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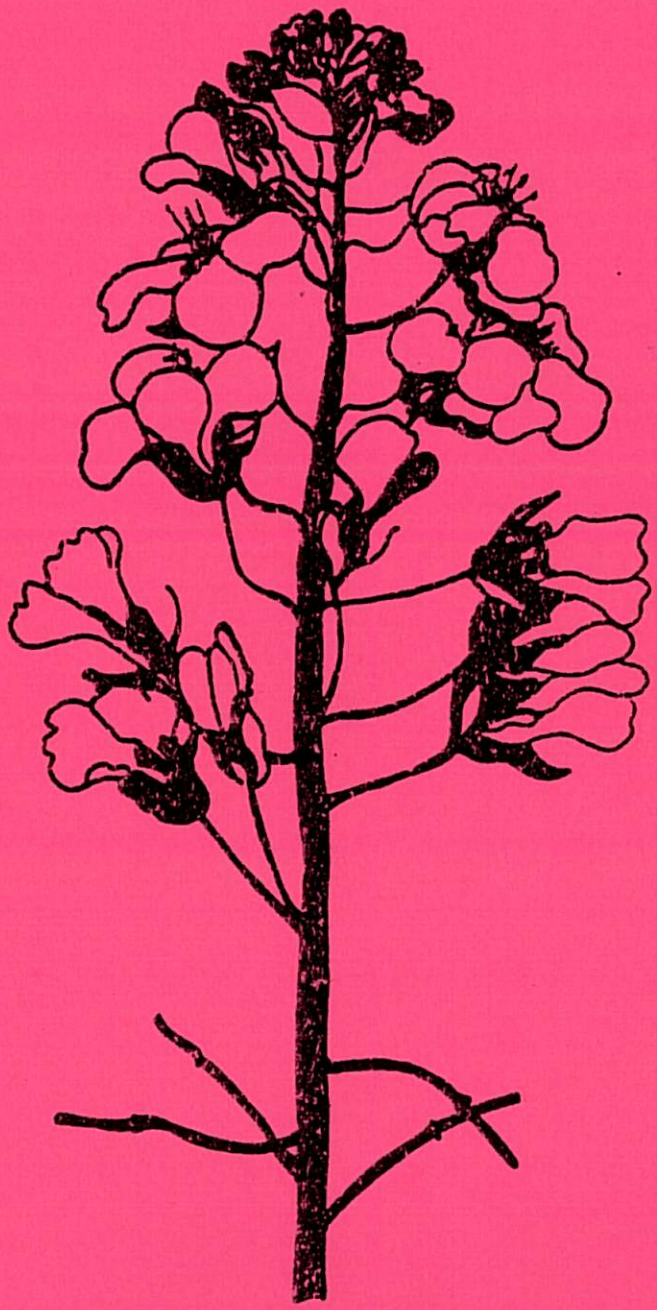
# CRUCIFERAE NEWSLETTER No. 8



November 1983

EUCARPIA

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## Editorial

We thank those readers who replied to the letter sent earlier in the year in which we requested up-to-date information concerning their address and professional interests. The distribution list has been amended to incorporate these responses and, during the year, the names of 61 new recipients have been added. The new distribution list has been produced as a separate booklet, accompanying this Newsletter. Future amendments will be notified in the Newsletter.

The costs of producing and mailing Cruciferae Newsletter No. 7, and of mailing the reminder to contributors, amounted to approximately £300 - considerably less than had been anticipated. Consequently, the balance in the fund is £516. This should adequately cover the costs of this issue although it will be necessary to raise more funds for the next issue.

In the past we have been reluctant to institute a charge to individual recipients because it would entail time-consuming administrative procedures and would increase costs. Instead we have been indebted to the generosity of commercial sponsors for our funding. We believe that we should continue with this system, at least for the time being, but we think it should be on a more flexible basis. Therefore, individual readers who would like to make a voluntary donation can be assured that their assistance in funding the Newsletter would be most welcome. To minimise bank charges donations should be sent in sterling by postal draft, or drawn on a British bank.

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

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

# BRASSICA CRETICA LAM. GERMPLASM COLLECTION IN GREECE

M. Gustafsson, C. Gómez-Campo and A. Zamanis

Three years ago, FAO/IBPGR officially recognized the importance of collecting germplasm of those wild n=9 Brassica species which are interfertile with cultivated B. oleracea L., and it started financing expeditions throughout their natural areas in the Mediterranean coasts (Gustafsson, 1982). Base collections are to be stored in the Universidad Politécnica (Madrid, Spain) crucifer seed bank, and duplicates in Tohoku University (Sendai, Japan) and in the appropiate institution of the country where the collection takes place. After the necessary multiplication (pressumably in 2-3 years for most samples) the material will be ready for distribution to interested scientists. A list of available material is expected to appear in the next issue of UPM catalog (see Gómez-Campo, 1978) which will appear by 1985.

In July 1982 an expedition was held to collect Brassica cretica Lam. seeds in Eubea, Attica and Peloponessos. In July 1983 a second expedition was covering the island of Crete as well as some additional areas in Eubea and Attica. Fourty-five seed samples were collected from as many different localities. As a by-product of these collecting activities, several new localities were discovered and a number of chorological, ecological and conservational observations were made. These are briefly summarized below.

Previous knowledge on the geographic distribution of this species has been summarized by Gustafsson (1982 bis). The following newly found localities should now be added.

- Eubea, 2 km. North of Mili in a ravine running eastwards.
- Eubea, 2 km. from Pigadia along well developed cliffs near the road.
- Eubea, 1, 5 km. from Pili in limestone cliffs.
- Viotia, cliffs near the beach of Psathes in the Chorinthos Gulf.
- Crete, Petres Valley, West of Rethimnion, 1 km. from the road.
- Crete, 1 km. South-West of the village Imbros, near Sphakion.
- Crete, Spili (Rethimnion province) just above the town.
- Crete, Spili, gorge 3 km. South of the village.
- Crete, gorge of Kotsifou, 1-2 km. North-West of Selia (Rethimnion)
- Crete, 5 km. North-West of Ano Vianos, Gorge near Panagia Monast.
- Crete, deep gorge above Moni Arvi in the South coast (Ano Vianos).
- Crete, Ambelo ravine, near Gonies (East Heraklion province).

In short, the area of B. cretica has been enlarged northwards in Eubea, the presence of this species in the coast of Chorinthos Gulf has been confirmed, and it has been found that the Heraklion province in Crete is as important in this respect as the West side of the island. Figure 1 summarizes our present knowledge on the area of B. cretica Lam. (Greek mainland and Crete; small islands are excluded) after the above localities were incorporated. Three recognized subspecies are considered.

The habitat of B. cretica most usually consists of the crevices of lime stone or dolomitic cliffs which are never far from the sea at altitudes ranging from 20 to 1,000 m. The habitat can therefore be referred to

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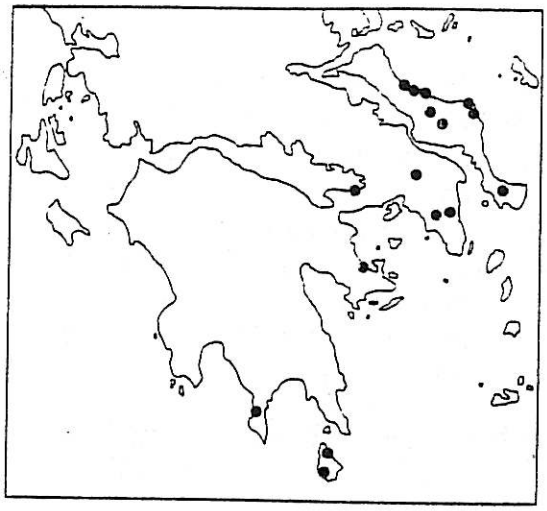

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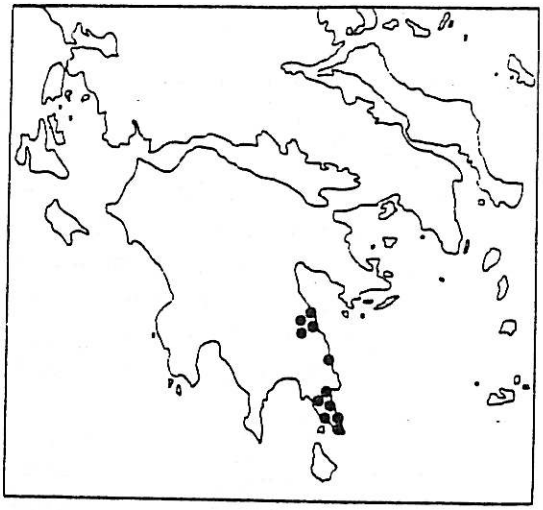

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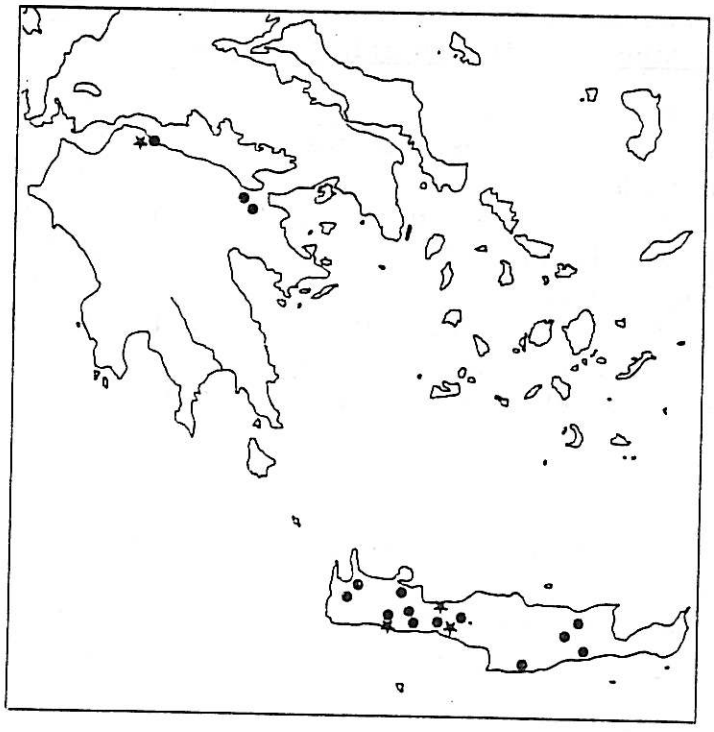

Legend:

- collecting sites
- * all plants inaccessible

the phytosociological class "Asplenietea rupestris". These cliffs must have enough water supply secured from adjoining land masses ; thus the drough which is associated to Southern aspects is usually avoided. In Crete, adequate situations are often obtained along the vertical walls of deep ravines.

Grazing by herbivores clearly represents a major restrictive factor for B. cretica distribution. The plants rarely grow below a distance of 2 or 3 meters from the soil and persistently prefer inaccessible positions where they can be out of reach of grazing goats or deer. As goats are especially abundant in many localities, germplasm collection for seed banking purposes is often difficult. Only two cases of plants growing directly on the soil were found (Diakoftos in N. Peloponessos and Kithera castle in Kithera Island); they both corresponded to places which were obviously protected from grazing goats.

Frequent companions of B. cretica were Ptilostemon chamaepeuce, Inula candida, Alyssum saxatile, Melica minuta, Erysimum senoneri, Coronilla juncea, Matthiola incana, Hypericum ericoides, etc. In Crete, also Salvia pomifera, Dianthus arboreus and Ebenus cretica were observed. Accidentally, the rocks are invaded by nearby ground species as Pistacia lentiscus, Quercus coccifera, Phlomis fruticosa, Calicotome villosa, Cercis siliquastrum, and, in the most thermic situations, Euphorbia dendroides, Thymus capitatus and Sarcopoterium spinosum.

From a conservational point of view, most B. cretica populations are abundant enough and they have such inaccessible positions that they do not seem subjected to an inmediate danger of extinction, even by human collectors. However, a few of them contained so small number of individuals (for instance, a single individual was seen in Petres Valley), that mere reproductive collapse might be fatal. Apart from the exploitation of goat herds (very often in semi-wild condition), other types of human action were rare. Even when the species was growing in cliffs near a village (as in Magheri, Crete), it was well known and somewhat respected by peasants.

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FIGURE 1. Populations of B. cretica Lam. investigated in 1982 and 1983. All known localities in Eubea, Attica, Peloponessos and Crete have been checked but four proved to be completely inaccessible.

# REDISCOVERY OF SINAPIS ARISTIDIS POMEL

Guy Baillargeon

Sinapis sect. ERIOSINAPIS Cosson (= Sinapis pubescens L. s.l.) reaches its maximum variability in the mountains of northern Algeria where members of this complex are fairly common in the natural permanent pastures and constitute an unnegligible part of the livestock's diet.

From June 26 to July 17, 1983, a field trip across the area between Alger and the tunesian border allowed me to collect herbarium material and seeds of most of the taxa of this group. One of them, S. aristidis Pomel, is known only for Djebel Debbagh and Djebel Thaya, two isolated mountains near the city of Guelma (36.29 N, 7.25 E). S. aristidis is a morphologically very distinct plant with thick, swallowed, spongious and indehiscent silicles which would be analogous to those of Raphanus sativus if they were not provided with suturated but strongly marked valves.

S. aristidis was discovered by Aristide Letourneux in 1861. From 1863 to 1867 the plant was offered as Heterocrambe aristidis Cosson et Durieu (nomen nudum) on the seed list of the Jardin-des-plantes de la ville de Bordeaux (France) and all the very rare specimens of this taxon in the herbaria seem to originate from the cultivation of these seeds. The most recent collection of wild material is apparently one from Hagemüller in 1880, kept in Paris (P-Co).

In its two classical localities S. aristidis is now found mostly under the protection of strongly thorned shrubs because of the severe overgrazing, or restricted to fissures, crevices, block-fields and scarps unaccessible to animals. On the Djebel Thaya I saw however many young rosettes and seedlings on well rotted manure near a cavern used as cattle-shed.

Gradual intergradation between S. aristidis and S. pubescens after some years of common cultivation is reported by Durieu (1867) but wild living plants from the two classical localities remain astonishingly morphologically marked more than a century after their first discovery. The new collection will allow to elucidate relationship between S. aristidis and the rest of the S. pubescens group.

Complete sets of herbarium specimens are kept in Berlin (B) and at the new herbarium of the Université des Sciences et de la Technologie d'Alger, Dar el Beida. Seeds will be preserved at the Dr. Gomez-Campo's germ plasm collection of crucifers after my work has been completed.

This travel was financed by the Biosystematics Research Institute, Agriculture Canada, Ottawa and the Berlin Botanical Museum. Cooperation from the Département de Biologie, U.S.T.A., Dar el Beida is also gratefully acknowledged.

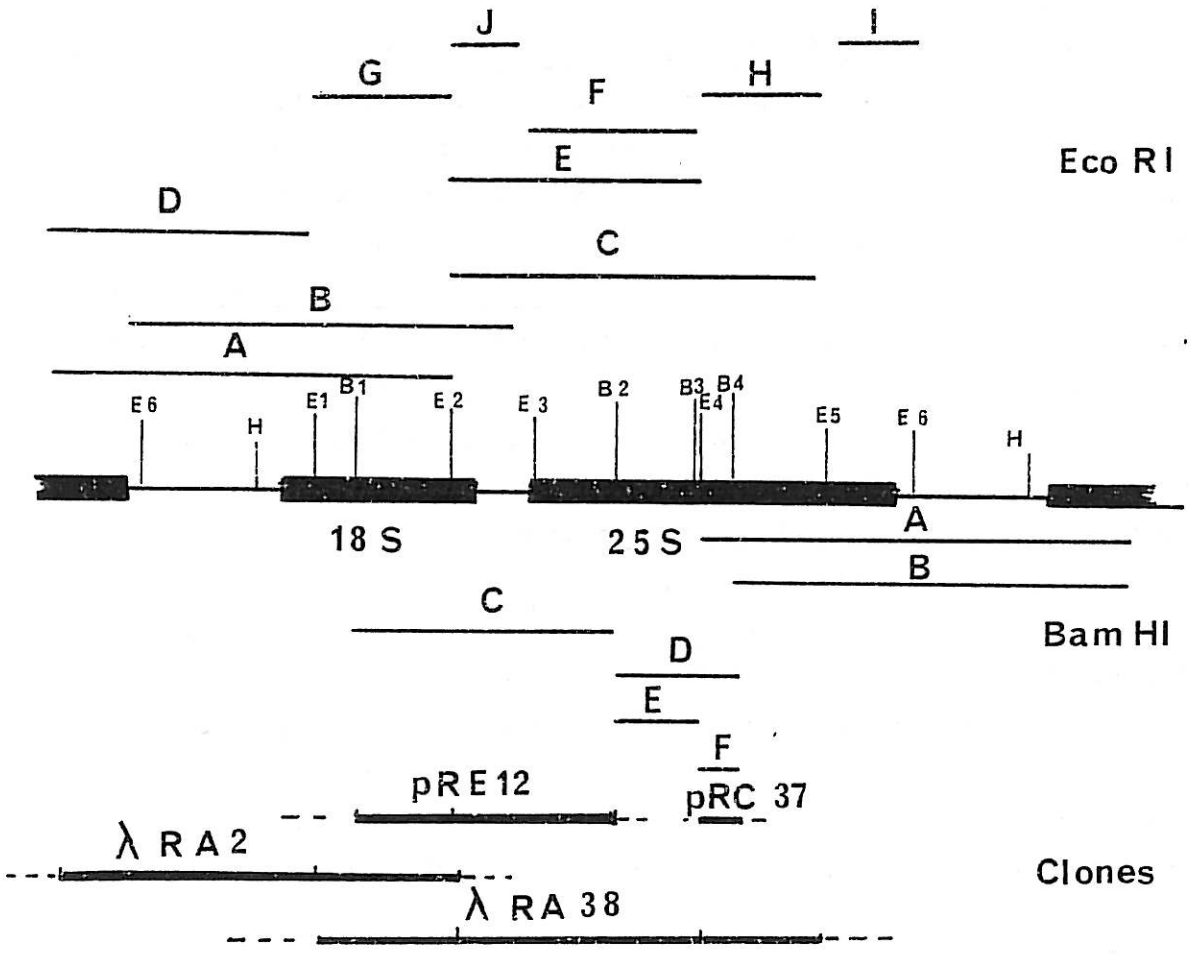
Durieu, M.C., 1867. Catalogue des graines récoltées en 1867. Jardin-des-Plantes de la ville de Bordeaux. (IDC microfiche).

