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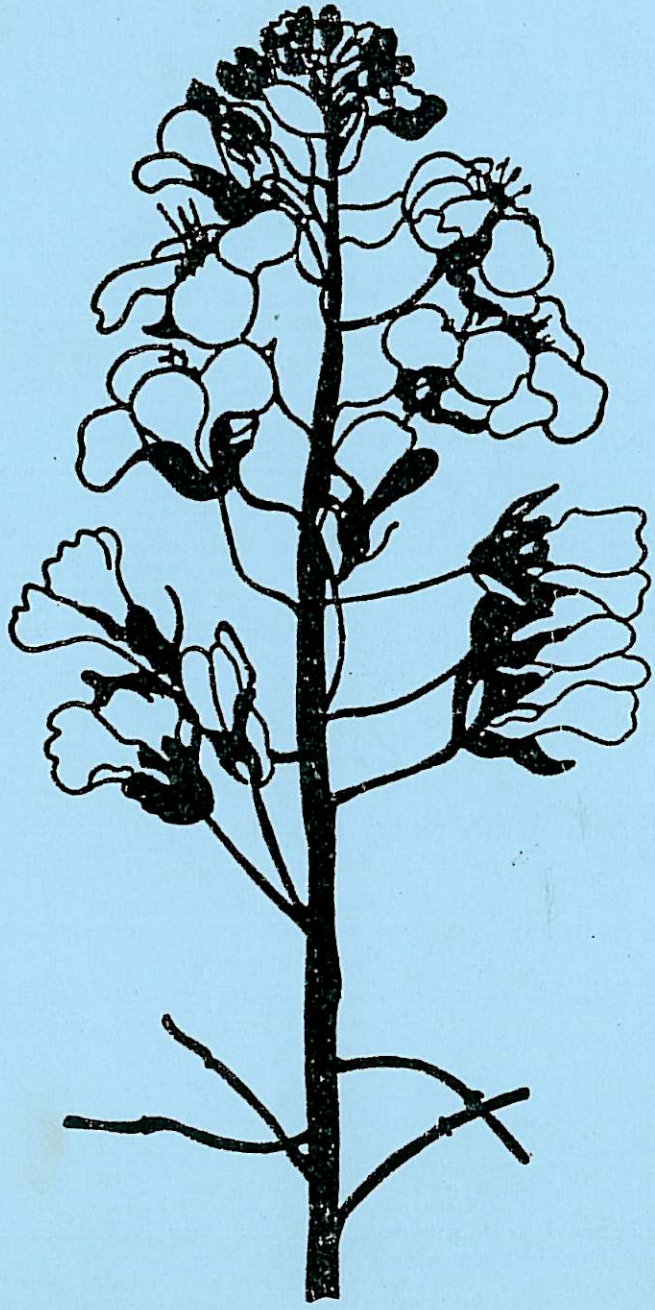
# CRUCIFERAE NEWSLETTER No.10



November 1985

EUCARPIA

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## Editorial

Ian McNaughton retired from the Scottish Crop Research Institute in February, 1985. He had been involved for several years with the production of the Newsletter as joint Editor and he will be missed as a colleague and friend by many of our contributors and recipients. Toby Hodgkin, also of SCRI, has agreed to assist with the editorship in his place.

The number of persons and/or organisations receiving the Newsletter has risen to 447. Many have had to be disappointed in their request for back numbers as most stocks of the earlier issues have been exhausted for some time. In order to satisfy these requests we have decided to re-copy the out-of-stock issues and they will be sold to recover costs of photocopying and mailing them. It would be appreciated if orders (form enclosed with this Newsletter) could be placed as soon as possible in 1986, although copying may not be completed until late in March. Also enclosed are the amendments to the mailing list.

There was a substantial increase this year in the number of papers received. This is a gratifying indication that the Newsletter is fulfilling a useful purpose, but it also means that we must watch production costs very carefully, as well as the length of individual contributions, in order to be able to maintain the current free distribution. Cooperation by intending authors in achieving this aim would be very helpful and this point will be emphasised in the revised instructions that will accompany the next call for papers.

The cost of producing and mailing Newsletter No. 9 and the call for papers for No. 10 was £620. After adding donations received during the year the balance in the Newsletter fund stands at £1840.

## Acknowledgements

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

Agrigenetics Corporation
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# CRUCIFER GENETICS COOPERATIVE

Paul H. Williams

The Crucifer Genetics Cooperative (CrGC) was established in November, 1982 in the Department of Plant Pathology at the University of Wisconsin, Madison for the purpose of acquiring, maintaining, and distributing seed stocks and pollen of various crucifers and of crucifer-specific symbionts. Emphasis is on genetic, chromosomal and cytoplasmic variants useful for expediting a wide range of research in breeding, cytogenetics, genetic engineering, molecular biology, plant physiology, entomology, phytopathology, ecology and other biological studies. Included among the crucifer symbionts would be parasites, pathogens and pests.

Seed, pollen, and symbiont cultures are received from those who have unique genetic stocks and who would like to share them with others interested in crucifer genetics. Annual summaries of CrGC activities are submitted through the EUCARPIA, CRUCIFERAE NEWSLETTER. All persons providing genes and stocks to the CrGC will be recognized as the source of those traits.

## CRUCIFER GENETICS COOPERATIVE RESOURCE BOOK

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

## OPERATION OF THE CRUCIFER GENETICS COOPERATIVE

In order to partially defray the costs of operating the CrGC, individual members are encouraged to pay a subscription fee of $25.00 covering a three-year period. Membership subscriptions should be made out to Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin. Upon receipt of the fee, subscribing members will receive the CrGC Resource

Book complete with all current IDs including current seed stock lists. Subscribing members will also receive a mailing list of the membership which includes a description of members' professional interests. All members, subscribing and non-subscribing, are entitled to seed stocks of the CrGC without charge. Non-subcribing members may also obtain individual IDs without a charge.

