fresh weight of button), depending upon variety. However a significant site/site variation has been found, probably due to factors such as soil type, climate, fertilizer etc., so that the same variety grown at different sites can have total glucosinolate levels differing by up to 200%.

Although the total level of glucosinolates is greatly affected by site effects, the relative abundance of the individual glucosinolates remains remarkably constant and enables a fingerprint "pattern" to be drawn. This pattern, together with that of the corresponding seed, may help to identify the individual cultivar. Preliminary results indicate that this is not limited only to Brussels sprouts.

The effect of breeding on the glucosinolate pattern of Brussesl sprout has also been examined, with that of the buttons of the F_1 hybrid being very close to that calculated from an equal contribution from both parents. This may indicate the possibility for removal of individual undesirable glucosinolates by breeding. The same relationship is not observed in seed.

Analysis of the glucosinolate patterns of 30 varieties of rapeseed of differing genetic origins has shown unexpectedly similar patterns (in contrast to the Brussels sprout reported above). However patterns from the leaves of 4 week seedlings of the same cultivars do show differences and suggest that the chemotaxonomic method may be applicable to rapeseed also.

THE ANALYSIS AND STRUCTURAL ELUCIDATION OF GLUCOSINOLATES

J. Eagles, R.K. Heaney and G.R. Fenwick

Glucosinolates, mustard oil glycosides, are common to members of the Cruciferae as well as occurring in certain other botanical families. Glucosinolates have been routinely identified after conversion to volatile degradation products (e.g. nitriles and isothiocyanates). However it has been recently found that the structures of intact glucosinolates may be obtained directly using electron impact - and chemical ionization - mass spectrometry. This work has been carried out in collaboration with Professor R. Gmelin, Institute of Pharmacognosy and Phytochemistry, Freie University, Berlin, German Federal Republic. Under these conditions the glucosinolates (and their desulpho analogues) yield spectra characterised by abundant ions corresponding to fragments containing the glucosinolate side chain. The method has been found to be applicable to simple mixture analysis. However, more complex mixtures of glucosinolates appear to be amenable to gc/ms analysis. It has been shown (see below) that such glucosinolate mixtures may be effectively separated (and quantified) by initial enzymatic desulphation and pertrimethylsilylation. Although normal gc/ms of such derivatives is disappointing in that little structural information is forthcoming, present work suggests that gc/chemical ionization ms shows considerable promise in this respect.

The varying natures of glucosinolates, - more accurately their breakdown products - are such that whilst certain may be considered desirable in products for human consumption (for example those contributing significantly to the taste/odour of the product) others exhibit undesirable physiological characteristics (for example anti thyroid activity). This means that methods for the analysis of total and individual glucosinolate analysis are very important.

A method for total glucosinolate analysis, using measurement of enzymically released glucose, has recently been developed which appears to offer the sensitivity, accuracy and speed required by the plant breeder. Using disposable micro-columns, glucosinolate analyses may be carried out on as little as 10 seeds and 40-60 analyses may be run daily.

Individual glucosinolates in Brassica seed and leaf material have been separated and quantified using the volatilization of intact glucosinolates described above. This has enabled the goitrogenic precursors, sinalbin, progoitrin, glucobrassicin and neoglucobrassicin, to be separated and directly for the first time. The method has been used extensively for the analysis of glucosinolate levels in Brassica vegetables and seed and also as a basis for the chemotaxonomic work described hereafter.

THE EFFECT OF CULTIVAR AND HARVEST DATE ON THE SMCO CONTENT OF KALE.

J.E. Bradshaw and R. Borzucki.

Today in the United Kingdom kale is mainly strip grazed *in situ*, using an electric fence, by dairy cattle during the autumn and early winter. If the kale is overfed the cattle may develop a severe haemolytic anaemia. Dr R. Smith and his colleagues at the Rowett Research Institute have provided good evidence for the anaemia resulting from the conversion of S-methylcysteine sulphoxide (SMCO), present in kale, to dimethyl disulphide by rumen micro-organisms. (See article by Dr R. Smith in Cruciferae Newsletter No. 3.) Hence there is now the possibility of removing the dangers of overfeeding kale by breeding new cultivars with lower SMCO contents.

Prior to starting such a programme, we carried out an experiment to investigate the effect of cultivar and harvest date on the SMCO content of kale. (We hope to publish the detailed results fairly soon.) The cultivars consisted of seven marrowstem kales (Condor, Giganta, Kestrel, Marrowstem, Merlin, Proteor and Vulcan), three thousand headed kales (Canson, Dwarf Thousand Head and Thousand Head), and two curly kales (Dwarf Green Curled and Tall Green Curled). Plants were harvested, by cutting at ground level, in the middle of September, November, January and March. Chopped samples were taken and stored in a cold room at -20°C until they were freeze-dried. The dried samples were then milled in a hammer mill and stored in plastic jars, with screw caps, in a deep freeze until analysed for SMCO content. This was done using the method developed by Dr A. Gosden at the Welsh Plant Breeding Station. (See article by Dr M. Allison and his colleagues in this edition of Cruciferae Newsletter.) Our results are summarised in the table below. There was no evidence for cultivar differences within the three varieties of kale.