# CHARACTERISATION OF NUCLEAR GENES CODING FOR RIBOSOMAL RNA IN RADISH

Michel DELSENY

Relatively few generic markers are available in Cruciferae when compared to cereals or leguminosae. The molecular biology methods now allow one to characterize and eventually isolate genes or DNA fragments of interest. Such possibilities are illustrated by the use chloroplast and mitochondrial DNA restriction patterns in studies on male sterility and in protoplast fusions (1, 2). We have initiated a study of the nuclear genes coding for ribosomal RNA in Radish (3, 4). The main results can be summarized as follows.

DNA coding for ribosomal RNA is made of $\sim$ 6500 copies of a 8000 bp unit. All the units are not all identical as indicated by the complexity of the restriction patterns. In order to understand how these units are organized we have cloned DNA fragments in plasmids or bacteriophages vectors. Using these recombinant DNA molecules as probes it has been possible to derive a restriction map which is shown in figure 1. On this figure we have indicated the major restriction frag-



ments and the recombinant clones which have been used. These clones are available on request. Analysis of a series of recombinant bacteriophages clearly demonstrated that the differences between the repeating units result from the absence or presence of a Eco RI restriction site at a specific position. Whether all the 6500 units are functional or not remains to be determined. However studies on DNA methylation suggests that certain types of units are more heavily methylated than others (5). Therefore it is likely that the different units are not equivalent to each other.

The heterogeneity of the ribosomal genes does not seem to be a characteristic of Cruciferae since two other species (Matthiola incana and Brassica pekinensis) have been reported to have homogeneous ribosomal genes (6). We have analysed two other species: Rapeseed (Brassica napus. cv Brutor) and Honesty (Lunaria annua) and found the first one heterogeneous and the second homogeneous. Interestingly radish, rapeseed and honesty exhibit different Eco RI patterns but rapeseed and radish, which are close relatives, have a more similar pattern. Eco RI fragments E, F, G, H, are common to both species whereas only G is shared with honesty.

Therefore restriction patterns of rDNA can be used as molecular markers in genetic studies with Cruciferae in the same way chloroplast and mitochondrial DNA restriction patterns have been used. Since rDNA units are repeated and clustered, in situ hybridization should allow to directly visualize this genes and help in cytogenetic studies. We are now analysing other cruciferae crops as well as wild relatives of radish in order to better understand how the heterogeneity is created and to which extent such markers can be used.

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# INHERITANCE OF HYPOCOTYL LENGTH AND COLOUR IN RADISH

N. Dayal and C. Prasad

Although the study of seedling characters bears considerable significance in systematics and applied genetics, there have been only a few reports on the subject (2,3). Radish is quite suitable for the genetical studies of seedling characters, which are sharp and distinct in this species. Seedling counts also give better segregation ratio than adult plant counts due to lesser effect of the environment on such characters. Here we report the study of inheritance of two seedling characters, length and colour of the hypocotyl, in radish.

## Hypocotyl length

For this mean hypocotyl length (mhl) has been scored in three varietal populations of radish, Raphanus sativus L., 'Japanese White' (JW), 'Kalamikati Red' (KR) and 'Jaunpur Giant' (JG) and their $F_1$ and $F_2$ hybrids, JW X JG and KR X JG. 100 - 300 seedlings were raised from each of them in controlled conditions. Measurements of hypocotyls were taken on the emergence of the first true leaf. A comparative study of the mhl showed a significant intervarietal difference in this parameter ($P > 0.001$) and that long hypocotyl is dominant to short. In $F_2$'s the mhl was either less than or equal to that of $F_1$'s. However, the distribution pattern of hypocotyl length varied noticeably in the parents and the $F_1$'s. The widest distributions occurred in the $F_2$'s, where both shorter and taller seedlings were found than in the parental and $F_1$ families (Table 1). The $F_2$ distributions indicate that polygenic segregation has occurred and that the apparent dominance of long hypocotyl may be attributed to interallelic and non-allelic interactions.

Table 1. Distribution of hypocotyl lengths in populations and hybrids.

| Materials | Classes of hypocotyl lengths (mm) | | | | | | | | | | | | | Mean ± SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | |
| KR | | | 1 | 3 | 5 | 13 | 15 | 30 | 16 | 10 | 5 | | | 48.6±0.90 |
| JW | | | 3 | 13 | 19 | 23 | 20 | 10 | 5 | 4 | 2 | 1 | | 41.7±0.86 |
| JG | 2 | 22 | 44 | 20 | 10 | 2 | | | | | | | | 26.0±0.51 |
| JWxJG($F_1$) | | 3 | 5 | 8 | 11 | 18 | 30 | 15 | 6 | 3 | 1 | | | 42.1±0.92 |
| JWxJG($F_2$) | 6 | 15 | 19 | 28 | 34 | 40 | 65 | 45 | 24 | 17 | 8 | 5 | 2 | 42.3±0.69 |
| KRxJG($F_1$) | | 2 | 4 | 5 | 9 | 12 | 18 | 30 | 15 | 3 | 2 | | | 45.3±0.94 |
| KRxJG($F_2$) | 3 | 9 | 14 | 24 | 54 | 84 | 48 | 25 | 17 | 14 | 7 | 3 | 2 | 41.0±0.60 |

## Hypocotyl colour

'Rainy Season Red' (RR) and KR have characteristic violet hypocotyl, found to breed true, due to the presence of anthocyanin pigments. For studying the inheritance of hypocotyl colour, plants of these varieties were crossed with JG having white hypocotyl. Colour was noted in ten days old

seedlings. The $F_1$ seedlings were found to have light violet (LV) hypocoyls, indicating incomplete dominance of coloured hypocotyls over the white ones. The $F_2$ seedlings segregated into three classes of hypocotyl colour : violet (V) as in RR/KR, light violet (LV) as in $F_1$ and white (W) as in JG (Table 2). A $\underline{X}^2$ test revealed the data to be very close to the expected ratio of 3 V : 1 W or more accurately, 1V :2 LV : 1 W, which is characteristic for monogenic segregation.

Table 2. Segregation of hypocotyl colour

| Crosses V X W | $F_1$ | $F_2$ | | | Total | P | Segregation ratio |
|---|---|---|---|---|---|---|---|
| | | V | LV | W | | | |
| RR X JG | LV | 78 | 145 | 77 | 300 | 0.80 | 1:2:1 |
| KR X JG | LV | 80 | 147 | 73 | 300 | 0.80 | 1:2:1 |

Differing conclusions on the inheritance of hypocotyl colour in radish have been reached from earlier studies. Sheppard (1) considered hypocotyl colour to be governed by two allelic pairs showing epistasis. The $F_1$ hybrids of red and white hypocotyl parents have purple hypocotyls which segregate into 9 red : 3 purple : 4 white. Tatebe (4), on the other hand, found 15 red : 1 white segregation in $F_2$. Here it is reported that hypocotyl colour, like the root colour, is governed by a single gene with two alleles, C and c. CC and Cc produce V and LV hypocotyls respectively while cc - W hypocotyls.

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# INHERITANCE OF COLOUR AND SHAPE OF ROOT IN RADISH

Narsinha Dayal

Radish, Raphanus sativus L., is well-suited for genetic studies for it grows rapidly and is economical on space. It is surprising, therefore, that despite this the mode of inheritance of only a few characters is known (1). The inheritance of root colour and shape has been studied on several past occasions with conflicting results and conclusions (2). Here the results of a dihybrid cross involving these two characters are reported.

In order to understand the inheritance of colour and shape of root a cross was made between 'Scarlet Globe' (SG), a cultivar of European origin, and the Indian cultivar 'Newar' (N). The former has coloured and round roots and the latter has white and long roots. A cross of SG and N gave coloured and oval roots in the $F_1$. The $F_2$'s, numbering 344 in all, segregated into six phenotypes as follows : 56 coloured and round, 136 coloured and oval, 70 coloured and long, 24 white and long, 42 white and oval 16 white and round. A $X^2$ test revealed the data to be very close to the ratio 3:6:3:1:2:1 ($P > 0.70$). Our results, thus, clearly, show that the colour and shape of root in these cultivars of radish are governed by two independent genes, one with complete and another with partial dominance. Gene C and its recessive allele c control the colour and gene L and l - the shape of the root. However, L is partially dominant to l and in heterozygous condition gives oval roots. The modification of the dihybrid ratio into 3:6:3:1:2:1 in lieu of 9:3:3:1 may be attributed to this. The phenotypes and the genotypes of the parents and the hybrids are shown below:-

Parents: CCll (Coloured, Round) × ccLL (White, Long)

Gametes: Cl, cL

$F_1$: CcLl (Coloured, Long)

$F_2$

| Male gametes → / Female gametes ↓ | CL | Cl | cL | cl |
|---|---|---|---|---|
| CL | CCLL Coloured Long | CCLl Coloured Long | CcLL Coloured Long | CcLl Coloured Long |
| Cl | CCLl Coloured Oval | CCll Coloured Round | CcLl Coloured Oval | CcLl Coloured Round |
| cL | CcLL Coloured Long | CcLl Coloured Oval | ccLL White Long | ccLl White Oval |
| cl | CcLl Coloured Oval | Ccll Coloured Round | ccLl White Oval | ccll White Round |

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# THE INHERITANCE OF AN APETALOUS CHARACTER IN CANOLA (BRASSICA NAPUS)

Gregory Buzza

After studying the crop physiology of rapeseed in England Neville Mendham returned to Australia and discussed the possibility of breeding a rapeseed without petals. His work had shown that at full flower the petals of a rapeseed crop reflects a lot of light and prevent it being used by the leaves and developing pods. He argued that a crop without petals should make more efficient use of light and should yield more. Naturally, at the time I did not think it likely that we could breed an apetalous variety. However it is surprising how often the planting of an idea increases the chance of its fulfillment.

In 1981 I found a canola plant which had a reduced number of petals. After inbreeding for several generations a line was produced which had virtually no petals. The flowers of these plants are normal except for the abscence of petals. The anthers are slightly shorter than many varieties but still well in the range found in canola varieties. This apetalous line (PL) occasionally has flowers with one or two petals.

To determine the inheritance of the apetalous character I crossed it (reciprocally) to a normal line RU6 and studied the F1 and F2 generations. I also made backcrosses of the F1 to both parents. The percentage of petals of an individual plant was determined by counting the number of petals on the first twenty-five flowers to open. There were no differences between reciprocal crosses so I assume the two parental lines had the same cytoplasm. The results of reciprocal crosses have been combined and are shown in the Table.

In the F1 most plants were normal but some plants had an occasional petal missing. The F2 had a wide range of variation with a few plants being similar to the apetalous parent and more than half being nearly completely petalled. About one quarter had an intermediate number of petals ie. 10 - 80%. The backcross to the apetalous parent also had a wide range of variation with about one quarter being apetalous (less than 10%), one half being intermediate (10 - 80%) and one quarter being mostly petalled (80 - 100%).

My interpretation of the data is that the character is controlled by two loci, ie. a normal plant would have four alleles for petals (PPPP) and the apetalous plant would have four alleles for abscence of petals (pppp). The F1 plants (PpPp) have 80 - 100% petals so alleles for petals have incomplete dominance. In the F2, segregation would be 1:4:6:4:1 for 0, 1, 2, 3 or 4 alleles for petals. This agrees with the data if we assume that all plants with two or more alleles for petals are greater than 80% petalled. In the backcross of the F1 to the apetalous parent the segregation would be 1:2:1 for 0, 1 and 2 alleles for petals. Again the data support the two loci hypothesis.

Although the original idea behind this work came from crop physiology, an apetalous variety may also reduce the incidence of sclerotinia as dehisced petals are a medium which sclerotinia ascospores use to penetrate the plant.

| LINE | NUMBER OF PLANTS | | | | | | | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PL | 6 | 4 | | | | | | | | | | | 10 |
| RU 6 | | | | | | | | | | | | 10 | 10 |
| F1 | | | | | | | | | | 1 | 5 | 14 | 20 |
| F2 | 2 | 2 | 6 | 2 | 3 | 2 | 1 | 5 | 6 | 9 | 33 | 25 | 96 |
| F1xRU6 | | | | | | | | | | 2 | 7 | 55 | 64 |
| F1xPL | 8 | 10 | 6 | 8 | 6 | 4 | 3 | 1 | 5 | 5 | 6 | 2 | 64 |

0 1-9 10-19 20-29 30-39 40-49 50-59 60-69 70-79 80-89 90-99 100

PERCENT PETALS

continued from page 13

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# CRUCIFERS AS MODEL FOR STUDYING THE CYTOGENETICS OF HETEROCHROMATIN

Narsinha Dayal

Once thought to constitute a stable, irreversible and genetically inert material, heterochromatin has now been found to carry out well defined functions in such diverse processes as chromosome pairing and segregation, position effect variegation, chromosomal rearrangements and gene regulation (1). Crucifers may be suitably modelled for studying the cytogenetics of constitutive heterochromatin for they contain dark staining heteropycnotic bodies, called chromocentres, in their interphase nuclei. Chromocentres represent pericentric constitutive heterochromatin and their mean frequency and distribution is quite constant for a population. The mean chromocentre frequency is either the same as or nearly the number of chromosomes. Localized heterochromatic chromocentres have also been considered as an adaptive character (2).

Over the past decade we have made extensive cytogenetical studies on chromocentres in radish. Our studies have shown that the mean chromocentre frequency is a function of the genotype and in some way related to the homo- and heterozygosity of the material. Highly homozygous inbred lines of radish have a higher mean and wider frequency distribution than their original varietal population and the interlineal $F_1$ hybrids (3). Significant interpopulational variation in this heterochromatin phenotype has been shown in the Indian radishes. The number and the distribution of chromocentres are specific for a particular population. There are populations with both low and high chromocentre frequency which may, respectively, be regarded as less or more heterochromatinized. In radish, chromocentre frequency has also been shown to be controlled polygenically (4), which is, interestingly, similar to that of another nuclear character, chiasma frequency (5). Chromocentres are not without influence. An increase in the amount of constitutive heterochromatin, as inferred from chromocentre counts, adversely affects the mean chiasma frequency and, hence, the genetic recombination in radish (6).

At present we are studying the effects of Y rays and colchicine on the chromocentre frequency in *Raphanus sativus* and several species of *Brassica*. Our preliminary studies show that Y rays and colchicine have a reducing effect on chromocentre frequency.

Cytogenetical studies on chromocentres in crucifers have immense potentialities in understanding the role of constitutive heterochromatin in various genetic functions. One would wish to know more about them, particularly in those cruciferous crops of which rare and valuable genetic collections are known and maintained.

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continued on page 12

# PRELIMINARY RESULTS FOR FODDER RAPE HYBRIDS

M. LEFORT-BUSON

In 1982, F1 hybrid seeds have been produced for a (5x5) complete diallel design including early maturing fodder rape selfed-lines : Cundy's, Arvor, 61.12B, R74 D, Pampa. It was a 4 replication-block design with 5-row plots, 3 m-long each ; the only 3 inner rows are taken into account in this study. The sowing and harvesting dates were respectively the 25th of August ant the 14th of November ; the sowing rate was about 8 kg/ha.

The comparaison of the F1 hybrids to their parental lines and to the standard (Furax) is briefly summarized here for the following characters : fresh yield, dry matter yield, and contents of dry matter, crude fibre, total carbohydrates, protein nitrogen, total nitrogen and ash.

RESULTS

Table 1 : AVERAGE VALUES OF F1 HYBRIDS AND PARENTAL LINES FOR DIFFERENT CHARACTERS

| CHARACTER | Mean value of Hybrids : Mh | Mean value of parental lines: Ml | Mh - Ml | Standard : Furax |
|---|---|---|---|---|
| fresh Yield, in T/ha | 52.17 ± 1.93 | 44.87 ± 3.79 | 7.30* ± 4.25 | 43.24 |
| dry matter yield, in T/ha | 4.45 ± 0.34 | 3.97 ± 0.15 | 0.48* ± 0.37 | 4.03 |
| % dry matter | 8.69 ± 1.52 | 8.91 ± 0.57 | - 0.22 ± 1.62 | 9.33 |
| % crude fibre | 17.25 ± 0.72 | 16.92 ± 0.39 | 0.33 ± 0.83 | 22.10 |
| % total carbohydrates | 14.85 ± 1.00 | 14.10 ± 1.28 | 0.75 ± 1.62 | 13.24 |
| % protein nitrogen | 11.82 ± 0.21 | 11.94 ± 0.55 | - 0.12 ± 0.59 | 11.31 |
| % total nitrogen | 23.25 ± 0.35 | 23.22 ± 0.75 | 0.03 ± 0.83 | 21.97 |
| % ash | 16.84 ± 0.17 | 16.99 ± 0.39 | - 0.15 ± 0.43 | 17.25 |

* Significant at 5 %

The average superiority of F1 hybrids over their parental lines is not very important for the dry matter yield (12.1 %) ; it seems to result first from a greater development of stem and leaves (16.3 % for the fresh yield) with a little decrease in dry matter content (table 1) : the decrease is not important but there is a significant negative correlation (-0.69) between fresh yield and dry matter content in the pool of F1 hybrids.

There is no heterosis for any of the nutritional characters studied (table 1)

For dry matter yield, the best F1 hybrid "Pampa x R74 D" (4.80 T/ha) is overyielding the best line "Arvor" (4.58 T/ha) only for 5 % (table 2). The parental lines "Arvor" and "Pampa" overyield the standard "Furax" (4.03 T/ha) and give the best F1 hybrids.

Reciprocal effects are not significant for both fresh and dry matter yields.

Table 2 : DRY MATTER YIELD IN T/ha

| | CUNDY'S GIANT | ARVOR | 61.12 B | R 74.D | PAMPA |
|---|---|---|---|---|---|
| CUNDY'S GIANT | 3.53 | 4.79 | 3.56 | 3.86 | 4.03 |
| ARVOR | 4.44 | 4.58 | 4.66 | 4.26 | 4.79 |
| 61.12 B | 4.14 | 4.65 | 3.69 | 4.41 | 4.68 |
| R 74.D | 4.32 | 4.62 | 4.47 | 4.24 | 4.57 |
| PAMPA | 4.50 | 4.73 | 4.77 | | 3.83 |

## DISCUSSION

Considering the results of this trial, for fodder rape hybrid vigor does not seem as important as it is for oilseed rape. However, these preliminary results have to be confirmed before discussing the economic interest of fodder rape F1 hybrids.

# FODDER CABBAGE AGRONOMY AND BREEDING

J.E. Bradshaw

Although cabbages have been grown for many years in the United Kingdom for feeding sheep and cattle, fodder cabbage has remained a minor crop compared with the two major fodder brassica crops, swede and kale. The old cattle cabbage cultivars, such as Late Purple Flat-Poll and Robinson's Champion, were traditionally sown in a seedbed, either in the autumn or early spring, transplanted in late spring at up to 90 cm by 90 cm spacing and cut and carted to housed livestock in early winter. It was thus a labour intensive crop.