## ACTIVITIES OF THE CRUCIFER GENETICS COOPERATIVE 1984-1985

Membership in the CrGC exceeds 300 representing 24 countries with professional interests in genetics, plant breeding, molecular, cell, population, and developmental biology, ecology, biochemistry, pathology and teaching. From 1984 through August 1985, 525 seed requests were filled. Since its initiation in 1982, there have been 122 requests for CrGC-1 to 7; 125 requests for CMS substitution stocks, 230 requests for multiple disease resistant 'Badger Inbred' cabbage and Chinese cabbage breeding stocks and 86 requests for CrGC-1. Currently there are 115 subscribing members and 4 sustaining members of the CrGC.

On May 30 and 31, 1985, the CrGC sponsored a Crucifer Genetics Workshop at the University of Wisconsin-Madison. One hundred and twenty attended the workshop. Thirteen oral presentations, 28 poster, and 5 discussion sessions were held, covering a wide range of topics including breeding, genetics, cell biology, molecular biology and bioindustrial and agronomic usage. A 26 page summary of the CrGC workshop presentations is available from the CrGC upon request.

Persons interested in participating in the CrGC should write Paul H. Williams, Crucifer Genetics Cooperative; Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706 [(608) 262-6496].

## REFERENCES

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# CLUBROOT NEWSLETTER

G. R. Dixon

In his Editorial to Clubroot Newsletter No. 1, Hille Toxopeus indicated how it had originated from a series of circular letters which he had sent out for the "clubroot discussion group - coding of races." From the work of this group the European Clubroot Differential (ECD) series developed. The Newsletter and ECD series have always been very closely interlinked, both being given great stimulation into activity by the Eucarpia Cruciferae 1974 Conference in Dundee, Scotland. As a consequence Clubroot Newsletter has largely functioned as a vehicle by which to circulate results from ECD tests. This has been an extremely valuable function since it ensured results were freely available to interested parties without impairing the abilities of individuals to use their results for more formal publications. The ECD series and data obtained with it has resulted in a large number of publications and considerably advanced our knowledge of the host-pathogen relationship between members of the Cruciferae and Plasmodiophora brassicae. Following ten years of intensive use it is likely that any technique or tool will itself require re-evaluation and perhaps redesigning. This is certainly now true of the ECD series. The question then arises as to how viable is the Clubroot Newsletter? Contributions other than those concerned with ECD data have been few and far between so that the publication is unlikely to be viable on its own. Following discussions at Cruciferae 1984 held at St. Andrews, Scotland, those who received Newsletter No. 14 (January 1985) were asked to vote on the suggestion of a merger with Cruciferae Newsletter. Results were:

| | |
|---|---|
| In favour of a merger | 16 (Yes) |
| Against a merger | 9 (No) |
| Abstentions | 4 |
| Leave it to you (!) | 1 |

The motion is therefore carried by 64% of those voting yes or no.

With the generous agreement of the Editors of Cruciferae Newsletter in future anyone with suitable results and information concerned with clubroot should submit it to them.

The Clubroot Working Group will continue as a means by which meetings of clubrooters can be organised and linked with larger congresses, symposia or conferences. I have been in contact with the organisers so that meetings can be held at:

1. International Horticultural Congress, California, U.S.A., 11-20 August 1986. Anyone not yet receiving information on this Congress should contact: Congress Secretariat, 22nd International Horticultural Congress, Attention Carolyn Norlyn, Campus Events and Information Office, University of California, Davis, CA 95616, U.S.A.

2. International Plant Pathology Congress, Kyoto, Japan 1988.

These arrangements should not inhibit anyone who wishes from holding other meetings concerned with clubroot. I am only too happy to help with such meetings and my mailing list can be sent out very quickly.

It is important to ensure that ECD seed continues to be available to those wishing to use it. A not inconsiderable amount of work has been done by Hille Toxopeus at SVP, Wageningen, The Netherlands, to ensure that everyone requesting seed received it quickly. It is now suggested that stocks of this seed should be moved to the Gene Bank, National Vegetable Research Station, Wellesbourne, Warwick, U.K. Negotiations between NVRS and SVP are almost complete, in the meanwhile requests for seed should be sent to me in the usual way.

Finally, I pay considerable tribute to Dr. Peter Mattusch, Institut für Pflanzenschutz im Gemüsbau, Hürth-Fischenich, Federal Republic of Germany. Peter took on the task of collating all ECD data as sent in by collaborating workers into a standard format for publication in Clubroot Newsletter. In addition to this time consuming task he has also organised publication of the last two editions of the Newsletter by courtesy of the German authorities.

# CHOI CROPS: A PROPOSED COMMON NAME FOR *BRASSICA RAPA* L.

Robert Prescott-Allen

In response to the call of Toxopeus, Oost and Reuling (1984) for a common name for *Brassica rapa* L., equivalent to cole crops for *B. oleracea* L., I suggest: choi crops.

Justification of this proposal is as follows:

1. A name of Chinese origin is appropriate, given that China is the region of varietal differentiation in *B. rapa*, corresponding to the role of Europe in the differentiation of *B. oleracea*.
2. Choi is Cantonese for vegetable (apparently excluding pulses except when sprouted), of which the brassicas are the most important in China. It is used in the names of *B. rapa* crops--for example, pak choi (ssp. *chinensis*), choi sam (vegetable heart, *B. parachinensis* Bailey), sieuw choi (ssp. *pekinensis*), yau choi (ssp. *oleifera*)--as well as in those of *B. juncea* (L.) Czern. (kaai choi) and other vegetables (Anderson and Anderson 1977).
3. Adopting useful words from other languages is common practice. Narrowing the meaning of the adopted word (in this case restricting the meaning of choi to *B. rapa* vegetables) is also common. For example, the meaning of the Latin *caulis* (stalk) was narrowed when it was used to form the English word "cole".
4. Choi is easy to pronounce in English (easier than the Mandarin ts'ai), and its spelling could be anglicized to choy if preferred. It can also be used readily in compound formations: for example, "radichoi" as a common name for x *Brassicoraphanus* genome AARR (*B. rapa* x *Raphanus sativus* L.), corresponding to "radicole" for x *Brassicoraphanus* genome RRCC (*R. sativus* x *B. oleracea*). Radichoi is thus also proposed here as a common name, in response to Oost (1984).