SMCO AS PERCENTAGE OF TOTAL DRY MATTER

	SEPTEMBER	NOVEMBER	JANUARY	MARCH	MEAN
MARROWSTEM	0.656	0.826	1.119	1.064	0.916
THOUSAND HEADED	0.758	0.871	1.183	1.317	1.032
CURLY	0.719	1.056	1.562	1.525	1.216
MEAN	0.692	0.876	1.209	1.204	0.995

Cultivar by harvest means had an average standard error of 0.072.

As the marrowstem kales are the highest yielding types for autumn use, and as amongst them are cultivars, such as Kestrel and Proteor, which have sufficient winter hardiness for use well into the winter, we intend to intercross the best marrowstem kales currently available, and to select for lower levels of SMCO whilst trying to maintain the high level of digestibility achieved in cultivars such as Condor, Merlin and Kestrel.

ANALYSIS OF QUALITY FACTORS IN BRASSICAS

M.J. Allison, I.A. Cowe and R. Borzucki

Chemistry laboratories that provide an analytical service to brassica breeders are asked increasingly to carry out new analyses as more problems concerning quality aspects of brassicas are identified. For example, recent worries about haemolytic anaemia, in ruminants fed exclusively, or largely on kale, have resulted in the development of methods for measuring the haemolytic factor, S-methyl cysteine sulphoxide, in a number of chemistry laboratories.

At our Station we have tried to cope with the increasing workload in numbers and kinds of analyses by automating methods and using quicker tests, where possible. Our system for estimating digestibility for example, is now based on an enzyme method (Jones and Hayward, 1973) in which pepsin-cellulase stages are used to digest forage material. With a minor modification (Allison and Borzucki, 1978) to their method we have found that digestibility results correlate very highly with results obtained using rumen liquor from sheep. Advantages of the pepsin-cellulase method are that it is quicker, more repeatable and filtration is easier and faster than in the rumen method.

For SMCO determinations on breeding material the method developed at WPBS (Gosden, 1979) has proved to be the most successful in our trials. The electrophoretic method developed by Whittle et al (1976) works well for a small number of samples, but when the number is increased to thousands of breeding lines, an automated method is preferable. Although the test developed by Gosden requires the inclusion of a separation column in an automated flow system, about 25 samples per day can be managed with ease. S-methyl cysteine sulphoxide is simply made by oxidising S-methyl cysteine so a standard curve can be easily and quickly produced for the estimation of unknowns, by automated analysis.

Thiocyanates derived from glucosinolates in brassicas can cause goitrogenic effects and again, thiocyanates are quickly and repeatably estimated by an automated method (Gosden, 1978). In this case the manifold system does not require a column and, thus, a high throughput is possible (about 100 samples per day). Myrosinase for the conversion of glucosinolates to thiocyanates etc., has to be extracted from mustard flour and this manual procedure is the only lengthy but infrequent, operation.

Nitrogen in brassicas is determined again by an automated system. Acid-hydrolysed material is analysed on a Technicon auto analyser II system using the Salicylate method recommended by the Association of Official Analytical Chemists.

More recently we have successfully calibrated an infra red (IR) analyser the Technicon 300 for the rapid (one sample per minute) and accurate prediction of nitrogen content in about 5g of milled kale material. Very high correlations ($r = 0.993$) were observed between manual and IR predicted values. Calibration of the Technicon 300 to predict nitrogen in other brassicas should also be possible.

Because of our success with fixed wavelength IR machines we acquired a continuous scanning infra red analyser, the Neotec 6350. This is a research instrument consisting of a monochromater linked

to a Nova III computer. Samples can be scanned and the IR absorbance values at 1400 wavelengths in the near infra red can be stored in the computer. The regression programme can then be used to find those wavelengths at which absorbance values can be used to predict the constituent of interest. Constants in prediction equations are derived by calibrating the machine with samples which have been carefully analysed. The manual analysis data is entered as ordinate values and the samples are scanned to obtain the predicted abscissa values. Once calibrated, different equations can be used to predict several parameters simultaneously on the same sample. It is our aim to provide a screening set of equations for nitrogen, SMCO, thiocyanates and digestibility for brassica material. Filters can also be made relatively cheaply for important wavelengths, so that quality factors can also be predicted by simpler fixed wavelength IR machines.

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1. Brassica oleracea
2. Brassica campestris (rape)
3. Brassica napus
4. Brassica other species
5. Other genera
6. Interspecific & intergeneric hybrids

Usage

a. Vegetables and condiments
b. Fodder
c. Green manure
d. Oil and protein

Pests, diseases and weeds

e. Animal
f. Weeds
g. Fungal
h. Bacterial
i. Viral
j. Mineral

Disciplines

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l. Pathology
m. Physiology
n. Taxonomy and Evolution (including resources and gene banks)
o. Genetics and cytology
p. Breeding
q. Mating systems
r. Nutrition and chemical composition
s. In vitro culture
t. Variety testing (including National Lists and Breeders rights)
u. Seeds, production and performance

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