However, in recent years there has been renewed interest in feeding cabbages to livestock, particularly as an alternative to soft turnips and swedes for finishing store lambs in late autumn and early winter, and a less labour intensive management system developed. The seed is drilled in situ with a precision drill, at a wide enough spacing to be left unsingled, and the crop divided into breaks with electrified net fence for grazing in situ. Because of their high yields in horticultural trials, interest has centred on cabbages bred for human consumption, and not specifically for ruminants.

Therefore in 1978, at the Scottish Crop Research Institute, it was decided to start a fodder cabbage breeding programme. The long term objective is an open pollinated cultivar (for both easy and cheap seed production) with a high dry matter yield, a high proportion of head (sheep have a preference for the head and outer leaves are often wasted), a high dry matter content (both to improve dry matter intake by livestock and for winter hardiness), low levels of S-methyl cysteine sulphoxide (SMCO, the haemolytic factor) and thiocyanate ion (a goitrogen), improved clubroot resistance and improved winter hardiness. Rapid early establishment and good standing ability once headed are also desirable. However, as resources are limited because fodder cabbage is a minor crop, the initial aim is to improve head dry matter yield and content by population improvement, from an initial population consisting of suitable autumn and winter culinary cabbages (eg round autumn, flat drumhead, winter white and January King types).

Having started this programme it was realised that there were three important agronomic questions to answer in relation to the assessment of breeding material. How late can the crop be sown without adversely affecting yield and heading ability? What is the optimal seed spacing in a crop to be left unsingled? Is a heavy top dressing of nitrogen fertiliser necessary? Two large experiments have been carried out and the results will be published in due course. The author's conclusions are that the crop should be sown before the end of May and preferably at the beginning of the month; 25 cm seed spacing in rows 50 cm apart is a satisfactory compromise between a large proportion of head, the possibility of gaps resulting from poor establishment, and total dry matter yield; and that applying 150, 75 and 75 kg/ha of N, $P_2O_5$ and $K_2O$ respectively in the seedbed is adequate as a further 150 kg/ha of N as a top dressing resulted in only a modest increase in yield.

The author would welcome information from readers about any interest in fodder cabbage in their countries, and in particular would like to hear from any other fodder cabbage breeders.

# THE INHERITANCE OF BOLT RESISTANCE IN AN INTERSPECIFIC CROSS SIBERIAN KALE (BRASSICA NAPUS) X CHINESE CABBAGE (B. CAMPESTRIS L. SSP PEKINENSIS) AND AN INTRASPECIFIC CROSS CHINESE CABBAGE X TURNIP (B. CAMPESTRIS L. SSP. RAPIFERA).

C. E. Mero and S. Honma

The inheritance of bolting in Chinese cabbage (Brassica campestris L. ssp. pekinensis) was investigaged by hybridizing Chinese cabbage with Siberian kale (B. napus), Chikale (B. campestris L. ssp. pekinensis x B. napus), and turnip (B. campestris L. ssp. rapifera). The inheritance model was developed from segregation ratios observed in segregating populations from these crosses after various durations of vernalization at 5°C and 16 hour daylength. Percent of bolters was determined by the sum of visible bolters and longitudinally cut plants with pointed apices, observed when the bolt-resistant parent either visibly bolted or reached a marketable size.

Differences in bolting between Chinese cabbage, Chikale, Siberian kale, and an $F_2$ population (Siberian kale x Chee Hoo) under natural field vernalization suggested (1) differences in bolting habit observed after artificial vernalization at a constant temperature (5°C) were similar to natural vernalization with fluctuating temperatures and (2) bolt resistance from Siberian kale was transferred to Chikale and the $F_2$ population.

Segregating for bolting response in the progeny from the crosses Siberian kale x Chee Hoo, Siberian kale x Nozaki Early, and Mandarin x Siberian kale suggested that bolting response was conditioned by a few major additive genes and that percent of bolters was dependent upon the Chinese Cabbage cultivar used. Differences in chromosome number between Chinese cabbage (n=10) and Siberian kale (n=19) produced varying degrees of fertility in the progeny. The observed segregations appear to have resulted from variable ploidy levels, random assortment and probable crossing over of chromosomes. Segregation in $F_3$ families from these crosses suggested one or more major genes.

Based on these results and the observed segregations for bolting response in the crosses Chinese cabbage x Chikale and Chinese cabbage x turnip, it appears that 4 major genes with modifiers conditioned bolting response. Genotypes for Chinese cabbage, Chikale, Siberian kale, and turnip were proposed. Cytoplasmic effects on the response to vernalization were also noted.

# NEW INTERSPECIFIC HYBRIDS BETWEEN B. CAMPESTRIS AND B. OLERACEA BY OVARY CULTURE IN VITRO

Nobumichi INOMATA

In recent studies, many interspecific hybrids between B. campestris and B. oleracea had been obtained in ovary culture in vitro ( Inomata 1977, 1978a, 1978b, 1979). In the previous papers, the female plants used in the experiment were B. campestris ssp. chinensis, japonica, oleifera, pekinensis and rapa, and the male plants were B. oleracea var. alboglabra, acephala, bullata, capitata, italica, gemmifera and gongylodes. Between these subspecies and varieties, many hybrids were obtained. The cytogenetical studies on the $F_1$ hybrids were reported (Inomata 1980).

The present paper deals with the production of interspecific hybrids between B. campestris ssp. dichotoma and ssp. trilocularis, and B. oleracea var. alboglabra and var. italica.

The materials used in the present experiment were B. campestris ssp. dichotoma cv. DS-2 and ssp. trilocularis cv. R-500 and cv. IB-1, and B. oleracea var. alboglabra cv. Senyo-shoyo and var. italica cv. De Cico. The culture medium and method were the same as a previous paper (Inomata 1978b).

The results are shown in Table 1. The frequency of the production of interspecific hybrids was very high like the previous papers (Inomata 1977, 1978a, 1978b, 1979). Morphological characteristics of leaf was intermediate between B. campestris and B. oleracea used in the experiment. Pollen fertility was examined in 15 $F_1$ hybrids. Twelve out of them were below 11%, with the mean of 3.7%. One was 44.4%. Two out of them were 92.1% and 98.8%, which might be due to spontaneous chromosome doubling.

The first meiotic division was examined in eight $F_1$ hybrids having 19 chromosomes. The results are shown in Table 2. Mode of the chromosome configuration at the frist meiotic division was $9_{II}+1_{I}$ (38.7%). Frequency of tri-, tetra- and pentavalents was 30.8%, 15.0% and 1.9%, respectively. Pentavalent was the most complex chromosome configuration. Mean pollen fertility, and the frequency of tetra- and pentavalents were slightly higher in the present experiment than that in a previous paper (Inomata 1980).

The hybrids obtained in the present experiment were easily infected by the virus, and some out of them died in flower season. The $F_1$ hybrids were crossed with B. napus, most plants obtained had 38 chromosomes (Inomata 1982). These $F_1$ hybrids may be also useful for the breeding of B. napus.

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Table 1. Production of interspecific hybrids between *B. campestris* and *B. oleracea* by ovary culture

| Cultivar of *B. campestris* used as a female parent | Cultivar of *B. oleracea* used as a male parent | No. of capsules examined (A) | No. of plants obtained (B) | B/A x 100 |
|---|---|---|---|---|
| R-500 | Senyo-shoyo | 30 | 11 | 36.67 |
| DS-2 | " | 30 | 35 | 116.67 |
| IB-1 | " | 37 | 7 | 18.92 |
| R-500 | De Cico | 30 | 10 | 33.33 |
| DS-2 | " | 30 | 7 | 23.33 |
| IB-1 | " | 33 | 10 | 30.03 |
| Total or mean | | 190 | 80 | 42.11 |

Table 2. Chromosome configuration in the first meiotic division of the $F_1$ hybrids with 19 chromosomes

| Plant No.[1] | No. of PMCs observed | $1_{III}+8_{II}$ | $1_{III}+7_{II}+2_{I}$ | $9_{II}+1_{I}$ | $8_{II}+3_{I}$ | Other types |
|---|---|---|---|---|---|---|
| | (%) | | | | | |
| 1 | 35 | 4 (11.4) | 0 | 18 (51.4) | 1 (2.9) | 12 (34.3) |
| 2 | 25 | 5 (20.0) | 0 | 12 (48.0) | 0 | 8 (32.0) |
| 3 | 36 | 0 | 5 (13.8) | 11 (30.6) | 9 (25.0) | 11 (30.6) |
| 4 | 35 | 0 | 1 (2.9) | 16 (45.7) | 2 (5.7) | 16 (45.7) |
| 5 | 30 | 2 (6.6) | 3 (10.0) | 11 (36.7) | 6 (20.0) | 8 (26.7) |
| 6 | 35 | 3 (8.5) | 5 (14.3) | 7 (20.0) | 10 (28.6) | 10 (28.6) |
| 7 | 35 | 7 (20.0) | 3 (8.6) | 12 (34.3) | 4 (11.4) | 9 (25.7) |
| 8 | 35 | 2 (5.7) | 0 | 16 (45.7) | 10 (28.6) | 7 (20.0) |
| Total or mean | 266 | 23 (8.6) | 17 (6.4) | 103 (38.7) | 42 (15.8) | 81 (30.5) |

1: 1 and 2 were obtained in the cross of cv. R-500 x cv. Senyo-shoyo. From 3 to 7 were obtained in the cross of cv. DS-2 x cv. Senyo-shoyo. 8 was obtained in the cross of cv. IB-1 x cv. De Cico.

Acknowledgement

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# ON THE WAY TO YELLOW SEEDED *BRASSICA NAPUS*
## I. CROSSINGS BETWEEN *BRASSICA OLERACEA* AND *B. CARINATA*

B. Barcikowska, M. Balicka, E. Zwierzykowska

Aiming at obtaining yellow seeded *Brassica oleracea* crossing components to get artificial *B. napus* with yellow seed coat, the programme of interspecific hybridization in the genus *Brassica* came into consideration and started in the year 1981. The female parent was *B. oleracea* var. *acephala* 'Normal', while the paternal plant was represented by the yellow seeded form of *B. carinata* (2n = 34), consisting, as it is well known, of the genomes *B. oleracea* (2n = 18) and *B. nigra* (2n = 16).

During the year 1981 there have been done 387 crossings, from which 173 seeds developed and 115 of them have been sown in the vegetation period 1982/83. These 115 seeds gave rise to 87 plants, mostly phenotypically intermediate, in comparison with the parental phenotypes. The pollen viability of these plants amounted on average about 20.0% and somatic chromosome numbers varied from 2n = 18 to 2n = 27.

The $F_1$ hybrids have been backcrossed with *B. oleracea* to get, after self pollination during two next generations, yellow seeded form *B. oleracea*, foreseen as parent in crossings with vigorous yellow seeded *B. campestris* forms, aiming at obtaining artificial yellow seeded *B. napus*.

# BREEDING CHLORO-TRIAZINE HERBICIDE RESISTANCE INTO RUTABAGA (*BRASSICA NAPUS* L.) AND CHINESE CABBAGE (*BRASSICA CAMPESTRIS* L.)

V. Souza Machado, A. Ali and J. Shupe.

The standard method to solve weed problems has been to search for new herbicides or try new formulations of old herbicides to deal with particular crop/weed situations. An alternative approach would be to alter the genotype of crops, so as to render them tolerant or resistant to existing proven registered herbicides. This has been attempted, to control broadleaf weeds in a broadleaf crop like rutabaga, using plant breeding techniques to transfer chloro-triazine herbicide resistance into the vegetable crop. A backcross method was used to transfer atrazine resistance intra-specifically from an annual rapeseed genotype (Beversdorf et al. 1980) to a biennial rutabaga genotype, using the cv. 'Laurentian' as the male pollen donor, since atrazine resistance was cytoplasmically inherited through the female parent (Souza Machado and Bandeen 1982). After four cycles of backcrossing, a suitable biennial genotype was produced with shoot/root ratios similar to the male pollen donor. Backcross progenies were monitored for atrazine resistance using *in vitro* techniques involving chlorophyll fluorescence (Ali and Souza Machado 1981). This project has since completed eight cycles of back-crossing. Programs are also in progress using the cv. 'York' and 'Fortune' as the male pollen donor to produce chloro-triazine resistant genotypes for subsequent field selection.

Field trials with heterozygous genotypes of A/Laurentian$^{6}$ rutabaga with triazine resistant cytoplasm were sown on May 27, 1982 in a Fox fine sandy loam at the Cambridge Research Station, of the University of Guelph, Ontario. A randomised block design was used, with 4 replications. Plots consisted of 3 rows 8 m long at a row spacing of 1 m. All treatments were applied with a 2 nozzle $CO_2$ powered sprayer fitted with HSS8004 orifice tips. Trifluralin at 2.0 kg/ha was applied ppi over the entire area. Treatments applied in Kg/ha were cyanazine 2.25 pre, cyanazine 2.25 pre and 1.75 post, cyanazine 2.25 pre and fluazifop/agral 0.25 post, cyanazine 2.25 pre and BAS 9052/assist 0.10 post, metribuzin 0.30 pre, prometryne 1.75 pre, blazine 3.25 pre, blazine 2.75 post and an unweeded check. The dominant weed in the check plots was shepherd's purse. All the herbicide treatments provided very good weed control, but crop injury was noted with metribuzin and prometryne. These trial results confirmed previous physiological studies with isolated chloroplasts (Souza Machado et al. 1978) that these genotypes are resistant to chloro-triazine herbicides and tolerant to methylthio-triazines.

Triazine resistance was also transferred into Chinese cabbage genotypes through intraspecific backcrosses, using the weed bird's rape (*Brassica campestris*) as the female parent (Malthais and Bouchard 1978) and the cv. 'Sunkiss' and 'Wondercross' as the pollen donors. Six cycles of backcrossing have been completed. Chlorophyll fluorescence analysis have confirmed the presence of chloro-triazine resistant trait in the backcross progenies.

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# CHROMOSOME COUNTS AND LEAF CHLOROPHYLL FLUORESCENCE OF ANTHER CULTURE DERIVED PLANTLETS OF ATRAZINE RESISTANT RUTABAGA GENOTYPES

V.Souza Machado, J. Shupe and W. Keller

Anther culture plantlets of atrazine resistant rutabaga genotypes produced after five cycles of backcrossing designated A/Laurention[5], were appraised for chromosome number and leaf chlorophyll fluorescence to confirm for chloro-triazine resistance. The number of plantlets evaluated were 66. They were transferred from B5 agar medium to jiffy blocks in a mist bed and later potted on with a standard greenhouse soil mix. Root tips were collected from the jiffy block stage and later 8 mm leaf discs from older plants established in pots. Chromosome counts indicated forty six plants each with a mean count of 38 and twenty plants each with a mean count of 19. Leaf chlorophyll fluorescence analysis using a Plant Productivity fluoremeter model SF-10 with leaf discs floated in $1 \times 10^{-4}$M atrazine and nil atrazine as a control in phosphate buffer solutions, indicated a mean fluorescence increase of only 10% over control following 4 hr. uptake, in all sixty six plants. This confirmed the presence of the atrazine resistant trait in all sixty six plants, including those with a haploid (19) chromosome count. The results serve to support the hypothesis that pollen from atrazine resistant genotypes, do carry the resistant trait, but as is evident in previous inheritance studies (Souza Machado and Bandeen 1982), is lost when 'double fertilization' occurs with an atrazine susceptible plant. Therefore the atrazine resistant trait is not transferred to the progeny, when the atrazine susceptible plant is the female parent.

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# CLUBROOT RESISTANT LOW GLUCOSINOLATE CABBAGES

C. Chong, M.S. Chiang and R. Crête

Clubroot disease of crucifers caused by Plasmodiophora brassicae Wor. is one of the most destructive diseases in Europe and in northern North America. In Canada, the most prevalent strains are races 2 and 6, coded as populations ECD 16/02/31 and 16/02/30, respectively, by Dr. Buczacki and other clubroot workers in Europe. Presently all commercial cabbages are susceptible to race 2, the most virulent strain in the Province of Quebec.

All cruciferous crops contain glucosinolates which form a number of breakdown products, the most prominent ones being isothiocyanates (mustard oils), thiocyanates, goitrin, and nitriles, liberated with tissue rupture or cell disorganization. These products contribute significantly to the flavour and pungency, and also to the toxic properties of cruciferous plants.

Recently, the Agriculture Canada Saint-Jean Research Station, Quebec and the Department of Plant Science of Macdonald College of McGill University worked jointly to transfer through interspecific hybridization of dominant gene responsible for resistance to P. brassicae race to cabbage (Brassica oleracea ssp. capitata, somatic chromosome number 2n = 18) from rutabaga (B. napus, 2n = 38) (Chiang et al. 1977). Cabbage lines resistant to both races 2 and 6 have already been developed from this project. To further improve the quality of these lines, the Saint-Jean Research Station again in cooperation with the same Department at Macdonald College are now selecting clubroot resistant cabbages that are also low in glucosinolates (Chong et al. 1981).

In recent studies, 59 commercial cabbage cultivars (all susceptible to clubroot) and 86 plants from selected breeding lines (all resistant to clubroot race 2) were analysed (Ju et al. 1980) for their composition of glucosinolates determined by quantifying their hydrolytic products, goitrin, volatile isothiocyanates, and thiocyanate. In cabbages indole glucosinolates form the bulk of the glucosinolate fraction. These yield thiocyanate as the major breakdown product.

In the commercial cultivars, the thiocyanate ion content (µg/g dry weight) ranged from 170 to 991 with a mean of 340 $\pm$ 162 (standard deviation). In comparison, the thiocyanate ion content in the breeding lines ranged from 34 to 1059 with a mean of 199 $\pm$ 118. While all commercial cultivars had thiocyanate ion content above 100 µg/g dry weight, with 24 in the range of 200-299 µg/g, seven of the breeding lines had thiocyanate ion content less than 100 µg/g and 43 had concentrations in the range 100-199 µg/g.

Among the clubroot resistant breeding lines, several were found to be low in goitrin or isothiocyanates. Four lines completely free of goitrin were also found as were cabbage with total glucosinolate content as low as 154 µg/g. These breeding lines will provide the germplasm for breeding new low glucosinolate and clubroot resistant $F_1$ cultivars.

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# BREEDING FOR RESISTANCE TO XANTHOMONAS CAMPESTRIS IN CAULIFLOWER AND BROCCOLI

Shigemi Honma

Hybridization between cauliflower and cabbage (cv Early Fuji) was made a number of years ago to transfer Black Rot (Xanthomonas campestris) to cauliflower. From the segregating population we were able to select cauliflower and broccoli types that showed high degree of resistance to the pathogen. To improve the quality of the broccoli heads the better lines were backcrossed to Spartan Early. These lines are being tested extensively for quality and resistance. For the cauliflower, interline crosses and backcrosses to commercial cultivars have been made. Selections for resistance and acceptable curd quality is being made. Some of the materials are nearing its final stages for release.