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Toxopeus, H., E.Oost and G.Reuling. 1984. Current aspects of the taxonomy of cultivated *Brassica* species. *Cruciferae Newsletter* 9: 55-58.

# A CULTIVAR GROUP CLASSIFICATION OF BRASSICA RAPA L.

Hille Toxopeus and Emiel H. Oost

This paper summarizes the progress made in our effort to develop a practical, yet scientifically sound infraspecific classification of B. rapa L. based on cultivar groups. Table 1 shows the present state of cv group formation as it was developed from the initial proposal (Toxopeus et al., 1984) on the basis of reactions and further studies. For a more detailed account of the scope and problems of cv group classification as exemplified by B. rapa L. reference is made to Oost (1985) and Oost and Toxopeus (in press).

We are not in favour of introducing the name 'choi' crops as a common name for B. rapa crops as suggested by Prescott Allen elsewhere in this Newsletter. This Cantonnese word means 'vegetable', it is not specific for B. rapa crops, not even for Brassica crops.

Table 1. Proposed cultivar group classification for Brassica rapa L. (syn. B. campestris L.)

| Name of cv group | shared character | use |
|---|---|---|
| **Vegetable Turnip** | turnip | vegetable |
| **Fodder Turnip** | forms rosette of leaves | fodder |
| **Winter Turnip rape** | biennial | oilseed |
| **Spring Turnip rape** | annual | oilseed |
| **Yellow Sarson** | annual, yellow seeds siliques usually multivalved | oilseed |
| **Pe Tsai** | heading, petioles winged | vegetable |
| **Pak Choi** | non heading, petioles conspicuous fleshy | vegetable |
| **Mizuna** | non-heading, leaves pinnate | vegetable |
| **Komatsuna** | thin, spatulate leaves | vegetable |
| **Brocoletto** | annual, flowering head enlarged | vegetable |
| Provisional cv groups | | |
| **Rapola** | annual, seeds 'double low' | oilseed |
| **Taku Tsai** | rosette of small blistered leaves, petioles conspicious, fleshy | vegetable |
| **Turnip greens** | ? | vegetable |

**Spring Turnip rape**
The cultivars of the Indian oilseed crops toria and brown sarson are included for lack of a shared character to separate them from the European and American forms. Mr Baillargeon from Canada has suggested that the 'double low'(seeds low

in erucic acid and low in glucosinolate content) cultivars of Spring Turnip Rape should be classified into a (provisional) cv group named Rapola.

**Yellow Sarson** (B. rapa subsp. trilocularis (Roxb.) Hanelt*)
This group was previously named Sarson. Breeding work is in progress to develop yellow seeded 'double zero' cultivars which, in the event, should probably be grouped in the Rapola cv group if this is confirmed.

**Pe Tsai** chinese cabbage, B. rapa subsp. pekinensis (Lour.) Hanelt*)
This group contains a rather large number of cultivars and although there is some variation in the formation of the head there is no reason at this time to split the group into smaller units.

**Pak Choi** (B. rapa subsp. chinensis (L.) Hanelt*)
We believe that there are grounds to propose a provisional cv group of the name Taku Tsai (or Ta Tsai?) the plants of which are considerably smaller than Pak Choi, forming an open rosette with numerous blistered leaves. Cultivars of this group are generally referred to B. narinosa Bailey, B. rapa subsp. narinosa (Bailey) Hanelt or B. chinensis var. rosularis Tsen et Lee.
The status of cultivars in B. parachinensis Bailey (mock pak choi, tsai sin) is not clear to us. The plants look like Pak Choi but they are apparently cultivated for their edible flowering stalk.

**Mizuna** (potherb mustard, B. nipposinica Bailey, B. japonica Makino nom. illeg., non B. japonica (Thumb) Sieb.*)
Dr Ohkawa proposed that the name Mizuna is given to this group, however, there is need to clarify the position of mibuna, a form that apparently produces entire leaves but is treated as part of this group (Anonymous).

**Komatsuna** (tendergreen, mustard spinach, B. pervirides Bailey*)
This appears to be a Japanese form of turnip greens.

**Brocoletto** (brocoletto di rapa, cyme, B. ruvo Bailey*).
A parallel variation of broccoli (B. oleracea L.)

The provisional group **Turnip Greens** is still not clear, who will enlighten us?

Those of you who are interested in this subject kindly give us comments and observations. Please remember that this classification is rooted in the International Code of Cultivated Plants and that it is dynamic. It is based on ample consideration, but open to improvement.

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* The latin infraspecific names are merely used to indicate overlapping taxonomic units, they are not in any way equivalent or synonymous with the corresponding cv group since they are essentially different from the botanic taxa.

# RAPHANUS BOISSIERI AL-SHEHBAZ, AN ILLEGITIMATE SYNONYM FOR QUIDPROQUO CONFUSUM GREUTER & BURDET.

Guy Baillargeon

The perfectly valid name *Quidproquo confusum* published by Greuter & Burdet (1983) for a plant endemic to Lebanon, Israel and Syria (Baillargeon, 1985) has been recently rejected by Al-Shehbaz (1985) who describes the taxon as a new species under the name *Raphanus boissieri*. This last name is illegitimate and cannot be used. Al-Shehbaz justifies his rejection of *Q. confusum* by referring to art. 37.1 of the Code (Voss & al., 1983) which states that a nomenclatural type must be designated to validate the name of a new taxon published on or after Jan. 1, 1958. But because Greuter & Burdet did not publish a new taxon at all, art. 37.1 does not apply to the present case. *Q. confusum* is a replacement name (*nomen novum*), an avowed substitute for an illegitimate later homonym published by Schulz (1919).

O.E. Schulz has been the first author to recognize that Boissier (1849) and Boissier (1867) was confounding two very different species under the name *Raphanus aucheri* (Boiss.) Boiss. This last name goes back to *Brassica aucheri* Boiss. (1842) for which the specimen *Aucher 203* (G) is the holotype (see Baillargeon, 1985, for a photograph). By putting the words "*quoad pl. Auch.*" (= as regards the plant of Aucher) after the combination *Sinapis aucheri* (Boiss.) O.E. Schulz, Schulz (1919 p. 135) clearly stated that the holotype *Aucher 203* was included under this name. Simultaneously, by putting the words "*excl. pl. Auch.*" after his use of the name *Raphanus aucheri*, Schulz (1919 p. 209) clearly stated that the specimen *Aucher 203* does not belong to this second species. According to art. 48.1 of the Code (Voss & al., 1983) when an author adopts a name referring to an apparent basionym (here *Brassica aucheri*) but explicitely excludes its type (here *Aucher 203*), he is considered to have published a new name and this new name must be ascribed solely to him (here *Raphanus aucheri* O.E. Schulz). In the present case, *Raphanus aucheri* O.E. Schulz (≡ *Q. confusum*) is a later homonym of *Raphanus aucheri* (Boiss.) Boiss. (≡ *S. aucheri*)

and is therefore illegitimate. The publication of the new name by Schulz is in the same time perfectly valid: There is a good Latin description, two illustrations of the fruit characters, and moreover six different specimens (syntypes) are cited. There is only the need for a new legitimate name. Greuter & Burdet (1983) were therefore perfectly justified in giving the plant a new specific epithet. This is a straightforward application of art. 72.1b of the Code (Voss & al., 1983). Baillargeon (1985, with a photograph) has already designated a lectotype (*Bornmüller 114*, W) from the six syntypes.

Simultaneously with the application of the new epithet, Greuter & Burdet (1983) created a new monotypic genus to accommodate the plant. As pointed out by Al-Shehbaz (1985), they did not provide any supporting evidence for the distinctness of *Quidproquo* from *Raphanus*, but such evidence is nomenclaturally not needed and the new generic name is valid. Those who disagree will have the choice to transfer the specific epithet "*confusum*" to the genus *Raphanus* (for example), or to any other genus judged to be appropriate. At the present time, the only evidence that *Q. confusum* would be better placed under *Raphanus* is the absence of valves in the fruit. This character could have evolved in two different genera as a result of convergence or parallelism. Apart from macro-morphological and distributional data, nothing is known about *Q. confusum*. It is therefore wiser to wait until more biosystematical data have been gained on this plant before burdening the literature with any new combination.

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# Classification of Leafy Vegetables in Brassica campestris L. in Japan

H. Yamagishi, S. Yui and M. Ashizawa

Last year Toxopeus et al. put forward the proposals on the classification and nomenclature of Brassica campestris L. Among B. campestris crops, leafy vegetables have developed mainly in China and Japan, and enormous variation can be found in these countries. On the other hand, it is supposed that for European workers the classification of them is very difficult because of no practical cultivations in Europe.

Here we will show the Japanese workers' traditional classificaion of leafy vegetables in B. campestris in order to compare with that of Toxopeus et al. And we will comment on their proposals and questions about the leafy vegetables. And finally we will describe the new breeding trends in Japan.

In this article we use the nomenclature 'B. campestris' to call the species following the traditional manner in Japan. Concerning the intraspecific variation in B. campestris, there are various opinions about the level of classification such as species, subspecies and variety. The Japanese Society for Horticulture Science use the word 'group' to classify in B. campestris. So we will also follow that here.

## 1. Botanical and agronomical classifications of B. campestris L.

On agronomical view point, we have three kinds of vegetables in B. campestris in Japan. They are Chinese cabbage, turnip and 'Tsukena'( in Japanese and 'Chinese mustard, salt green or mustard green etc.' in English). This agronomical classification does not agree with the botanical one. For example, in pekinensis group there are continuous variations of plant type from complete heading to non-heading. Among them heading and semi-heading types are classified into Chinese cabbage and dealt as one of major vegetables. And non-heading type is classified into 'Tsukena'. Also in rapifera group there are wide variations in the root and leaf shape. Among them we classify 'Komatsuna', the root of which doesn't become so large, into 'Tsukena', while we call the root swollen types as turnip even if we use only the leaves of them. Further we have both leafy vegetable and oil crop in campestris group. Usually in Japan we define 'Tsukena' as 'Non-heading leafy vegetables in B. campestris L.'

## 2. Classification of 'Tsukena' in Japan

Table 1 shows the classification of 'Tsukena' by Japanese workers and its relation to the 'cultivar group' of Toxopeus et al.

Usually 'Tsukena' is divided into seven groups and each group has many land races in it. In Table 1 we show also the typical varieties in each group.

Although 'Tsukena' is one of oldest vegetables in Japan, the introduction age is different group to group. Komatsuna, Natane and Mizuna groups have very old history in Japan. Especially Mizuna group is said to have developed its unique characteristics in Japan. On the other hand, Taisai, Saishin and Taasai groups are relatively new faces. In these groups new varieties such as 'Pak-choi' in Taisai group were introduced as so-called 'Chinese vegetables'recently, and are expanding their cultivation area rapidly.

Table 1 Classification of leafy vegetables in B. campestris and its relation to that of Toxopeus et al.[a]

| Scientific group name | Japanese group name | Typical varieties | Cultivar group of Toxopeus et al. |
|---|---|---|---|
| *campestris* | Natane | Shigatsuna<br>Hatakena<br>Kanzaki-hanana | Winter turnip rape |
| *rapifera* | Komatsuna | Kukitachina<br>Komatsuna | -------[7] |
| *japonica* | Mizuna | Mizuna<br>Mibuna | -------[5] |
| *chinensis* | Taisai | Seppaku-taisai<br>Shigatsu-shirona<br>Pak-choi | Pak-choi |
| *parachinensis* | Saishin | Koosaitai<br>Saishin | Brocoletto[6] |
| *narinosa* | Taasai | Hisagona<br>Kisaragina<br>Taasai | |
| *pekinensis* | Hikekkyu-Hakusai | Mana<br>Hiroshimana<br>Oosaka-shirona | Pe-Tsai |

a Classification is based on mainly Nagayoshi(1982) and Ashizawa(1982).