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# ROOT MAGGOT INJURY TO B. NAPUS RAPIFERA CULTIVARS

KENNETH G. PROUDFOOT & RAY F. MORRIS

Field trials were conducted in 1981 and 1982 to assess differences in susceptibility of rutabaga (swede) cultivars and breeding lines to injury by larvae of the cabbage root maggot fly (Delia radicum L.).

Single 5 m rows were grown in 1981 and duplicate 5 m rows in 1982. After harvest, roots were assessed for damage and rated on the scale: clean 0, slight 1, moderate 2, severe 4. Mean scores for cultivars and breeding lines included in both years are in Table I:

Table I

| | 1981 | 1982 |
|---|---|---|
| Chignecto | 1.9 | 2.1 |
| #6-80 * | 1.5 | 2.3 |
| #5-80 * | 1.5 | 2.6 |
| Fortune | 3.4 | 3.2 |
| #1-80 | 2.7 | 3.4 |
| #2-80 | 3.2 | 3.4 |
| #3-80 | 3.7 | 4.0 |
| Kiri | 3.2 | 4.0 |

Gry was included in the 1981 trial and scored 2.4, whilst Wilhelmsburger was included in the 1982 trial and scored 2.9. Eales' Green Top, a local strain, derived from Wilhelmsburger, scored 1.8 in the 1981 trial.

* Green Topped lines derived from Gry & Wilhelmsburger.

Results from a similar trial but with 4 replications of 10 m rows are shown in Table II:

Table II

| | 1981 | 1982 |
|---|---|---|
| Wilhelmsburger | 2.4 | 1.7 |
| Eastern Laurentian | 3.0 | 2.5 |
| Purple King | 3.7 | 3.1 |
| York | 3.1 | - |
| Laurentian | 3.3 | - |
| Fortune | 3.3 | - |
| Altasweet | 3.5 | - |
| Monarch | 3.5 | - |
| Macomber | 3.6 | - |

These trials confirm the greater resistance of the green topped cultivar Wilhelmsburger (Swailes 1959) and lines derived from it. Except for the Chignecto cultivar, purple top lines and cultivars are susceptible or very susceptible to injury.

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# BREEDING OF CHINESE CABBAGE PARENTAL LINES RESISTANT TO CLUBROOT

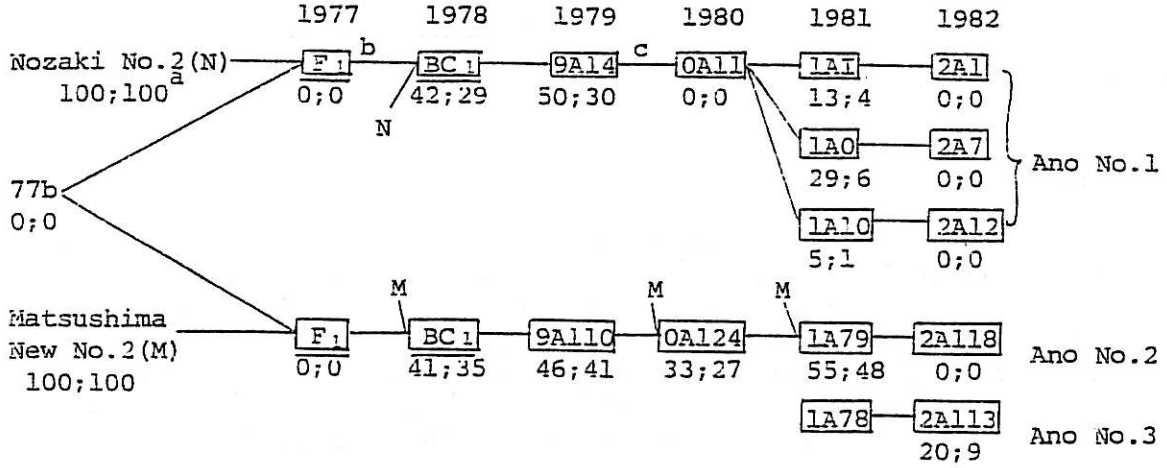
H. YOSHIKAWA, M. ASHIZAWA, K. HIDA and H. YAMAGISHI

The productions of *Brassica* vegetables have been severely threatened by clubroot( *Plasmodiophora brassicae* WORONIN ) during about twenty-five years in Japan. We began Chinese cabbage breeding for the resistance in 1973 in combination with the exploitation of a simple and reliable inoculation method( Yoshikawa; 1981 ). Unfortunately we couldn't find any resistant varieties in Asian vegetables belonging to *Brassica campestris*( Chinese cabbage, turnip and mustard green ), but we could use European turnip as a resistant material and now have established parental lines of Chinese cabbage resistant to clubroot.

In 1977 European resistant turnip '77b'( selected line from ECD02;AAbbCC ) was crossed with Chinese cabbage varieties 'Nozaki No.2' and 'Matsushima New No.2'. Chinese cabbage parents were backcrossed to the hybrid and in the later generations backcrosses or self-fertilizations were conducted. In the early generations( $F_1$ and $B_1$ ) selections of resistance and leaf shapes were done simultaneously in a glasshouse, and in the later generations emphasis was put on the selection of leaf quality and productibity in the field. By these selections the shape and quality of the progenies rapidly became closer to Chinese cabbage type, keeping the resistance. As a result three parental lines of Chinese cabbage resistant to clubroot were established( Fig. 1 and Table 1 ).

In the course of these breedings it was found that the resistance of '77b' was considered to be governed by a single dominant gene and a few modifier genes( Table 2 ). Leaf characters had no genetic correlations with clubroot resistance. The details of these breeding progresses will soon be published in Bull. Veg. & Ornam. Crops Res. Stn.

Nagano prefectural agriculture station also bred the resistant Chinese cabbage and Nozawana( turnip but for pickles of leaves ) varieties using European turnip. European turnip is now being used as the resistant material for the breeding of many traditional *B. campestris* vegetables in several parts of Japan.




a X;Y: X=percent of diseased plants Y=disease index
b □: simultaneous selection of resistance and leaf shape
c □: separate selection in glasshouse and field

Fig. 1. Breeding progresses of Chinese cabbage parental lines resistant to clubroot

Table 1. Reactions of Chinese cabbage parental lines to clubroot[a]

| Line or variety | Number of plants with disease index | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Ano No.1 | 294 | 18 | 5 | 2 |
| Ano No.2 | 65 | 8 | 2 | 1 |
| Ano No.3 | 42 | 6 | 5 | 3 |
| New Azumasanto[b] | 0 | 10 | 18 | 19 |
| 77b | 39 | 1 | 0 | 0 |

a tested by insertion method in June 1983
b susceptible Chinese cabbage variety

Table 2. Frequency distribution of clubroot resistance in crosses between Matsushima New No.2 and 77b

| Generation and progeny | | Number of plants with disease index | | | | Mean disease index | Segregation ratio observed (expected) R(3):S(1)[a] |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | | |
| $P_1$ | Matsushima New No.2(M) | 0 | 0 | 0 | 22 | 3.00 | |
| $P_2$ | 77b(b) | 32 | 0 | 0 | 0 | 0.0 | |
| $F_1$ | M × b | 44 | 0 | 0 | 0 | 0.0 | |
| $F_2$ | M × b | 247 | ( 5 | 6 | 35) | 0.42 | 247(220):46(73) |
| $BC_1$ | (M × b) × M | 101 | (12 | 9 | 50) | 1.05 | 101(86):71(86) |
| $BC_2$ | (M × b) × b | 174 | ( 0 | 0 | 0) | 0.0 | 174(174):0(0) |

[a] R: resistant plants (disease index=0); S: susceptible plants (disease index = 1,2,3)

# INOCULATION OF SECONDARY EMBRYOIDS OF OILSEED RAPE WITH SINGLE RESTING SPORES OF *PLASMODIOPHORA BRASSICAE*

Eileen Scott and Chiang-Shiong Loh

## Introduction

Populations of *Plasmodiophora brassicae* may comprise a range of pathotypes; this heterogeneity causes problems when characterising material using the European Clubroot Differential Series. The composition of clubroot populations could be determined by characterising isolates derived from single resting spores (Jones *et al.*, 1982). Such single spore isolates of *P. brassicae* would be useful in screening breeding material for resistance to clubroot disease.

Clubroot disease has been produced in seedlings of Chinese cabbage and oilseed rape, grown in compost, following inoculation with single resting spores of *P. brassicae* (Buczacki, 1977; Jones *et al.*, 1982; Tinggal and Webster, 1981; E. Scott, unpublished). This method, however, takes up much time and space, and the success rate is low. Tissue cultures of oilseed rape have been successfully infected with mass spore suspensions (Sacristan and Hoffman, 1979). It was therefore decided to investigate the possibility of producing single spore isolates of *P. brassicae* in tissue culture.

## Methods

Secondary embryogenic tissues derived from anther culture of winter oilseed rape (cultivar Primor; Loh and Ingram, 1982) were maintained on the basal medium of Murashige and Skoog (1962) supplemented with sucrose (2% w/v) and Difco agar (0.8% w/v), and incubated in a growth cabinet at 22°C with continuous light (70 µmol $m^{-2}$ $S^{-1}$ PAR). Three hundred secondary embryoids (2-5 mm in length) were subcultured individually on to fresh medium in 5 cm petri-dishes. After seven days 200 embryoids, which had developed roots with root hairs exposed on the surface of the agar, were selected for inoculation.

A population of *P. brassicae* obtained from the National Institute for Agricultural Botany (from Trawsgoed, Wales) was used to inoculate seedlings of the oilseed rape cultivar Jet Neuf in the glasshouse. Firm, intact clubs on roots of 42-day-old plants were used to prepare a contaminant-free resting spore suspension. The gall tissue was first surface-sterilised and washed (Buczacki, 1980), then all subsequent procedures were performed aseptically in a laminar flow cabinet. After washing, the tissue was homogenised in sterile distilled water and the suspension filtered through three layers of sterile muslin. The spores were washed six times by centrifugation, resuspending only the beige spore mass each time. The final pellet was suspended in sterile distilled water and examined microscopically for the presence of contaminants. This stock suspension was stored at 4°C for up to 4 days.

A drop of spore suspension, diluted to approx. $10^3$ spores $ml^{-1}$, was spread on 3% Difco purified agar in a 9 cm petri-dish and single, well-isolated spores located by scanning under a microscope. An agar

disc (approx. 1 mm diameter), bearing a single spore, was marked out using a spore punch mounted on the microscope. The disc was then transferred, using a sterilised needle, to the root hairs of an embryoid; the root had previously been moistened with a drop of sterilised root leachate (prepared by growing Chinese cabbage in nutrient solution). After inoculation cultures were sealed with parafilm and incubated in the dark for 48 h. Embryoids were examined at weekly intervals.

## Results and Discussion

Of the 200 embryoids inoculated, four developed galls, up to 3 mm in diameter after 4 weeks, on the roots. Sections from one gall were examined using the light microscope; clubroot plasmodia were observed in the root cells.

This preliminary study has shown that clubroot symptoms can be produced in tissue cultures of oilseed rape following inoculation with single resting spores of P. brassicae. It is hoped to repeat the work, on a larger scale, to follow the development of symptoms over a longer period and to assess the ability of resting spores produced in tissue culture to infect seedlings in the glasshouse.

## Acknowledgement

We thank Dr. D.S. Ingram for confirming the presence of clubroot plasmodia in tissue culture.

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# THE EFFECT OF INSECT DAMAGE ON THE INCIDENCE OF INFECTION BY *PHOMA LINGAM* IN WINTER OILSEED RAPE

P. Newman and H. Plumridge

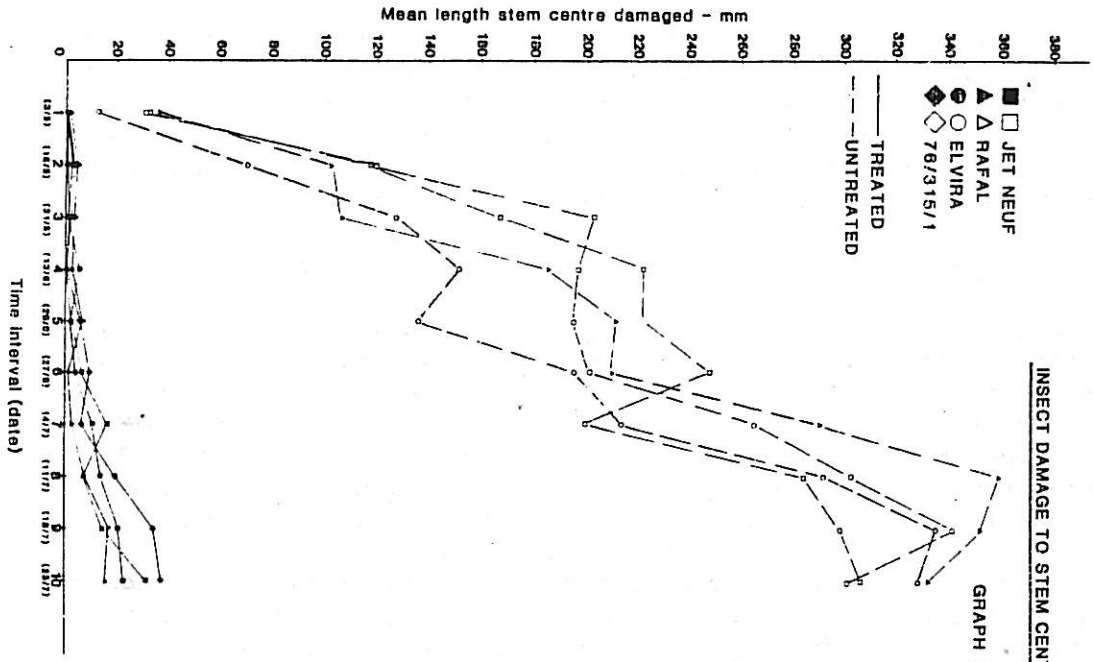
The effect of insect damage on the resistance of four cultivars was examined in a field trial in 1982-83. Two of these cultivars were susceptible to canker (Elvira and 76/315/1) and two were resistant (Jet Neuf and Rafal). The trial was split into two blocks, one of which was left untreated and the other treated with 30g 100 $m^{-1}$ row Carbofuran ('Yaltox', Bayer UK Ltd.) in autumn and spring. Each cultivar was replicated three times within each treatment block.

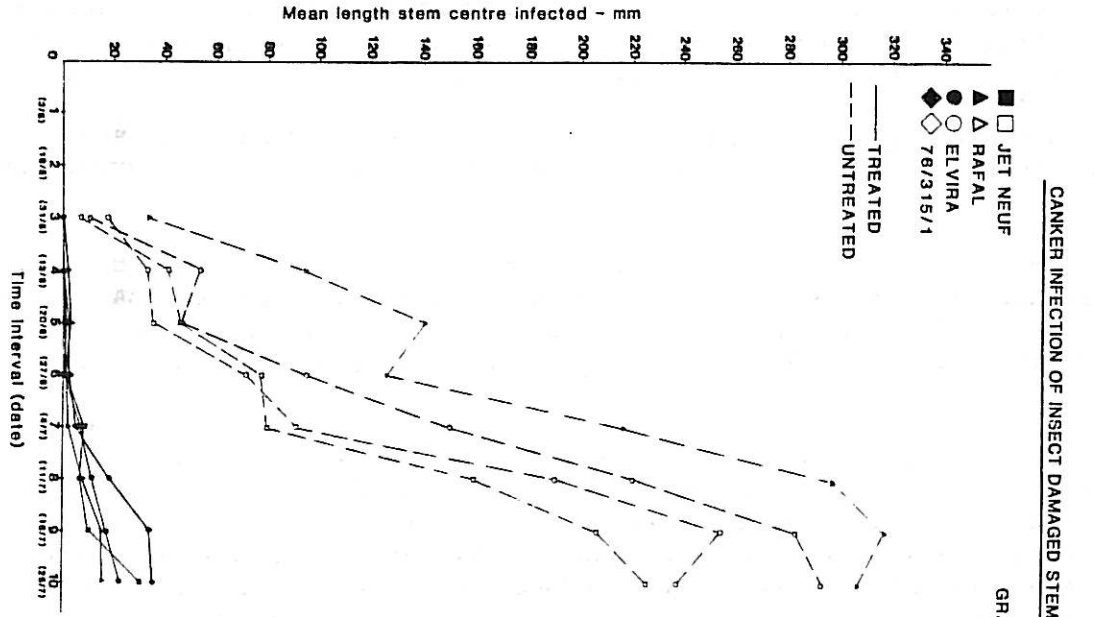
At each sampling date 20 plants were taken at random from each plot. Thus the results in Graphs 1 and 2 represent the means of 60 plants of each cultivar and treatment. Individual plants were split longitudinally along the main stem, from just above the crown (which was taken as the basal 5 cm) to the region of the lower pods, and the total length of the stem centre tunnelled by insects and/or infected with canker was measured. The insect species present were also noted; flea beetle larvae were observed at sample times 1 to 4, and weevil larvae at times 3 to 6.

Considerably less insect damage was found on the treated than on the untreated plots (Graph 1) and the pattern of canker infection (Graph 2) reflected that found for insect damage. Nearly all the cankers on the high parts of the stem (i.e. excluding the basal, crown cankers) were associated with insect damage and a very high proportion of the damaged tissues was infected. There were few stem cankers on the insecticide- treated plots. The correlation between insect damage and canker infection on insect-damaged tissue was highly significant ($r = 0.95$, $p = 0.001$).

Rafal, considered a resistant cultivar on the basis of crown canker infection, had consistently more internal stem canker infection than the other three cultivars. This suggests that the resistance mechanism in Rafal does not operate when stem tissue is damaged. There were no significant differences for internal stem canker infection between cultivars in the treated block. It was also noted that 76/315/1 had less insect damage than the other cultivars at the start of the season, though by July had reached a similar level to the others (Graph 1).

It seems, therefore, that by controlling insect larvae it is possible to reduce considerably the incidence of canker on the upper parts of stems. Although these stem cankers are not considered to be as damaging as crown cankers, they probably have some effect on yield, and in particular cause premature senescence, further weakening stems already damaged by insects. The results also suggest there may be differences in insect susceptibility between cultivars, although this requires confirmation.