3. Several comments on the proposals and questions of Toxopeus et al.

1) The common name of B. japonica Makino is Mizuna in Japanese and there are two sub-groups in it, Mizuna and Mibuna. The former has pinnate leaves but the latter has slender and entire leaves. Both sub-groups don't have head. So their 'shared character' must be corrected. The most remarkable characters of this group is high tillering habit.

2) Saishin group is an annual flowering type and we eat young inflorescences and stems. 'Brocoletto' is supposed to be Saishin, but its flowering head is not so large as broccoli. It doesn't need low temperature to bolt. On this bolting habit, it resembles annual turnip rape, but leaf characters are completely different from it. Although usually Saishin is dealt as an independent group( parachinensis ), some workers think it as a sub-group in Taisai( chinensis ).

3) According to the description of Bailey(1930), Brassica pervirides Bailey is estimated to be Komatsuna. Komatsuna is considered to have developed from turnip but only leaves are used. Leaf blade is oblong and dark green. The tap root doesn't become large.

4) B. narinosa Bailey corresponds to Taasai. This name 'Taasai' is a Chinese one. In Japan we have a little bit old varieties such as 'Hisagona and Kisaragina' introduced about fifty years ago from China. They have similar characteristics to Taasai recenly introduced as one of Chinese vegetables. So we use here the name 'Taasai'. Taasai has

a rosette or semi-rosette plant type and many leaves. Leaf blade is round and very dark green.

4. Inter-group hybrids of 'Tsukena'

In B. campestris there are no barriers for the inter-group reproduction. So several varieties have been developed from the inter-group crosses. Table 2 shows the examples of them. These varieties are pure-bred ones and have more or less similar characteristics to one of the parents. Recently there are new movements to use the inter-goup hybrids directly as $F_1$ hybrid varieties. This new trend will make the classification of leafy vegetables in B. campestris more and more difficult.

Table 2 Example of varieties derived from inter-group crosses in B. campestris[a]

| Variety | Cross combination |
|---|---|
| Nagasaki- Hakusai | *pekinensis x narinosa* |
| Oosaka-shirona | *pekinensis x narinosa* |
| Vitamine-na | Oosaka-shirona x *narinosa* |
| Oosakina | *rapifera x japonica* |
| Yukina | *rapifera x japonica* |

a From Nagayoshi(1982) and Aoba(1979)

5. References

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## X Brassicoraphanus Sageret, cultivargroup Raparadish

Hille Toxopeus

The name X Brassicoraphanus Sageret was identified as the correct scientific name for all bigeneric hybrids containing Brassica and Raphanus genomes (Oost 1984)

Recently genetically stable allopolyploid plantmaterial was developed at the SVP, originally derived from crossing several forms of Brassica rapa L. and a non-bulbing form of Raphanus sativus L.: oelrettich*, with the genome formula AARR (Dolstra, 1982). The seed fertility of this material was substantially improved in the past years and it will soon be released to breeding firms.

It is proposed that this potential new crop plant is called Raparadish in the sense of a cultivargroup. Raparadish was developed for use as a fresh fodder crop for sowing in late summer after crops such as winter and spring barley, rather like certain non-bulbing cultivars of the cv group Fodderturnip (B. rapa L.) (Toxopeus and Oost 1985).

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* Translated 'oilseedradish', a greenmanure crop, not an oilseed crop, not to be confused with fodderradish which is bulbing and has different origins.

# COLLECTING OF BRASSICAS IN NORTHWESTERN SPAIN

A. Ordás and J. J. Baladrón

Brassica crops are very important in Galicia (northwestern Spain). Practically all farmers in the region grow at least one of the brassicas. Traditionally they have saved their own seedstocks. In the last few years, however, many farmers have abandoned their local varieties and shifted to commercial cultivars. Consequently genetic erosion has begun to take place.

A program to collect brassica landraces was started by the Misión Biológica de Galicia in January 1985. The collecting area has been restricted to Galicia for the time being. So far 110 samples have been collected. Most of the accessions were of small size and therefore will be available for distribution after they are multiplied.

A crop known locally as "nabicol" posed some problems. Gallástegui (2) reported that its chromosome number was 36. Our countings, using seedling root tips stained with acetic orcein, have determined 38 chromosomes. It, then, seems to be a form of Brassica napus similar, as it is used as a leaf vegetable, to the "Sibirischer Grünkohl" cited by Andersson and Olsson (1).

Kale is the brassica fodder crop most widely extended. People in the region are accustomed since ancient times to consume leaves of turnips as vegetables (turnip greens) and so a great deal of the turnip cultivars are specialized in such direction. In the south of the Atlantic Coast "nabicol" is almost invariably grown for this use instead of turnips.

Most collecting has been carried out in the central and southern zones of the region. Further expeditions will concentrate on the northern areas, where turnips for fodder are an important crop.

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# SEED DISPERSAL MECHANISMS IN THE TRIBE BRASSICEAE

C.Gómez-Campo and M.Gustafsson

Fruit structure is rather constant throughout the Crucifer family, and nothing seems to indicate, in general, that siliques or silicules are especially efficient from the point of view of seed dispersal. Perhaps the most striking exception is supplied by some Cardamine species (tribe Arabideae), whose valves become suddenly enrolled at dehiscence and throw the seeds a few meters away. The tribe Brassiceae (where Brassica,Erucastrum,Diplotaxis, Sinapis,Eruca and forty-five other genera are included) shows an evolutionary tendency toward fruit segmentation or heteroarthrocarpy (1) which is based on the differentiation of a seed-bearing cavity or beak in the stylar region. But apart from the rich implications of heteroarthrocarpy -sometimes well established, sometimes only hypothetical- several other subtle adaptations aimed at improving seed dispersal occur. These are briefly summarized below.

Anemochory.

Winged seeds are present in some desert-adapted genera as Savignya and Oudneya, as well as in Euzomodendron, a genus growing on gypsaceous soils in SE Spain (1). Vestigial wings are found in the seeds of some species of Moricandia, Vella, Boleum and Eruca ; though they have little effect on dispersion, their phylogenetic significance is high. Winged fruits (or parts of them) can be found in Fortuynia, Zilla, Fezia, Cordylocarpus, Psichyne and Schouwia.

Light spongy fruits as those of Raphanus sativus or the beaks of Coincya rupestris or Brassica macrocarpa, can also be considered an adaptation to anemochory. The low density and high cross-section of these diaspores favours their transportation by wind, sometimes along considerable distances.

During our expeditions to collect germplasm of n=9 Brassica species, we have repeatedly observed that some seeds are often imprisoned within the valve at dehiscence. Valves become slightly curved inwards and they can grasp one or a few seeds while most other are released. As wind strokes are a major triggering agent for dehiscence, the valves are blown away in the right moment and they can carry some seeds as in an airship. This behaviour is common for most observed populations of B.cretica (an almost obligate rupicolous species) (2), but it can also be found in populations of B.incana, B.montana and even of wild B.oleracea.

Pendulous siliques of Diplotaxis harra sway on their stalks until an

air stroke produces dehiscence and disperses seeds and valves. Here, the valves are almost flat and clasp no seeds at all, but they can obviously play a role in pushing them forwards.

Mechanical devices.

Contact by passing animals or by other branches or stems may also be important in triggering fruit dehiscence. Infrutescences which are especially rigid in all their parts as in Sinapis,Vella,etc.,can be interpreted under this point of view. Their fruits are additionally provided with long sword-like beaks acting as tangent organs, and dehiscence is accompanied by elastic movements that impel the seeds away.

Under this heading we should include the ability of sphaerical seeds to bounce off and to reach longer distances from mother plant than elliptic or flat seeds can do. A clear evolutionary tendency can be observed toward sphaeric seeds in many members of the tribe (Brassica,Sinapis,Succowia,etc.) and this has been interpreted as a way to improve the effi - ciency of seed dispersal.

Hidrochory,Zoochory and Geocarpy.

Spongy diaspores of the seaside taxa Cakile maritima and Crambe maritima, can be viewed as an adaptation to be transported by sea water. Not in vain Cakile is the only genus of its tribe that has naturally migrated into the New World. However, wind might at least be as important as water for the short distance dispersion of these species.

The tendency to reduce fruit length in the sub-tribe Vellinae, reaches an extreme situation in Boleum, where the whole fruit becomes indehiscent and is released as a dispersal unit. The valves are covered with stiff hair that can easily adhere to the fur of animals.

Ants do not seem to play any important role in the dispersal of the Brassiceae (3) but this subject should deserve further investigation.

The habit of burying the fruits before they become free from the mother plant -as in peanut plants- can also be found in the tribe Brassiceae. It is partially present in the genus Raffenaldia from N.Africa and fully developed in Morisia from Corse and Sardinia.

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# TAXONOMIC STUDIES IN CULTIVATED SPECIES OF SUB-TRIBE BRASSICINAE

P.R.KUMAR[1] AND R.C.YADAV[2]

In the family Cruciferae, the species belonging to the sub-tribe Brassicinae include the largest number of economic plants which are grown either for oilseeds or vegetables. There has been a confusion about the precise nomenclature of plant species belonging to sub-tribe Brassicinae. The term rapeseed and mustard used most commonly is quite vague for all oil bearing Brassicas. Distinction between various types of Brassicas at seed and leaf stages sometimes becomes difficult because of their close resemblance and lack of knowledge of diagnostic characters to precisely separate them. In an attempt to overcome the problem of nomenclature, a study was initiated during 1984-85 at this laboratory with improved cultivars of the following species of sub-tribe Brassicinae.

Brassica juncea cv. RH-30, Seeta

Brassica campestris

var. toria (Indian rape) cv. T-9, PT-303
var. sarson (Yellow sarson) cv. YS-151, B-9
var. dichotoma(Brown sarson) cv. Pusa Kalyani, BSH-1

Brassica carinata cv. HC-1, HC-2

Brassica napus cv. HNS-3, HNS-5

Eruca sativa cv. RTM-1, T-27

At the first instance, 22 morphological characters were studied in five species. The variation in different morphological characters observed in five species are presented in table 1 and 2. Brassica napus(cv. HNS-5) had the thickest(1.46 cm) stem followed by HNS-3, whereas the Eruca sativa (cv. RTM-1)had the least thickness(0.52 cm). Brown sarson, Yellow sarson and toria had more or less the same thickness. The Yellow sarson (cv. YS-151) had the thickest pod(0.72 cm) whereas B. napus(cv. HNS-3) had the lowest pod thickness(0.26 cm). B. carinata (cv. HC-2)had the highest number of nodes(18,0) and B. juncea(cv. RH-30) had the lowest number of nodes. L ikewise, B. carinata (cv. HC-1) had the widest angle between branches (36.3°)while RH-30 had narrow angle (22.0°). B.campestris cultivars had no significant difference amongst themselves. Cultivar YS-151 of Yellow sarson was having the widest pod angle (51.9°) followed by HNS-3 (46.9°) whereas Eruca sativa cultivars

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had narrow pod angle(7.2°). B. campestris cultivars had, in general, longer beaks than other species. Brown sarson (cv. BSH-1)was having the longest beak(1.78 cm) followed by PT-303 of toria(1.76 cm). B. carinata cultivars were having the maximum height (154.8, 150.1 cm) from rest of the cultivars and Eruca sativa(cv. RTM-1) was the shortest (88.9 cm). In case of the height of the main shoot, B. juncea(cv. RH-30) had the longest main shoot while B. carinata(cv. HC-2)had the shortest. Contrary to this, B. carinata(cv. HC-2)had the maximum number of primary branches(8.0) while B. juncea(cv. Seeta) possessed minimum number of primary branches (4.5). Similary, secondary branches were found to be the maximum (15.9) in case of B. carinata(cv. HC-2) while minimum in Yellow sarson (cv. YS-151) . B. napus(cv. HNS-5) had the maximum number of pods on main shoot(62.2) whereas Eruca sativa(cv. RTM-1) had the minimum number of pods(15.30). Likewise, B. napus cultivars had the longest pods(4.97 & 4.56 cm) while both cultivars of Eruca sativa RTM-1 and T-27 were having the shortest pods(1.65 cm). About the seeds per pod, the Yellow sarson cultivar YS-151 possessed the maximum number of seeds per pod(19.32) followed by HNS-5 (17.06) of B. napus. B. juncea(cv. RH-30) possessed the lowest number of seeds per pod(8.60).