INSECT DAMAGE TO STEM CENTRE
Mean length stem centre damaged - mm
JET NEUF
RAFAL
ELVIRA
76/315/1
TREATED
UNTREATED
Time Interval (date)
GRAPH 1


CANKER INFECTION OF INSECT DAMAGED STEM CENTRE
Mean length stem centre infected - mm
JET NEUF
RAFAL
ELVIRA
76/315/1
TREATED
UNTREATED
Time Interval (date)
GRAPH 2

# PHYSIOLOGIC SPECIALISATION IN ALTERNARIA BRASSICAE

G.S. Saharan and A.K. Kadian

Alternaria brassicae (Berk.) Sacc. causes leaf spot and blight disease in most of the crucifers throughout the world. The pathogen produces brown to black lesions on the stem, leaves, pods and seeds of the infected plants. The damage caused by this pathogen in different crops varies from 25-50 per cent depending on the time of appearance, environmental conditions and status of resistance in a particular crop. The present paper deals with existence of physiologic races in A. brassicae.

Eight isolates of A. brassicae, each from a different host species/varieties, were cross-inoculated to a range of crucifers, including those from which they had originally been taken (Table 1). Out of eight isolates of A. brassicae collected from different host species/varieties and cross-inoculated on differential test plants, the isolates 1, 3 and 4 were grouped into one group, the isolates 2 and 5 into second group and isolates 6, 7 and 8 formed third group on the basis of their differential reaction to the test host (Table 1). Therefore, it appeared that these isolates consisted of three distinct races of the fungus which were tentatively designated as race RM1 from rape seed and mustard group of crops, race RM2 from B. campestris var sarson and Eruca sativa and race V3 from radish, cabbage and cauliflower crops. Rape seed and mustard group of crops and radish were susceptible to all the three races and were of little value in differentiating the various isolates. The differential reaction of different races were observed only on cabbage and cauliflower. Cabbage was resistant to all the five isolates from rape seed and mustard group of crops constituting race RM1 and RM2. Cauliflower was resistant to race RM2 (isolates obtained from B. campestris var sarson and Eruca sativa). Race V3 (isolates from radish, cabbage and cauliflower) infected all the eight test host indicating wide virulence of this race.

No correlation was established with the morphology of conidia, since a large amount of variation was observed between the races designated and amongst the isolates tested. It seemed, conidial size is a function of nutrition from host individual of A. brassicae rather than a characteristic of pathological variation.

It is clear from Table 2 that there was variation in the incubation period (5-14 days) of all the three races in different hosts. Each of the originally sampled host differentiated its own isolate by exibiting shorter incubation period ie, races RM1 and RM2 had 5-6 days of incubation period in rape seed and mustard group of crops but longer in vegetable crops (8-14 days). However, race V3 showed longer incubation period in all the test host but nevertheless it had wide virulence.

Table 1. Physiologic races of Alternaria brassicae

| Differential host | Reaction to isolates from | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1. Brassica juncea | S | S | S | S | S | S | S | S |
| 2. B. campestris var. sarson | S | S | S | S | S | S | S | S |
| 3. B. campestris var. dichotoma | S | S | S | S | S | S | S | S |
| 4. B. campestris var. toria | S | S | S | S | S | S | S | S |
| 5. Eruca sativa | S | S | S | S | S | S | S | S |
| 6. Raphanus sativus | S | S | S | S | S | S | S | S |
| 7. Brassica oleracea var. botrytis | S | R | S | S | R | S | S | S |
| 8. Brassica oleracea var. capitata | R | R | R | R | R | S | S | S |
| Race designate | RM1 | RM2 | RM1 | RM1 | RM2 | V3 | V3 | V3 |

R = Resistant; S = Susceptible; 1 = B. juncea; 2 = B. campestris var. sarson; 3 = B. campestris var. dichotoma; 4 = B. campestris var. toria; 5 = Eruca sativa; 6 = Raphanus sativus; 7 = B. oleracea var. botrytis; 8 = B. oleracea var. capitata.

Table 2. Incubation period of Alternaria brassicae races in different crucifers

| Host | Incubation period (days) of race | | |
|---|---|---|---|
| | RM1 | RM2 | V3 |
| Brassica juncea | 6 | 6 | 8 |
| B. campestris var. sarson | 6 | 5 | 8 |
| B. campestris var. dichotoma | 5 | 5 | 8 |
| B. campestris var. toria | 6 | 6 | 9 |
| Eruca sativa | 6 | 5 | 8 |
| Raphanus sativus | 8 | 8 | 8 |
| Brassica oleracea var. capitata | 0 | 0 | 10 |
| B. oleracea var. botrytis | 14 | 0 | 9 |

# EPIDEMIOLOGY OF ALTERNARIA LEAF SPOT OF CHINESE CABBAGE

S.S. Karwasra and G.S. Saharan

Alternaria leaf spot of chinese cabbage (Brassica pekinensis L) caused by Alternaria brassicae (Berk.) Sacc. was reported by Karwasra & Saharan (1982) from India. Since then it has become one of the major destructive disease of this important fodder crop causing considerable foliage damage resulting into poor yield. In the present paper the role of environmental factors was studied for their influence on the epidemic development of disease under field conditions.

## Materials and Methods

On randomly selected ten plants, 25 leaflets per plant were tagged and numbered on 10.1.83. The observations were recorded on the number and size of Alternaria spots/leaflet. The subsequent observations were recorded at an interval of 12 days on the marked leaflets till their senescence on 21.3.83. The environmental factors viz, minimum temperature °C (MNT), maximum temperature °C (MXT), per cent relative humidity (RH) and rainfall (MM) prevalent during the preceding 12 days of date of observation were recorded and analysed for their role in influencing the disease development in terms of number and size of spots/leaflet.

## Results and Discussion

The periodic and cumulative increase in number and size of Alternaria leaf spot/leaflet is given in Table 1. It is clear from the Table that the size of Alternaria leaf spot/leaflet varied from 1 to 9.33 MM. The maximum periodic increase in size of spots/leaflet was recorded on 2.2.83 and minimum on 26.2.83. The periodic increase in the number of spots/leaflet varied from 0.37 to 9.33. The maximum period increase in number of spots/leaflet was recorded on 10.3.83. It is evident from Fig. 1 that the environmental factors like temperature, relative humidity and rainfall favoured increase in number of spots/leaflet during 26.2.83 to 10.3.83. During this period 23.2 - 25.2°C MXT, 8.2-11.07°C MNT, 61.5-75% RH and 1.0-1.5 mm rainfall favoured the development of disease in terms of increase in number of spots/leaflet at a fast speed. However, the disease progress was limited during 22.1.83 to 13.2.83 because of low temperature (2.2-20°C) and relative humidity (62-69.5%).

The maximum number and size of spots-leaflet were 31.33 and 9.33 mm respectively. No correlation was established between increase in number and size of spots/leaflet. The increase in number of spots or infection points/leaflet was influenced greatly by the prevailing environmental conditions during that period. However, there seemed to be little bearing on the size of spots due to minor fluctuations in environment, since periodic increase in size of spots was recorded in the present study.

## References

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Table 1. Increase in number and size (MM) of Alternaria leaf spot of chinese cabbage

| Date of observation | Increase in number of spots/leaflet | | Increase in size of spots/leaflet | |
|---|---|---|---|---|
| | Periodic | Cumulative | Periodic | Cumulative |
| 10.1.83 | 1.0 | 1.0 | 1.0 | 1.0 |
| 22.1.83 | 6.33 | 7.33 | 1.66 | 2.66 |
| 2.2.83 | 0.37 | 7.70 | 2.34 | 5.0 |
| 13.2.83 | 2.96 | 10.66 | 1.0 | 6.0 |
| 26.2.83 | 4.34 | 15.0 | 0.66 | 6.66 |
| 10.3.83 | 9.33 | 24.33 | 1.34 | 8.0 |
| 21.3.83 | 7.0 | 31.33 | 1.33 | 9.33 |

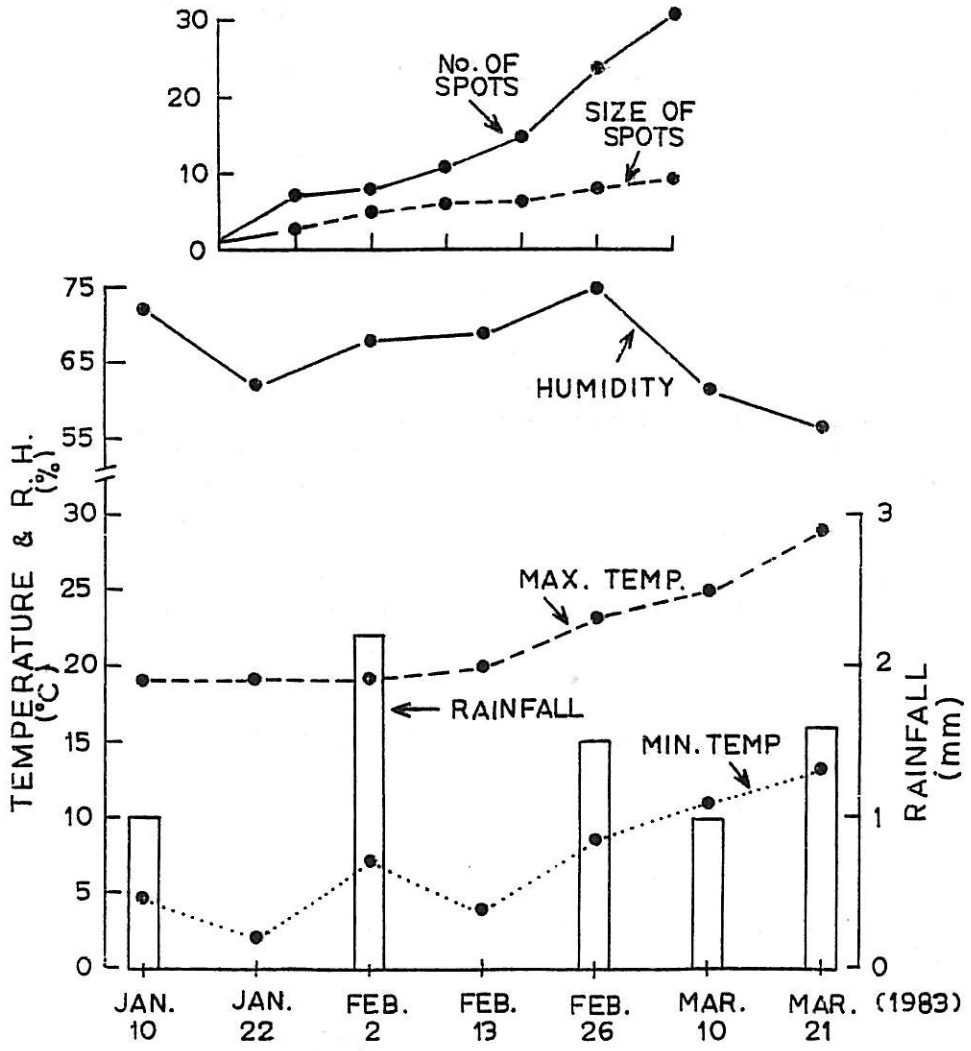


Fig.1.

# INCIDENCE OF *ALTERNARIA* INFECTION IN OIL SEED RAPE (*BRASSICA NAPUS* L.) CROPS IN SCOTLAND IN 1983

Kothanur P.R. Prasanna and J.H. Lennard

In a survey of oil seed rape crops in Scotland in 1982 *Alternaria brassicae* (Berk.) Sacc. was found to be the major cause of leaf spotting (Prasanna and Lennard, 1982a). In a similar survey carried out in 1983 45 crops were assessed at two stages in summer. In addition seven other *Brassica* crops were examined at the first assessment (five Brussels sprouts, one cauliflower and one swede). Prior to this survey, observations were made on 13 overwintering oil seed rape crops in December 1982: *A. brassicae* was found in six crops.

From the more general survey at flowering (June 1983) 73 per cent of oil seed rape crops were infected with *A. brassicae* and 16 per cent with *Alternaria brassicicola* (Schw.) Wiltshire, which was not recorded during December (Table 1). Of the other *Brassica* crops, all showed the presence of *A. brassicae* and three of the seven fields had some *A. brassicicola*.

Table 1. Incidence of *Alternaria brassicae* and *A. brassicicola* in oil seed rape and other *Brassica* crops in south-east Scotland

| District | Number of fields inspected | Number of fields infected with *Alternaria* | |
|---|---|---|---|
| | | *A. brassicae* | *A. brassicicola* |
| Border | 13(7) | 11(7) | 2(3) |
| Lothian | 17 | 12 | 3 |
| Fife | 9 | 6 | 1 |
| Perth, Kinross and Angus | 6 | 4 | 1 |

Figures in brackets refer to other Brassica crops

The incidence of seed infection is given in Table 2. *A. brassicae* was again the most prevalent species, being present in 47 per cent of the samples as opposed to 36 per cent for *A. brassicicola*. With both species, the infection level was usually low (1-5 per cent): with more severely infected samples all except one were associated with *A. brassicae*.

Table 2. Incidence of *Alternaria brassicae* and *A. brassicicola* in seed samples of oil seed rape in south-east Scotland

| District | Number of field samples | Number of samples in different percentage seed infection classes for *A. brassicae* (and *A. brassicicola*) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1-5 | 6-10 | 11-15 | 16-20 | 21-50 |
| Border | 13 | 4(10) | 1 (3) | 3 | 1 | 2 | 2 |
| Lothian | 17 | 10 (9) | 7 (7) | 0 | 0(1) | 0 | 0 |
| Fife | 9 | 7 (6) | 2 (3) | 0 | 0 | 0 | 0 |
| Perth, Kinross and Angus | 6 | 3 (4) | 3 (2) | 0 | 0 | 0 | 0 |
| Total | 45 | 24(29) | 13(15) | 3 | 1(1) | 2 | 2 |

The pattern of incidence of *A. brassicae* in relation to district was similar to that of the previous year, the highest incidence and most severe infections occurring in the Border district. However the general incidence of this species was less than in 1982, 53 per cent of crops being free from seed infection as opposed to 20 per cent in 1982. This lower incidence may be attributable in part to the widespread use of spray applications of iprodione in 1983: 36 of 45 fields sampled were known to have been treated. However, of six crops known to be unsprayed three samples were free from infection and only one showed severe infection (29 per cent). With spray applications, seven of the 36 samples still showed over 5 per cent seed infection. A further factor which may account for the lower incidence of *A. brassicae* in 1983, compared with 1982, may have been the drier weather during the main growing season (Table 3): although May showed a relatively high rainfall the subsequent months, particularly July and August, were dry. Both *A. brassicae* and *A. brassicicola* require free water for infection (Humpherson-Jones and Hocart, 1983).

Table 3. Monthly temperature and rainfall in south-east Scotland (1983)

| District | Daily mean temperature °C | | | | Monthly rainfall mm | | | |
|---|---|---|---|---|---|---|---|---|
| Station | May | June | July | August | May | June | July | August |
| Border | 9 | 13 | 18 | 16 | 60 | 48 | 28 | 30 |
| Lothian | 9 | 12 | 17 | 16 | 135 | 65 | 10 | 37 |
| Fife | 8 | 13 | 18 | 16 | 80 | 48 | 28 | 30 |
| Perth | - | 12 | 17 | 16 | 75 | 82 | 10 | 14 |

*A. brassicicola* was infrequent in 1982, but showed a more widespread incidence in the present year: this may be associated with its high temperature requirement (Degenhardt, Petrie and Morrall, 1982). Humpherson-Jones and Hocart (1983), working with cabbage, indicated an optimum infection temperature of 25°C compared with 15°C for *A. brassicae*. The more frequent occurrence of *A. brassicicola* in the Lothian district compared with other districts (Table 2) may relate to the more intensive growing of *Brassica* vegetable crops in market gardens. The present survey indicated a more common occurrence of this fungus in *Brassica* vegetables than in oil seed rape (Table 1) and a survey of seed of vegetable *Brassicas* showed a widespread incidence of *A. brassicicola* (Prasanna and Lennard, 1982b).

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# RESPONSES TO INFECTION BY *ERYSIPHE CRUCIFERARUM* Opiz ex L. Junell

J.M. Munro and J.H. Lennard

The development of powdery mildew (*Erysiphe cruciferarum*) and host tissue response were studied for three isolates of the pathogen, from three different *Brassica napus* swede cultivars, inoculated onto leaf discs of three cultivars of *B. napus*, Doon Major, Ruta Otofte and Barsica, and one line of *Raphanobrassica*, RB 25/8. The production of plants, inoculation procedure and incubation methods were similar to those reported previously (Munro and Lennard, 1982) and observations were made 5 days after inoculation. Mildew development was assessed on a 0-5 scale, and the leaf discs were then processed for microscopic examination using incident fluorescent light and transmitted bright-field illumination. Following clearing, leaf discs were transferred to aniline blue, counter-stained in trypan blue and then mounted in aniline blue. Under the microscope a varying number of fluorescent sites were seen in the outer walls of infected tissue: these and numbers of necrotic host cells associated with colonies were counted.

The extent of mildew development, the numbers of fluorescent sites and numbers of necrotic cells per colony varied significantly with cultivar (Table 1). Doon Major showed the greatest amount of mildew development and RB 25/8 the least; with the latter there was little progress beyond the appressorial stage. Mycelium growth was moderate on Ruta Otofte and sparse on Barsica. Fluorescent sites may be considered as penetration points and cultivars showed the same order of ranking for numbers of fluorescent sites as for mildew development. On the other hand, necrotic cell numbers per colony were least with Doon Major, the most susceptible cultivar, and were also very low with RB 25/8, regarded as the most resistant. With cultivars other than Doon Major, necrotic cell number increased with increased disease development. Doon Major - *E. cruciferarum* may be considered a compatible combination of host and biotroph, where infected host cells are maintained alive over a protracted period. With the other three cultivars varying degrees of resistance were evidently associated with a necrotic response, the greater the level of resistance the fewer the number of cells affected. With cereal mildew (*Erysiphe graminis*) resistance may manifest itself by the death of infected epidermal cells (Cherewick, 1944) or by mesophyll collapse (White and Baker, 1954). In the later case the degree of resistance was reported to be a function of the rapidity of response: with higher levels of resistance collapse occurred earlier and was confined to fewer cells. A similar type of relationship with respect to rate and extent of development of necrotic tissue and resistance is suggested in the present work for the moderately or highly resistant cultivars.

The differences between isolates were significant only in the case of fluorescent site number, which also evidenced an interaction between isolate and cultivar. This would indicate that counts of fluorescent sites may provide a more sensitive index of host-pathogen relationships than assessments of mildew development. There were no significant differences between isolates in numbers of fluorescent sites per colony for Doon Major or RB 25/8, the most susceptible and most resistant cultivar respectively. However with both Ruta Otofte and Barsica, which showed intermediate levels of resistance, one isolate showed significantly more penetration points per colony than the other two isolates.