A perusal of data presented in table 2 revealed that B. napus (cv. HNS-3) had the longest pedicel(16.98 mm) and Eruca sativa(cv. RTM-1) had the smallest pedicel(2.80 mm). While Eruca sativa (cv. RTM-1) had the longest (10.50 mm) sepals and Brown sarson (cv. BSH-1) and toria (cv. PT-303) had the smallest sepals(6.68 mm). Similarly, for petal length, Eruca sativa(cv. T-27) had the longest (20.68) and B. juncea (cv. RH- 30 had the smallest petals (9.93 mm). In breadth also, Eruca sativa(cv. RTM-1) had the maximum breadth (8.68 mm) while B. juncea (cv. Seeta) had the lowest petal breadth(5.38 mm). Similarly, Eruca sativa (cv. RTM-1) had the biggest claw(10.68 mm) while Pusa Kalyani of Brown sarson had the smallest claw(3.73 mm). There was no significant difference amongst B. campestris cultivars so far their floral morphology was concerned. Stamens of both the Eruca sativa cultivars were the longest (12.60 mm) while B. napus (cv. HNS-5) had the smallest stamens(7.10 mm). Similarly, cultivars Eruca sativa (cv. T-27 and RTM-1) were having the maximum anther length. Cultivar B-9 of Yellow sarson had the longest ovary(5.60 mm) while toria(cv. PT-303) had the smallest ovary. Cultivar T-27 of Eruca sativa had the longest style(5.89 mm) while toria(cv. PT-303) and Brown sarson(cv. Pusa Kalyani) had the shortest style length (2.15 mm). In addition to these morphological attributes, investigations on seed, root and leaf characters are in progress.

Table: 1 Plant Morphological Characters in *Brassica* species

| Sr. No. | Cultivars | Stem thickness (cm) | Pod thick-ness (cm) | No. of Nodes | Angle between branches | Pod Angle | Beak length | Plant height (cm) | Main shoot height (cm) | Primary branches | Secondary branches | Pods on main-shoot | Pod length (cm) | Seeds/ pod. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ***Brassica carinata*** | | | | | | | | |
| 1 | | | | | | | | | | | | | | |
| 1. | HC-1 | 0.84 | 0.36 | 14.4 | 36.3 | 19.3 | 0.42 | 154.8 | 44.1 | 7.7 | 14.8 | 18.6 | 3.16 | 10.22 |
| 2. | HC-2 | 0.76 | 0.36 | 18.0 | 31.9 | 20.3 | 0.52 | 150.1 | 41.2 | 8.0 | 15.9 | 21.9 | 3.35 | 10.98 |
| | | | | | | ***Brassica napus*** | | | | | | | | |
| 3 | HNS-3 | 1.26 | 0.26 | 13.6 | 25.7 | 46.9 | 1.34 | 133.3 | 49.7 | 6.8 | 1.30 | 60.9 | 4.56 | 15.96 |
| 4. | HNS-5 | 1.46 | 0.36 | 9.6 | 25.6 | 41.8 | 1.30 | 136.2 | 60.3 | 5.5 | 3.70 | 62.2 | 4.97 | 17.06 |
| | | | | | | ***Brassica juncea*** | | | | | | | | |
| 5. | RH-30 | 0.76 | 0.46 | 8.0 | 22.0 | 29.0 | 1.00 | 137.7 | 65.4 | 5.0 | 7.50 | 33.0 | 3.47 | 8.60 |
| 6. | Seeta | 0.68 | 0.36 | 8.2 | 23.9 | 23.1 | 0.74 | 125.8 | 62.0 | 4.5 | 10.00 | 32.7 | 3.06 | 10.78 |
| | | | | | | ***Brassica campestris* var. Yellow sarson** | | | | | | | | |
| 7. | YS-151 | 0.86 | 0.72 | 11.8 | 29.4 | 51.9 | 1.64 | 104.4 | 50.1 | 6.6 | 1.00 | 31.2 | 3.10 | 19.32 |
| 8. | B-9 | 0.86 | 0.58 | 10.4 | 25.1 | 34.9 | 1.44 | 108.0 | 57.6 | 6.7 | 1.40 | 38.0 | 3.06 | 11.64 |
| | | | | | | ***Brassica campestris* var. Brown sarson** | | | | | | | | |
| 9. | BSH-1 | 0.90 | 0.46 | 11.8 | 25.0 | 43.9 | 1.78 | 114.6 | 47.8 | 6.6 | 3.7 | 27.8 | 4.03 | 13.56 |
| 10. | Pusa Kalyani | 0.88 | 0.52 | 12.0 | 29.4 | 40.1 | 1.68 | 129.3 | 60.7 | 6.5 | 3.7 | 40.4 | 4.20 | 16.58 |
| | | | | | | ***Brassica campestris* var. toria** | | | | | | | | |
| 11. | T-9 | 0.68 | 0.44 | 9.6 | 24.2 | 34.3 | 1.42 | 96.7 | 49.3 | 5.6 | 5.4 | 39.1 | 3.42 | 13.52 |
| 12. | PT-303 | 0.68 | 0.40 | 11.6 | 25.5 | 30.5 | 1.76 | 94.4 | 51.7 | 6.1 | 5.8 | 30.1 | 3.80 | 14.70 |
| | | | | | | ***Eruca sativa*** | | | | | | | | |
| 13. | RTM-1 | 0.52 | 0.56 | 10.2 | 26.4 | 7.2 | 0.74 | 88.9 | 53.3 | 5.9 | 9.00 | 15.3 | 1.65 | 12.58 |
| 14. | T- 27 | 0.54 | 0.58 | 11.0 | 25.3 | 10.2 | 0.76 | 103.3 | 60.7 | 5.9 | 5.90 | 22.5 | 1.65 | 12.86 |