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Table 1 Mildew development and tissue responses produced by three isolates of *Erysiphe cruciferarum* on four Cruciferous hosts 5 days after inoculation.

| Cultivar | Mildew Development (0-5 scale) | Number of fluorescent sites per colony | Number of necrotic cells per colony |
|---|---|---|---|
| Doon Major | 2.9 | 14.4 | 0.3 |
| Ruta Otofte | 1.8 | 2.1 | 2.4 |
| Barsica | 1.0 | 1.0 | 1.1 |
| RB 25/8 | 0.2 | 0.3 | 0.5 |
| SED ± (DF = 99) | 0.2 | 0.4 | 0.3 |

# A METHOD FOR ESTIMATING SPORE NUMBERS OF ERYSIPHE CRUCIFERARUM USING INFRA-RED REFLECTANCE ANALYSIS

Elaine A. Doughty, Cynthia J. Williamson and I.A. Cowe

Differences between cultivars in rate of spore production by Erysiphe cruciferarum are important as a measure of host resistance and in relation to the development of powdery mildew epidemics on brassica crops. Several methods have been used to measure spore numbers of foliar pathogens; recently, Asher et al. (1982) reported that spore samples from four cereal foliar pathogens could be counted with high precision by infra-red reflectance spectrophotometry using a Neotec 6350 Research Composition Analyser. In this note we report how the method of Asher et al. (loc. cit.) has been adapted for estimating spore numbers of E. cruciferarum.

## Calibration

Glasshouse grown plants of swede cv. Doon Major heavily infected with powdery mildew were used as the source of conidia. Leaves on which the pathogen was sporulating profusely were removed from the plants and washed in a 100 ppm solution of Tween 80. The resulting spore suspension was filtered through four layers of muslin then passed through an Acrodisc filter system[/] with a pore size of 5 µm. Any leaf exudates and particles smaller than 5 µm were discarded with the filtrate. The spores were then resuspended by injecting 5 ml of Tween 80 solution through the Acrodisc filter.

The spore suspension was adjusted to a concentration of $60 \times 10^3$ spores per ml and a dilution series was prepared with thirteen samples between $1 \times 10^3$ and $60 \times 10^3$ spores per ml. Spores from each dilution were counted on a haemocytometer slide from ten separate samples and three 0.5 ml subsamples were transferred to the centre of Whatman GF/A glass micro-fibre discs as were blank subsamples of the Tween 80 solution. The filter discs were dried for 12 hours at c. 40°C then transferred to individual Petri dishes and stored in a desiccator until they were mounted in sample cups for scanning by the Neotec 6350.

Four wavelengths 1880, 2016, 2310 and 2464 nm gave the highest multiple correlation between spore concentrations estimated by infra-red reflectance and by direct counts using the haemocytometer slide. The correlation coefficient was 0.992 with a standard error for calibration of $2.365 \times 10^3$ and additional wavelengths did not improve this correlation significantly.

Using these four wavelengths, the equation $N = K_0 + K_1W_1 + K_2W_2 + K_3W_3 + K_4W_4$ expressed the relationship between spore numbers estimated by the two methods. N = number estimated in any one sample : $K_1$, $K_2$, $K_3$ and $K_4$ are partial regression coefficients with the values -238.20, 22431.87, -38712.46, -10473.64 and 24072.79 respectively; and $W_1$, $W_2$, $W_3$ and $W_4$ are optical density values at 1880, 2016, 2310 and 2464 nm respectively. Asher et al. did not obtain satisfactory estimates of spore numbers when spores were washed from leaves, and they suggested that this may have been due to the presence of leaf exudates; the use of the Acrodisc filter system appeared to overcome this problem with powdery mildew on swedes.

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Estimation of spore numbers on eight swede cultivars

Ten plants of each of the cultivars Acme, Bangholm, Doon Major, Magres, Marian, Merrick, Ruta Øtofte and Seefelder were grown in a glasshouse and inoculated at the 2-3 leaf stage. After three weeks the plants were sprayed lightly with water to remove old conidia; after a further twelve days, sixteen samples were taken, each derived from five plants of one cultivar. The last fully mature leaf was removed from each plant, the five leaves from each sample were bulked and washed in 100 ppm Tween 80 solution. The sixteen spore suspensions thus obtained were filtered, and three 0.5 ml subsamples were taken as described for the calibration. Three subsamples from each sample were counted on a haemocytometer slide so that the accuracy of spore numbers predicted by infra-red reflectance analysis could be assessed. The total leaf area of each sample was also measured.

The number of spores of E. cruciferarum from eight swede cultivars estimated using infra-red reflectance analysis and by haemocytometer counts

| Cultivar | Sample | Haemocytometer counts | | Infra-red reflectance analysis | |
|---|---|---|---|---|---|
| | | Mean of 3 direct counts* | Number of spores per 100 mm$^2$ leaf | Mean of 3 predicted counts* | Number of spores per 100 mm$^2$ leaf |
| Acme | (a) | 56.5 | 10.82 | 92.19 | 17.65 |
| | (b) | 13.7 | 2.57 | 32.14 | 6.09 |
| Bangholm | (a) | 14.5 | 3.30 | 30.48 | 7.00 |
| | (b) | 11.7 | 2.44 | 18.31 | 3.82 |
| Doon Major | (a) | 61.5 | 11.89 | 94.99 | 18.36 |
| | (b) | 51.7 | 8.32 | 78.22 | 12.59 |
| Magres | (a) | 15.0 | 2.02 | 22.33 | 3.00 |
| | (b) | 11.2 | 1.63 | 16.64 | 2.43 |
| Marian | (a) | 9.7 | 1.80 | 11.76 | 2.18 |
| | (b) | 2.7 | 0.49 | 7.68 | 1.39 |
| Merrick | (a) | 3.4 | 0.43 | 7.57 | 0.95 |
| | (b) | 3.7 | 0.61 | 6.08 | 1.00 |
| Ruta Øtofte | (a) | 7.1 | 1.15 | 10.13 | 1.65 |
| | (b) | 4.3 | 0.81 | 11.25 | 2.12 |
| Seefelder | (a) | 4.6 | 0.74 | 7.63 | 1.23 |
| | (b) | 2.7 | 0.47 | 0.75 | 0.13 |

* Each count x $10^3$ spores per ml.

The correlation coefficient between the two estimates of spore numbers was 0.965 with a standard error for prediction of 14.455 x $10^3$. A higher correlation (r = 0.991) was obtained when the mean estimate for

each sample was adjusted for leaf area. A clear separation was obtained between the two most susceptible cultivars which were sporulating profusely, and the other six cultivars. There were only small differences in ranking order between cultivars which produced small numbers of conidia, these differences probably reflect the lack of precision in estimating low spore numbers from haemocytometer counts.

The authors wish to thank D.C. Cuthbertson for his technical assistance.

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# [$^{14}$C] RADIOLABELLING STUDIES IN OILSEED RAPE

Harris, S.R., Williams, B.L. and Simpkins, I.

A study of the developing siliqua of Brassica napus L., cv Victor revealed a patter of dry matter accumulation, fatty acid content and composition similar to that reported previously (Appelgvist, 1968; Norton and Harris, 1975), indicating three major phases in seed development. An active glycerol kinase was demonstrated in the developing seed but not sn-glycerol phosphate dehydrogenase. The photosynthetic competence of detached siliquae, harvested 35 days post anthesis, was demonstrated by incubating them with $^{14}CO_2$ for periods up to six hours in illuminated sealed perspex chambers, when label was recovered in the aqueous, lipid and residue fraction of both seeds and hulls. Translocation of photosynthate from hull to seed was shown with sucrose as the major labelled product in the aqueous fraction. Substantially more label was incorporated into the water soluble fractions of both hulls and seeds than the lipid fraction throughout the period of incubation. Some $^{14}[CO_2]$ was incorporated into lipids however predominantly into the glycerol moiety of the neutral fraction of the seed but the hull lipid fraction never became significantly labelled. A small amount of free labelled glycerol was detected in seeds. Substantially the same labelling patterns were observed when detached rape siliquae were incubated with either [U-$^{14}$C] sucrose or [U$^{14}$C] glycerol.

When rape leaf discs were incubated with [U-$^{14}$C] glycerol and the label followed into the lipid components, most of the label was recovered in the glycerol moiety of the glycolipid and phospholipid fraction. Similar leaf disc experiments using [H$^{14}CO_3^-$] revealed an early appearance of label in the galactose moiety of the glycolipid and later in the glycerol and fatty acid components.

Rape chloroplasts prepared under conditions demonstrated to be suitable for showing high rates of $CO_2$-dependent $O_2$ evolution in peas, were not capable of showing $CO_2$-dependent $O_2$ evolution and when incubated with $^{14}HCO_3^-$, label was not incorporated into lipid. When rape chloroplasts were incubated with [$^{14}$C] acetate, [$^{14}$C] glycerol or [$^{14}$C] glycerol phosphate however, some label was incorporated into lipid but much more weakly than with pea chloroplasts.

A fuller treatment of methods, results and a discussion of results can be found in Harris (1983).

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# GERMINATION OF B. NAPUS RAPIFERA SEED AT LOW TEMPERATURES -

KENNETH G. PROUDFOOT

The ability of seeds to germinate at low temperatures is of importance in areas where weather conditions are less than ideal at seeding. Seeds of swede (rutabaga) are known to germinate at temperatures as low as -0.3 °C (Roeggen 1982). Differences between cultivars in germination percentage at low temperatures have been reported for tomatoes (Kemp 1968) and for oilseed B. napus and B. campestris (Acharya et al. 1983).

In May 1983 the germination potential at 5°C of rutabaga seeds from different breeding lines was examined. Results at this temperature were compared to results of germination at 22°C. At both temperatures, 100 seeds of each line were placed in petri dishes containing 0.8% agar.

Seeds at room temperature (22°C) germinated within 2 days, but no germination was recorded until the 7th day at 5°C. A sample of the results obtained are tabulated below:

| YEAR SEED PRODUCED | LINE CULTIVAR | GERMINATION at 22°C AFTER | | | GERMINATION at 5°C AFTER | | |
|---|---|---|---|---|---|---|---|
| | | 2 days | 6 days | 10 days | 7 days | 10 days | 14 days |
| ? | Laurentian (A) | 5 | 78 | 88 | 0 | 0 | 2 |
| ? | Vige | 90 | 95 | 97 | 0 | 16 | 74 |
| 1980 | #6-80 | 79 | 94 | 95 | 0 | 3 | 45 |
| 1980 | #7-80 | 74 | 95 | 97 | 0 | 2 | 15 |
| 1982 | Fortune | 95 | 97 | 97 | 4 | 18 | 85 |
| 1982 | R.S.T. | 56 | 79 | 85 | 8 | 28 | 73 |
| 1982 | I.A.P.-82 | 94 | 96 | 96 | 2 | 15 | 74 |
| 1982 | #2-82 | 96 | 99 | 100 | 10 | 49 | 86 |

These results confirm that differences exist between cultivars in germination at below optimum temperatures, and also in rapidity of germination. Differences between age of seed are also a factor in determining percentage germinating at the lower temperature. Six samples produced in 1980 ranged in germination from 15 to 45 (mean 30) at 5° and 14 days compared to a range of 69 to 89 (mean 81) for 5 samples produced in 1982.

Germinated seeds from the 5°C plates were planted into flats and later transplanted into the field and roots have been retained for seed production. Seedlings were not subjected to low temperatures following germination but establishment and development at low temperatures could be of critical importance in the field.

Further confirmation of differences between cultivars was obtained in a second trial where 2 seed lots of Laurentian, Fortune and York were compared. Differences between seed lots of the same cultivar were also evident.

| CULTIVAR | Germination at 22°C After 3 days | 10 days | Germination at 5°C After 9 days | 14 days |
|---|---|---|---|---|
| Laurentian (A) | 22 | 83 | 1 | 5 |
| Laurentian (B) | 95 | 98 | 10 | 49 |
| York | 98 | 98 | 3 | 11 |
| Fortune | 93 | 94 | 45 | 80 |

Further studies in germination and development at low temperatures appear warranted, particularly where cultivars are to be precision seeded and near perfect stands are required to produce a highly uniform product, and weather conditions following seeding are unpredictable.

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# POLLINATION AND REPRODUCTION IN ZERO ERUCIC WINTER - RAPE WITH LOW GLUCOSINOLATE CONTENT

W. Odenbach, M. Grünberg-Villaroel, C. Beelitz-Kunze

Fertilization and seed set were studied in three 0-0-winter rape varieties, NPZ ST 1657/79, DSV ST 154/81, Librador and Garant and Jet Neuf as standard varieties.

Remarkable differences in pollengermination in vivo could be observed. All testcrosses with Jet Neuf showed normal pollengermination, whereas in ST 154/81 only 82% of the testcrosses showed normal pollengermination. In ST 1657/79 normal pollentube growth could be observed in only half of the testcrosses. In vitro tests confirm these observations (Tab. 1).

TAB. 1 1983 POLLENGERMINATION IN VITRO I%I

| | germinated | non$^{+}$ germinated | FDA fluorescent pollen |
|---|---|---|---|
| Jet Neuf | 74 | 22 | 74 |
| ST 1657/79 | 6o | 39 | 33 |
| ST 154/81 | 82 | 11 | 84 |

FDA = Fluoresceindiacetat
\+ = well developed oval pollen

Only 33 % FDA flourescentpollen could be found in this variety. Comparing shape and growth of pollentubes in the style of ST 1657/79 a high amount of irregularities was observed (uneven growth, nodular in shape).Pollentubes growing most straight were found in the variety Garant.Observing ovule development about 5 days after pollination in siliques from ST 1657/79, nearly half of the ovules were less in size and a remarkable part of them was connected with irregular growing pollentubes. Such irregularities were also observed in the other varieties analyzed here, but in a less amount. May be, these facts point at the transitional stage from a selfincompatible to an autogamous species. At the same time these findings can reflect specific genotypic plasmatic interactions, as ST 1657/79 based on normal European rape cytoplasm (S-type), whereas in ST 154/81 the F-type cytoplasm from Bronowski is present.

On the other hand, from all varieties tested in the 1982 trial, ST 1657/79 showed the highest seed set (Tab. 2). Siliques of the 5th side branch showed the same number of ovules as those from the main shoot. There were practically no differences in the number of ovules in relation to plant density or growing in cages.

Remarkable fertility reduction was found in the lower part of the 5th side branch. This cannot be explained by reduced pollination by bees. The same fertility reduction was observed under growing the plants in a cage free of bees.

Tab. 2 1982 SEED SET +I%I UNDER TWO WAYS OF POLLINATION

| | mainshoot | | $5^{th}$ side branch | |
|---|---|---|---|---|
| | a | b | a | b |
| Jet Neuf | 72 | 53 | 6o | 44 |
| Garant | 77 | 49 | 68 | 6o |
| ST 1657/79 | 88 | 63 | 69 | 49 |
| Librador | 75 | 54 | 64 | 61 |
| ST 154/81 | 85 | 5o | 7o | 52 |

a = planted normally, b = planted in cages

\+ = seed set potential ( = no. ovules/ silique) of ten siliques from the middle of the shoot = 1oo %

# QUANTITATIVE ANALYSIS OF GLUCOSINOLATES BY HPLC - PRELIMINARY OBSERVATIONS

E.A. Spinks, G.R. Fenwick and W.T.E. Edwards

The analysis of glucosinolates, mustard oil glycosides, has increased in importance over the last decade due initially for the need to monitor levels in rapeseed and its products and more recently because of concern over the amounts of these natural toxicants in crops and produce intended for human consumption (1).

There are many methods, both direct and indirect, available for the analysis of glucosinolates (2). In recent years the method generally used for the estimation of individual glucosinolates in rapeseed and elsewhere has involved enzymatic desulphation, volatilization, separation by GC and quantification using appropriate standards. Two particular shortcomings of the method are the breakdown of ω-methylsulphinylalkyl glucosinolates (typified by the behaviour of 3-methylsulphinylpropyl glucosinolate, glucoiberin) yielding multiple peaks and the failure of the method to deal with a recently described ring-hydroxylated indole glucosinolate (3).

To overcome these problems Minchinton et al. (4) have recently described a method for the qualitative analysis of desulphoglucosinolates using high performance liquid chromatography. The full potential of this method however, will only be realised if the method can be placed on a quantitative footing. To this end we have developed, independant of other workers, a method for the separation and quantitative analysis of these compounds. The method, summarised in Figure 1, gives excellent separation of the thirteen glucosinolate standards examined, in particular separating the early running glucoiberin from progoitrin (2-hydroxy-3-butenyl glucosinolate) and glucocheirolin (3-methylsulphonylpropyl glucosinolate). The method has been applied to the analysis of seed, root and leaf extracts and in agreement with Minchinton et al. (4) it is clear that neither ω-methylsulphinylalkyl- nor ring-hydroxylated indole glucosinolates behave anomalously. Retention times are reproducible (for example glucobrassicin varies by $\pm$ 0.04 min at 17.64 mins).

With the aid of suitable pure standards (5), glucosinolate response factors have been determined and applied for the quantitative analysis of glucosinolates in Brussel sprouts. Rather surprisingly, good correlation (0.962) was obtained between GC and HPLC estimation of glucoiberin, suggesting that the problems associated with the GC analysis of this compound may have been overstated. The correlation coefficients for progoitrin, sinigrin, gluconapin and glucobrassicin were respectively 0.957, 0.996, 0.970 and 0.994. Excluding hydroxy indole glucosinolate (absent in GC analysis) the correlation between total glucosinolates as determined by GC and HPLC was 0.977.

Response factors were found to vary much more widely for HPLC than for GC and moreover would be expected to differ between instruments. Thus the availability of standards is of crucial importance in the development of the method. The time of analysis, 30 minutes, is comparable to that required for GC analysis. However this may be speeded up considerably by suitable modification of the programme, an important requirement in the analysis of large numbers of samples, e.g. from breeding lines; moreover the method may be automated. No attempt has been made to examine these variables since they are not requirements for our present work. Instead attention is being paid to linking the chromatograph directly to a mass spectrometer thus enabling the chemical composition of complex glucosinolate mixtures to be resolved more effectively.

FIGURE 1

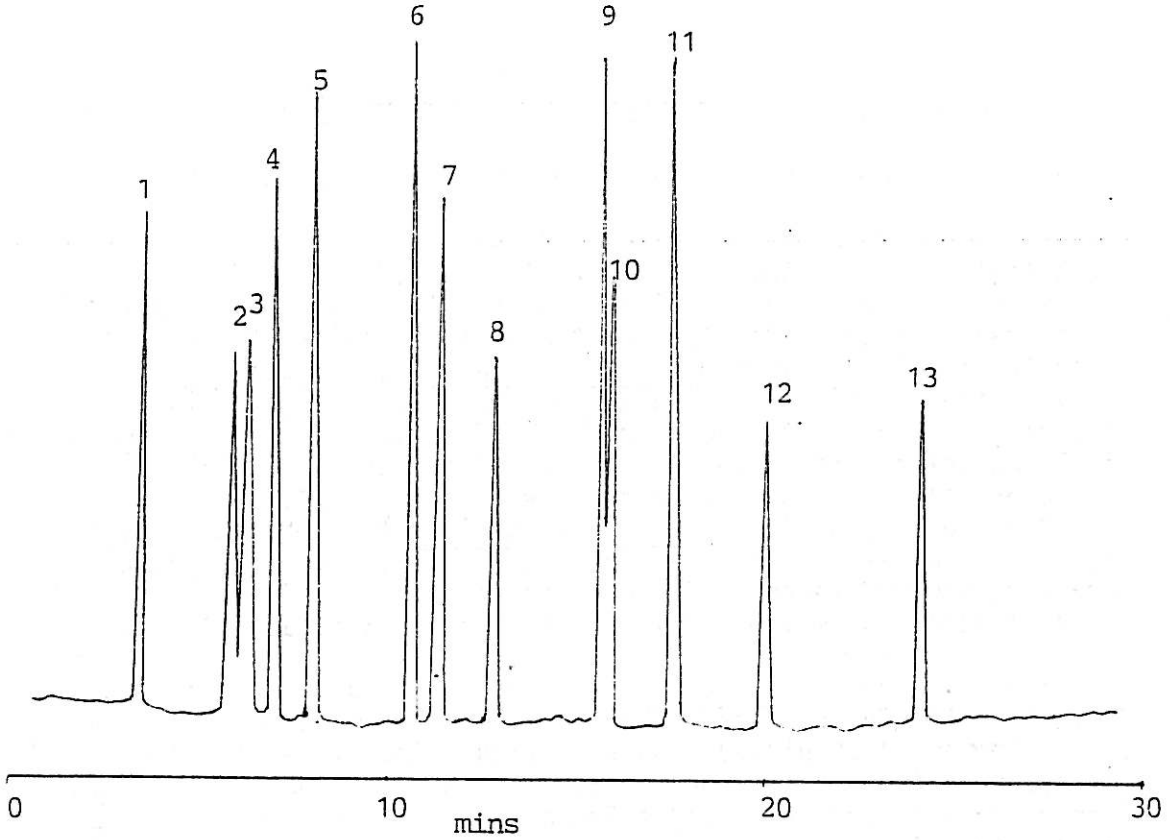


Fig. 1. HPLC chromatogram of 13 desulphoglucosinolate

The conditions were as follows: Spherisorb 5 ODS 2 column 250 x 4.6 mm, flow 1.5 ml/min; solvent A water, solvent B 20% acetonitrile in water; programme (i) 1 min at 99% A (ii) 20 minute linear gradient to 1% A:99% B (iii) 5 min linear gradient to 99% A : 1% B (iv) equilibration for 10 mins. The compounds were monitored at 230 nm.

Key

| | | |
|---|---|---|
| 1 | Methyl glucosinolate | (Glucocapparin) |
| 2 | 3-Methylsulphinylpropyl glucosinolate | (Glucoiberin) |
| 3 | 3-Methylsulphonylpropyl glucosinolate | (Glucocheirolin) |
| 4 | 2-Hydroxybut-3-enyl glucosinolate | (Progoitrin) |
| 5 | 2-Propenyl glucosinolate | (Sinigrin) |
| 6 | p-Hydroxybenzyl glucosinolate | (Glucosinalbin) |
| 7 | But-3-enyl glucosinolate | (Gluconapin) |
| 8 | Hydroxyindole glucosinolate | |
| 9 | Benzyl glucosinolate | (Glucotropaeolin) |
| 10 | 4-Methylthiobutyl glucosinolate | (Glucoerucin) |
| 11 | 3-Indolylmethyl glucosinolate | (Glucobrassicin) |
| 12 | 2-Phenylethyl glucosinolate | (Gluconasturtiin) |
| 13 | 1-Methoxy-3-indolylmethyl glucosinolate | (Neoglucobrassicin) |

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# RELATIVE GLUCOSINOLATE CONTENTS OF RAPESEED SEED AND SEEDLINGS

Helen Adams, John G Vaughan, Robert K Heaney and G Roger Fenwick

The identification of seeds of Brassica cultivars based upon morphological and anatomical information presents some difficulty.

Work carried out at the Food Research Institute and reported in Cruciferae Newsletter No 5[1] demonstrated the possibility of identifying Brussels sprout cultivars (Brassica oleracea L. var gemmifera) by studying the relative distribution of the four main glucosinolates occurring in the vegetative tissue. Differences in the relative amounts of glucosinolates found in sprout seeds indicated a possible use of this characteristic for distinguishing between cultivars[2].

Rape seed (Brassica napus L. var oleifera) has assumed great economic importance in Britain in recent years, current production exceeding 650,000 tonnes. The crop poses problems of cultivar identification for which a similar biochemical approach might prove useful.

62 accessions of B. napus representing 49 cultivars were obtained from the following sources:- Professor G S Tokumasu, Japan; Dr R K Downey, Agriculture Canada; National Institute of Agricultural Botany, Cambridge, U.K; The Swedish Seed Association, Svalöf; Statens Forsøgsstation, Denmark; Ringot France and INRA Station d'Amelioration des Plantes, France.

Air dried, defatted seed meal was analysed for the six main glucosinolates, gluconapin, glucolobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin and neoglucobrassicin by gas chromatography[3]. Figure 1 shows the upper and lower limits for the percentage each glucosinolate is of the total glucosinolate content of single zero oilseed rape and forage rape. Not only are the two different types of rape indistinguishable one from the other, but within each type no cultivar exhibited a 'glucosinolate profile' which could unequivocally distinguish it from any other cultivar.

The situation for "double zero" (low glucosinolate) oil seed rape is slightly different. Here it would appear that the cultivars can be split into two groups; those in which the percentage of progoitrin is less than that of neoglucobrassicin, and those with higher levels of progoitrin than neoglucobrassicin.

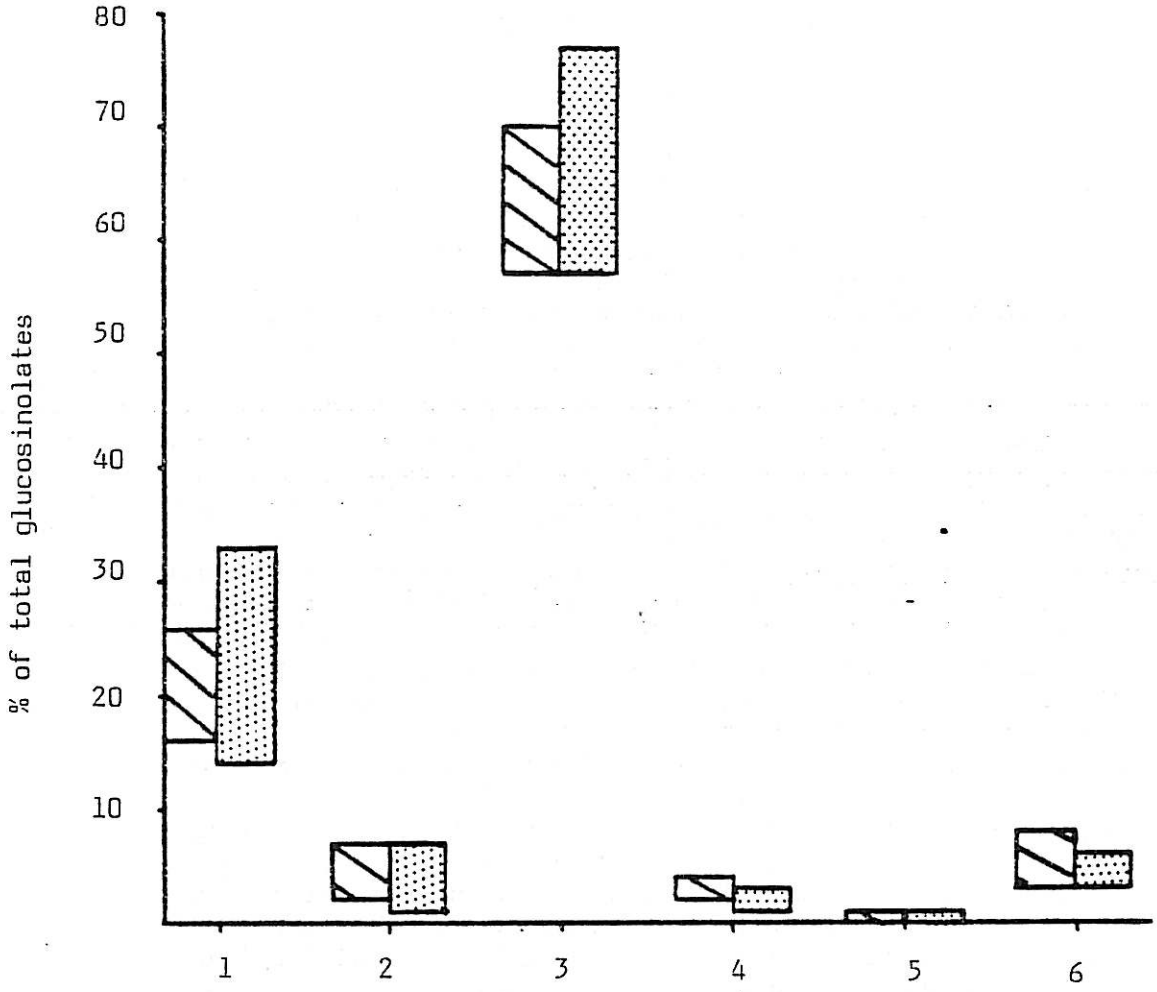
For several cultivars two or three accessions were available and comparisons made within each cultivar indicated that, as would be expected, the glucosinolate composition was more consistent when glucosinolates were represented as percentages than when actual levels were compared.

These findings are in agreement with earlier work in which 29 samples of high glucosinolate oilseed rape supplied by NIAB were analysed for the 6 main glucosinolates. All were shown to have similar 'glucosinolate profiles'. A further 13 samples having a more diverse background (some being of Polish origin and some of Japanese origin) and supplied by Dr K F Thompson, Plant Breeding Institute, Cambridge, U.K. showed the same basic distribution with no sample falling outside the extremes of the samples, previously supplied by NIAB.

Thus while seed glucosinolate patterns can be used to separate high or low glucosinolate rapeseeds they offer little potential for the identification of different rapeseed cultivars.

It was thought possible that leaves of rape seedlings might reflect the differences found in Brussels sprouts, and though more difficult to define ontogenetically they might prove to be a useful chemotaxonomic aid.

FIGURE 1




The upper and lower levels of the six measured glucosinolates expressed as a percentage of the total glucosinolates. Figures are obtained from 22 cultivars of oilseed rape and 11 cultivars of forage rape.

Key

1 gluconapin
2 glucobrassicanapin
3 progoitrin
4 gluconapoleiferin
5 glucobrassicin
6 neoglucobrassicin

high glucosinolate oilseed rape

forage rape

The top leaves of 4 week old seedlings of 32 cultivars of high glucosinolate oilseed rape were thus analysed, although some differences did exist between cultivars the results were difficult to reproduce in subsequent plantings. Thus the technical difficulties of such an approach to cultivar identification seem likely to outweigh any possible benefits.

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# THE PHYTOTOXICITY OF THIOCYANATE ION TO WEEDS

Bernard B. Bible

As part of a broader study of the possible allelopathic properties of cruciferous crop residues, three weed species were treated with thiocyanate ion to determine weed uptake of thiocyanate and effects on growth. Thiocyanate is a common constituent of cruciferous plants that is known to be phytotoxic (Ju et al.; Wu and Basler, 1969), and it could be one of the agents responsible for the allelopathic effects of crucifers previously reported (Bell and Muller, 1973; Campbell, 1959).

Field collected plants of Chenopodium album (lambsquarter), Portulaca oleracea (purslane) and Amaranthus retroflexus (pigweed) were transplanted to eight liter plastic pots containing a sandy loam, field soil. The weeds were grown in the greenhouse with two lambsquarter, two purslane and two pigweed plants in each pot.

Thiocyanate treatments, consisting of four thiocyanate concentrations (0, 5, 25, 125 mg/L) obtained by varying amounts of KSCN in nutrient solutions, were soil applied on seven occasions (100 ml of solution per application) beginning on September 14, 1982 and ending on October 5. Content of thiocyanate ion and plant weight were determined for foliage, stems and reproductive parts of the three weed species.

Seed heads of lambsquarter and leaves of purslane accumulated the highest amounts of thiocyanate ion, whereas stem tissue of all three weed species had the lowest thiocyanate ion accumulation. Increasing levels of thiocyanate in the treatment solution resulted in decreased growth of the three weed species. Accumulated contents of thiocyanate ion in the leaves of purslane, pigweed and lambsquarter that corresponded to significant reductions in plant growth were 150, 600, and 1,200 µg/g D.W. respectively. The uptake and accumulation of soil applied thiocyanate by the weed species and the resulting inhibition of growth suggests the capacity of thiocyanate to act as an allelopathic agent of crucifers.

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# EFFECT OF CRUCIFER LEAF EXTRACTS ON WEEDS

Bernard B. Bible and Barbara Stiehl

The release of thiocyanate ion by plant tissues, has been reported in a wide variety of cruciferous plants. Little evidence, however, is available concerning the possible allelopathic effects of thiocyanate ion.

Seeds of Chenopodium alba (lambsquarter) and Portulaca oleracea (purslane) were planted on March 16, 1983 and transplanted on April 15 to 1.25 liter pots containing sandy loam soil. The weeds were grown in the greenhouse with two lambsquarter and two purslane plants per pot.

Fresh leaves of tomato, cabbage and cauliflower were collected and extracted with water, 1 part leaf tissue; 2 parts water (weight:volume) in a blender, while radish leaves were extracted with, 1 part leaf tissue: 1 part water (weight:volume). The leaf extracts were tested for their thiocyanate ion content and held in a cooler at 5° C. until use. The leaf extracts were soil applied on six occasions (125 ml of each extract per application) beginning on April 28, 1983 and ending on May 20. The extract treated weeds were compared to control plants treated with water.

The leaf extract treatments had no significant effect on the growth of the lambsquarter and purslane plants compared to the control plants. However, lambsquarter and purslane plants from pots treated with crucifer leaf extracts did accumulate small amounts of thiocyanate ion (Table 1). The weeds treated with tomato leaf extract and the water control did not accumulate thiocyanate. Apparently, the amounts of thiocyanate in the cruciferous leaf extracts were not high enough to result in sufficient thiocyanate accumulation to inhibit growth.

Table 1. Influence of plant extracts on analyzed contents of thiocyanate ion in different tissues of lambsquarter (Chenopodium album) and purslane (Portulaca oleracea).

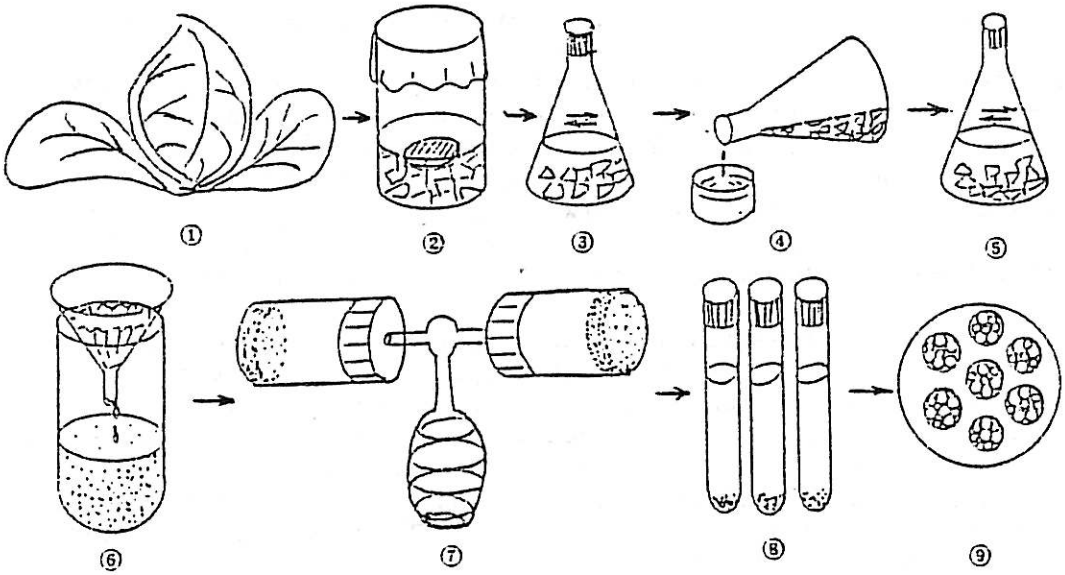
| Extract | | Thiocyanate ion (µg/g D.W.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Lambsquarter | | | Purslane | | |
| Treatment | µg SCN per ml | Leaves | Seed head | Stem | Leaves | Seed head | Stem |
| Control | - | nd[a] | nd | nd | nd | nd | nd |
| Tomato | trace | nd | nd | nd | nd | nd | nd |
| Cabbage | 16 | 67 | nd | 8 | 29 | nd | 4 |
| Cauliflower | 20 | 32 | nd | nd | 10 | 3 | nd |
| Radish | 6 | 15 | 6 | 8 | nd | 2 | 2 |

[a]nd is none detected

# PLANT REGENERATION FROM MESOPHYLL PROTOPLAST OF HAKURAN (INTERSPECIFIC HYBRID, *B.CAMPESTRIS* X *B.OLERACEA*)

K. Oosawa and K. Takayanagi

Protoplast manipulation is an essential technique for cell fusion as a tool for somatic hybridization. We have tried to prepare more active protoplasts from mesophyll tissue in *Brassica* vegetables. Among them Hakuran (cv. Shoren) produced active protoplasts and regenerated efficiently after 6 to 7 month-culture. Procedure for protoplast preparation is shown in Figure below.




1) Mesophyll tissue from mature leaves.
2) Leaves cut into pieces 1 $cm^2$ size were immersed in the enzyme solution in vacuum.

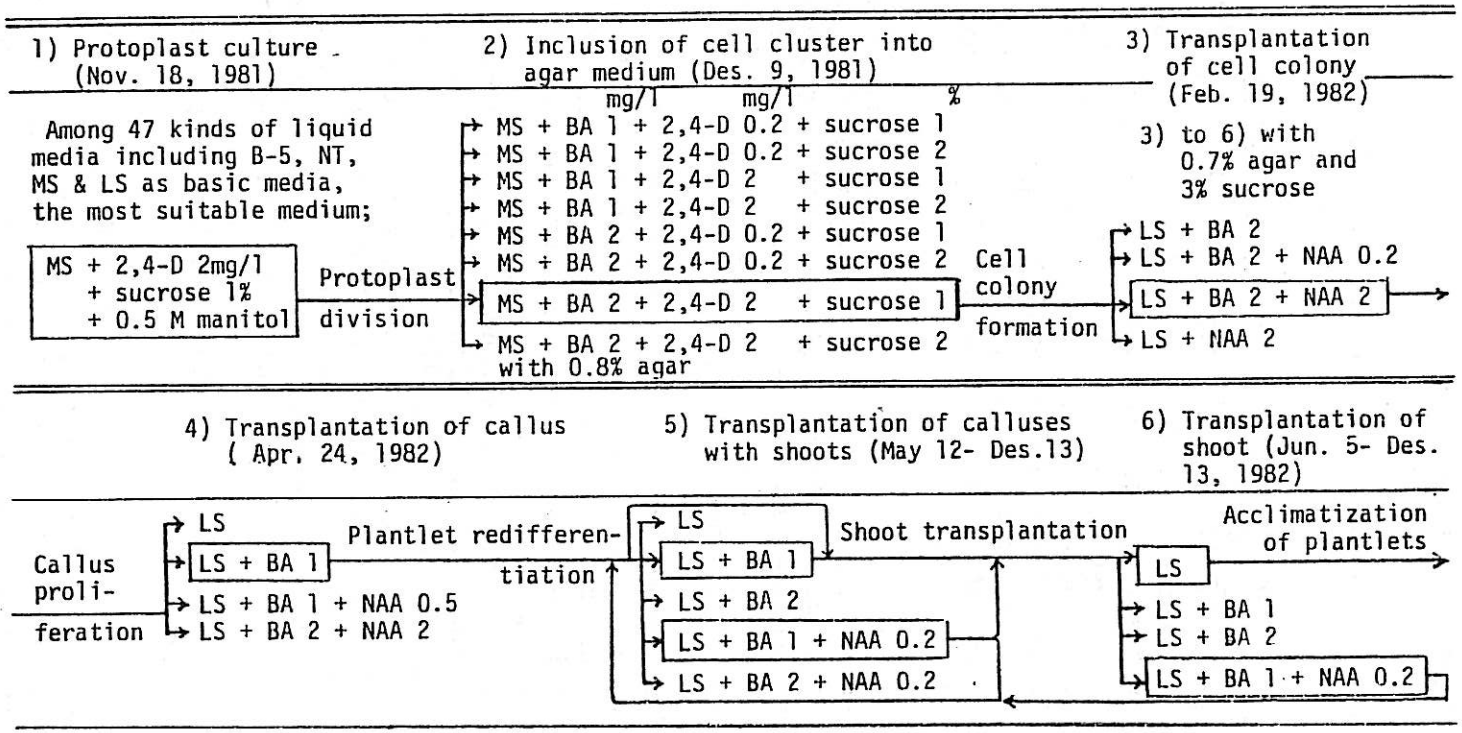
Enzyme mixture
- 0.1% Pectolyase Y 23
- 1.0% Driselase
- 1.0% Cellulase Onozuka RS
- 0.5M Mannitol

3) Incubated at 25°C for 5 to 10 min. with stirring 100-120 st/min.
4) Discarded enzyme solution and changed with the fresh enzyme mixture.
5) Stirred again at 25°C for 15 to 30 min., 60-80 st/min.
6)-8) Filtrated and centrifuged at 800 r.p.m.(100 xg), 2 min.
9) Washed and purified with 0.5M mannitol solution.

Protoplast culture (5 x $10^4$cells/ml) was successfully conducted with the media shown in Table 1. The most suitable medium was required at each step. Cultural environment was at 20°C, 12 hour-light (3,000lux) -12 hour-dark condition.

We could establish plant regeneration method from mesophyll protoplast of Hakuran. Now in the field we grow many Hakuran plants of which characteristics will be compared. Several plants regenerated from protoplast were already forming head and bearing normal flowers in the green house.

Table 1. Selection of suitable culture medium for plant regeneration from protoplast.




MS; Murashige & Skoog's basic medium, LS; Linsmaier & Skoog's basic medium, BA; 6-Benzyl aminopurine, NAA; Naphthalene acetic acid, 2,4-D; 2,4-Dichlorophenoxiacetic acid.

# REGENERATION OF PLANTS FROM ANTHERS OF CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA*)

C.G. Kuo, Susan Delafield, Calvin Chong and M.S. Chiang

Plants have been developed from anthers of cabbage, *B. oleracea* L. var. *capitata*, and broccoli, *B. oleracea* L. var. *italica* (Kameya and Hinata, 1970; Quazi, 1978) through the formation of pollen callus. However, the direct embryogenesis of cultured anthers is preferable since this development pathway requires less time and avoids the problem of aneuploidy (Keller et al., 1975). Although isolated anthers in some *Brassica* species have been successfully cultured to yield plantlets through direct embryogenesis (Keller et al., 1975; Keller and Armstrong, 1977 and 1978; Loh and Ingram, 1981; George and Rao, 1982), there is no report of the regeneration of plants from anthers of cabbage, *B. oleracea* L. var. *capitata*, through this development pathway.

As part of a research program to develop homozygous clubroot resistant and low glucosinolate cabbage, anthers of 14 breeding selections were cultured. These selections were obtained from the cross of rutabaga, *B. napus* L. and cabbage, and possessed clubroot resistance as well as different levels of glucosinolates. The anther culture method of Keller et al. (1975) was adopted with some modifications.

The plants were first grown at the Ste. Jean Research Station of Agriculture Canada, and then transferred to the greenhouse of Macdonald College prior to anther culture. Buds, collected at the onset of flowering before elongation of the inflorescence axis, were surface sterilized with filtered calcium hypochloride solution followed by 3 rinses with sterile, demineralized water in a laminar flow work bench. Five anthers of each bud were cultured in the basic $B_5$ medium, with 100 mg/l serine, used for *B. campestris* and *B. napus* (Keller et al, 1975; Keller and Armstrong, 1977). The 6th anther of each bud was fixed in Carnoi's solution and subsequently stained with propionic carmine in an attempt to identify pollen stages. Petri dishes with the cultured anthers were sealed with parafilm and subjected to elevated temperatures prior to culture at 25°C in total darkness. Three elevated temperatures were utilized: 35°C for one day, 30°C for 3 days, and 35°C for 1 day and then 30°C for 6 days. There were 100 anthers per elevated temperature per breeding selection. A total of 4200 anthers were cultured.

Anther-derived embryoids were moved from total darkness to 16-hour/day of fluorescent light (25°C) for 1 week, prior to transfer to hormone-free $B_5$. Plantlets with normal development rooted on the $B_5$ medium and were transplanted to peat pellets for acclimatization in environment with high humidity. Abnormal embryoids were transferred to a modified MS medium (Keller and Armstrong, 1977) for shoot production. Any resulting shoots were moved to the hormone free $B_5$ medium for rooting. After acclimitization all the plants were moved to a growth cabinet.

Currently 3 established plants of a breeding selection with clubroot resistance and low glucosinolate content were obtained. One of them was detected to be diploid by a chromosome count of a rooting squash. Other plantlets are being acclimatized and more than 20 shoots are being rooted.

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# USE OF ANTHER CULTURE IN BRUSSELS SPROUT BREEDING

D J OCKENDON

Now that anther culture is becoming widely used in rape breeding for the production of 'instant' inbred lines, (Renard & Dobsa 1980, Hoffmann et al 1982), there is increasing interest in the application of this method to other Brassica crops. Anther culture has been more fully studied in B. napus and B campestris than B. oleracea, but a detailed account of anther culture in green sprouting broccoli has recently been published by Keller & Armstrong (1983). This note reports the first successful production of embryos and regenerated plants from anther culture in Brussels sprouts. A more detailed account is in preparation.

## METHODS

The methods and media used were essentially the same as those of Keller & Armstrong (1983) and Keller et al (1975). Donor plants were raised in a glasshouse (10-20°C) or in a growth room (10°C night/17°C day). Buds about 4-5 mm long with a petal/anther ratio of 2/3 to 4/5 were removed from each plant before, or soon after the first flower opened. The buds were surface sterilised, and the anthers removed, taking care to remove as much of the filament as possible. Thirty anthers were placed in 9 cm petri dishes sealed with Nescofilm. Immediately after culturing the anthers were given a thermal shock at 30° or 35°C, thereafter cultures were kept in the dark at 25°C.

As soon as embryos emerged from the anthers they were transferred to Gamborg's B5 medium without growth substances and cultured in the light at 20°C. After 3-4 weeks the green thalloid-like structures which developed from the embryos were transferred to B5 medium with 2.0 mg/L benzyladenine to promote formation of shoot meristems. When these appeared, they were dissected out and transferred to B5 medium with dimethylallylaminopurine at 0.3 mg/L to encourage shoot growth. Well developed shootlets were recultured on B5 medium without hormones, and when good roots had developed were transferred to Jiffy 7 peat pellets in propagators for 3-4 weeks before being put in Levington compost in pots in a glasshouse.

## RESULTS

Embryos appeared 3-6 weeks after the start of anther culture and could easily be distinguished from the callus tissue which sometimes developed from the cut filament. The embryos varied greatly in size (1-5 mm long) and in stage of development (globular to torpedo). The largest embryos did not necessarily give the best regeneration, but regeneration was generally better with embryos which appeared early than with those which appeared later. Some embryos remained albino when cultured in the light, some produced callus, but about 1/3 gave green, differentiated structures from which plantlets could be obtained.

Table 1. Embryo yields from three cultivars of Brussels sprouts. The donor plants were raised in a glasshouse (GH) or growth room (GR).

| Plant | Location of donor plant | No. anthers cultured | No. embryos | Embryo yield per 100 anthers |
|---|---|---|---|---|
| cv Pinnacle x Nym $F_2$ | GH | 360 | 0 | 0 |
| " | GH | 210 | 104 | 49.5 |
| " | GR | 300 | 46 | 15.3 |
| cv Nym $F_1$ | GH | 420 | 1 | 0.2 |
| " | GH | 450 | 171 | 38.0 |
| " | GR | 1140 | 186 | 16.3 |
| cv Gower $F_1$ | GH | 480 | 556 | 115.8 |
| " | GH | 690 | 14 | 2.0 |
| " | GR | 690 | 403 | 58.4 |
| " | GR | 210 | 750 | 357.1 |

A sample of results is given in Table 1 to indicate the very great variation in response to anther culture. All these results were obtained with a treatment of 16 hr at 35°C, followed by 24 hr at 30°C, and thereafter at 25°C. Treatments of 48 hr at 35°C and 14 days at 30°C gave almost no embryos, indicating that the temperature and duration of the thermal shock treatment is of critical importance. Generally, donor plants grown in a growth room gave better results than those in a glasshouse, probably because of better control of conditions. cv Gower generally gave higher embryo yields than cv Nym, but even cv Gower gave very poor yields on some occasions.

About half of the plants regenerated were diploid, with most of the others haploid, although occasional triploid or tetraploid plants were found. Determining the ploidy level of the regenerants is not always straightforward. Root tips of different ploidy level are often found in the same plant, and the ploidy of the flowering shoots of a plant does not necessarily correspond with that of its root tips. Haploid and diploid shoots differ in guard cell lengths but there is considerable overlap, making it difficult to determine the ploidy level with certainty by this method. Once the shoots flower the difference is usually clear, with haploids having no anthers or pollen with very low stainability and no seed set, while diploids have pollen with high stainability and good seed set.

The diploids obtained so far have all arisen spontaneously. It is possible that these diploids arose from unreduced pollen grains, but these seem to be rare in B. oleracea. Provided that the diploids are spontaneously doubled haploids they will be homozygous. Selfed progenies will be raised from the spontaneous diploids to check that this is the case. When the haploids have been firmly identified as such they will be converted to diploids using colchicine, and these diploids will certainly be homozygous.

## DISCUSSION

The maximum embryo yield for Brussels sprouts (357 per 100 anthers) is similar to that for broccoli (270 per 100 anthers), but for Brussels sprouts the optimum thermal shock treatment was 16 hr at 35°C whereas for broccoli it was 48 hr at 35°C (Keller & Armstrong 1983). In both cases yield varied considerably between experiments.

Keller & Armstrong (1983) attributed their variation largely to genotypic differences, but it is difficult to distinguish between genotypic and non-genotypic variation. Although there was great variation in response within both of the $F_1$ hybrid Brussels sprouts tested, nevertheless a consistent genotypic effect was found, with cv Gower giving on average about three times more embryos than cv Nym. This genotypic effect could only be established reliably by repeated tests. More genotypes will therefore be studied to see how they differ in their response.

Although non-genotypic and genotypic variation is considerable, the results with anther culture show that for some Brussels sprouts genotypes it is relatively straight forward to produce large numbers of inbred lines for use in breeding programmes. The methodology of anther culture in Brussels sprouts is not yet as well developed as it is in rape, but shows great promise.

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# CHROMOSOMIC ANALYSIS OF REGENERATED PLANTS FROM PROTOPLASTS OF B. NAPUS AFTER FUSION TREATEMENT

C. PRIMARD - F. EBER

Several cytoplasmic male sterility systems are under studies in B. napus in order to create hybrid varieties. The introduction of the cms japanese radish cytoplasm of Ogura in the genus Brassica (oleracea, napus and campestris) gave very complete cms plants but having two major defects ; one of these is a thermosensitive chlorophyll deficiency, maternally inherited with the male sterility trait.

As it is now possible to regenerate whole plants from mesophyll protoplasts of in vitro grown plants of B. napus, two fusion experiments wereperformed (Pelletier & al, 1983).

I - Fusion between protoplasts of B. napus var. Brutor and protoplasts of B. napus var "C" with cms and deficient radish cytoplasm. Out of 131 regenerated plants able to flower, 7 showed a new combination of cytoplasmic traits : 5 were male sterile and normally green and 2 half fertile and green. The others were of either one or the other parental types.

II - Fusion between protoplasts of B. napus var "C" with cms and deficient radish cytoplasm and protoplasts of B. napus var "TOWER" with B. campestris cytoplasm having triazine resistant chloroplasts. Out of 83 regenerated plants able to flower, only one was male sterile and normaly green.

Studies of DNA mt and ch showed that the complete cms and green plants had the chloroplasts of the fertile parent of fusion. Observations over three generations indicated that these new cytoplasmic associations were stable.

The chromosomic analysis was done on a sample of regenerated plants of the parental type and on the plants having the hybrid cytoplasm and their first progeny. Observations were made at the meiosis stage in young anthers as described by Rousselle and Eber.

Concerning the chromosome number, among 20 plants of parental type, 4 plants were not diploïds (38 chromosomes), but aneuploïds with 39, 40, 41, 54 chromosomes ; the first one excepted, these plants exhibited some abnormalities in their developments. In some cases, the young regenerated shoots wer subcultured in vitro and so formed clones of 2 to 4 plants of the same genotype. Out of 9 clones, one is heterogenous with one diploïd and one aneuploïd plant (39); the others are diploïds.

It is a general observation that in vitro tissue culture of B. napus tends to induce aneuploïdy. The technique of regeneration from protoplast has to be as quick as possible. Among the plants resulting from a fusion event, 2 are diploïds and are true cybrids (only one nucleus was kept in the fusion product). One is aneuploïd with 63 chromosomes and the other 3 are tetraploïds ; the nuclear markers (erucic acid content and the base petal width) were not accurate enough to make sure either that nuclear fusion occured or that these ploïdy levels are a consequence of tissue culture (just refering to the cytoplasmic traits, we call them cybrid plants).

Concerning the chromosome behavior in all regenerated plants, we could observe a great number of cells having a pair of univalents ; but this also occurs in the parental plants coming from seedlings, even in the pure line var. "Brutor".

The progeny of the non diploïd cybrids, obtained by back-cross with the fertile diploïd line, covers a range of chromosome number from 42 to 68. Fixation by accelerated generations at the diploïd level is undertaken and seems without any problem ; these cybrids are now included in breeding programs. The second major defect studied now is the stabilisation of genes of fertility restauration.

Progeny of the diploïd regenerated plants will be analysed in the field to detect any variation of the expression of few caracters.

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Acknowledgement

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# A MEDIUM FOR GERMINATING BRASSICA POLLEN IN VITRO

T. Hodgkin

Hodgkin, Marr and Wiseman (1982) obtained a marked increase in the percentage germination of Brassica oleracea pollen in hanging drop cultures by including 3.5 mM ammonia in solution in the culture medium. They suggested that the improvement was due to the high pH of the medium (increased from pH 5.5 to pH 8.6). This note reports some results of continuing experiments at SCRI to define the optimum pH for germination and to compare different buffers.

The basic germination medium used contained 0.585 M sucrose, 2.54 mM Ca $(NO_3)_2$, 1.62 mM $H_3BO_3$, 0.99 mM $KNO_3$ and 0.88 mM $MgSO_4.7H_2O$. Under our conditions this medium has pH 5-6 and gives 20-30% germination. Germination percentages were calculated from counts made after incubation of pollen in hanging drops for 4 h at 20°C or 25°C, tubes with a length greater than the diameter of the pollen grain being scored as having germinated. Including Tris or Phosphate buffers (10-40 mM, pH 7-9) in the medium gave no significant increase in percentage germination but a number of the "Goods" series of buffers (Good et al., 1966) gave a marked improvement in germination when incorporated in the medium. The best results were obtained using 20 mM TAPS buffered to pH 8 with M NaOH. Increasing the molarity of the buffer caused a sharp drop in percentage germination and tube length as did lowering or raising the pH outside the range 7.6 to 8.4 (Table 1).

Table 1. Percentage germination of Brassica oleracea pollen at varying pH after 4 h incubation at 20°C (mean of 3 experiments).

| | Control | Buffered with TAPS | | | | |
|---|---|---|---|---|---|---|
| pH | 5.5 | 7.2 | 7.6 | 8 | 8.4 | 8.8 |
| | 30 | 40 | 65.5 | 87 | 70 | 38 |

Roberts et al. (1983) have recently reported that they obtained satisfactory germination of Brassica oleracea pollen using a germination medium which contained 1mM Tris and had pH 9.0. In comparative experiments at SCRI the medium of Roberts et al. gave 60-70% germination, significantly lower than that obtained using a TAPS buffered medium (80-90%). Preliminary results suggest that a TAPS buffered medium is also suitable for germinating B. campestris and B. napus pollen.

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