Table 2: Floral Taxonomy in Brassica species

| Sr. No. | Cultivars | Pedicel length (mm) | Sepal length (mm) | Petal length (mm) | Petal breadth (mm) | Claw length (mm) | Stamen length (mm) | Anther length (mm) | Ovary length (mm) | Style length (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Brassica carinata | | | | |
| 1. | HC-1 | 7.73 | 7.80 | 13.68 | 6.93 | 5.18 | 8.53 | 2.95 | 5.18 | 2.28 |
| 2. | HC-2 | 7.40 | 8.00 | 14.53 | 7.30 | 5.50 | 7.20 | 2.80 | 4.90 | 2.25 |
| | | | | | | Brassica napus | | | | |
| 3. | HNS-3 | 16.98 | 8.05 | 13.30 | 7.80 | 5.00 | 10.30 | 3.00 | 4.10 | 2.20 |
| 4. | HNS-5 | 16.20 | 7.60 | 12.60 | 6.70 | 5.10 | 7.10 | 2.70 | 4.40 | 2.20 |
| | | | | | | Brassica juncea | | | | |
| 5. | RH-30 | 8.45 | 7.20 | 9.93 | 5.58 | 4.45 | 8.03 | 2.23 | 3.88 | 2.78 |
| 6. | Seeta | 9.28 | 6.93 | 10.60 | 5.38 | 4.58 | 8.40 | 2.18 | 4.90 | 2.90 |
| | | | | | | Brassica campestris var. Yellow sarson | | | | |
| 7. | YS-151 | 13.38 | 7.0 | 10.70 | 6.33 | 3.98 | 7.88 | 2.23 | 4.23 | 2.78 |
| 8. | B-9 | 13.98 | 7.05 | 10.88 | 6.43 | 4.00 | 8.33 | 2.53 | 5.60 | 2.93 |
| | | | | | | Brassica campestris var. Brown sarson | | | | |
| 9. | BSH-1 | 9.93 | 6.88 | 10.45 | 6.78 | 4.05 | 8.03 | 2.53 | 3.90 | 2.15 |
| 10. | Pusa Kalyani | 10.95 | 7.05 | 10.43 | 7.70 | 3.73 | 8.53 | 2.25 | 4.15 | 2.28 |
| | | | | | | Brassica campestris var. toria | | | | |
| 11. | T-9 | 11.05 | 7.33 | 12.30 | 7.53 | 4.08 | 7.58 | 2.73 | 4.15 | 2.45 |
| 12. | PT-303 | 10.33 | 6.88 | 11.85 | 7.05 | 3.98 | 8.25 | 2.58 | 3.63 | 2.15 |
| | | | | | | Eruca sativa | | | | |
| 13. | RTM-1 | 2.80 | 10.50 | 20.40 | 8.68 | 10.68 | 12.60 | 3.28 | 4.45 | 5.53 |
| 14. | T-27 | 2.93 | 10.05 | 20.68 | 8.20 | 10.0 | 12.55 | 3.30 | 4.93 | 5.89 |

# GENOME EVOLUTION IN BRASSICA: USE OF MOLECULAR MARKERS AND CYTOGENETIC STOCKS.

Carlos F. Quiros, Shahryar F. Kianian, Oswaldo Ochoa and Dave Douches

Coulthart and Denford (1) reported that the enzyme coding locus PGI produced specific isozymes useful to mark the chromosomes of genomes a and c. Furthermore, the isozymes of both genomes were present in the natural allotetraploid B. napus, confirming its hybrid origin. Arus (2) was able to trace B. oleracea specific isozymes distributed in five independent loci, into B. napus. Coulthart and Denford (3) based on the genomic formulas postulated by Robbelen (4) a=AA B C DD E FFF; b=A B C DD EE F; c=A BB CC D EE F, suggested the location of several isozyme loci on chromosomes which were either disomic or multisomic in B. oleracea and B. campestris. In addition to enzyme coding loci, chloroplast DNA restriction sites have been used to confirm the relationships between species of Brassica, supporting the triangle of U (5,6,7).

We have expanded these efforts by sampling additional isozyme loci and ribosomal genes (rDNA) for all six species forming part of the triangle. Our findings indicate that most isozyme loci provide enough information to confirm the U triangle, in spite of the variability observed for the diploid species. We found allozymes which were specific to each diploid species and which could be followed in the three natural allotetraploids. The table below lists the enzyme coding loci diagnostic for the confirmation of each allotetraploid hybrid.

| Isozyme loci | B.ol | B.np | B.cp | B.jc | B.ng | B.cr | B.ol |
|---|---|---|---|---|---|---|---|
| PGI | A | AB | B | BC | C | | |
| GOT | A | AB | B | BC | C | CD | D |
| 6PGD | A | AB | B | BC | C | CD | D |
| LAP | A | AB | B | BC | C | | |
| MDH | | | B | BC | C | CD | D |
| PRX(ANOD) | | | | | C | CD | D |
| PRX(CATD) | A | AB | B | | | | |
| TPI | A | AB | B | BC | C | CD | D |
| PGM | | | | | C | CD | D |

Letters indicate phenotypes.

The level of electrophoretic variability between diploids and tetraploids was strikingly different. While the diploid species were polymorphic for most of the loci inspected, the allotetraploids were very consistent in phenotype within and between accessions. Their phenotypes were in most cases fixed for heterozygosity. For the enzyme 6PGD, heterozygosity was detected in B. oleracea indicating that homologous loci might be present in at least disomic condition.

We also made a preliminary survey for restriction fragment length polymorphisms in rDNA. Genomic DNA was digested with EcoRI, Southern blotted and probed with a clone of wheat rDNA which contained both the 18S and 25S transcribed and the nontranscribed regions (pTA71, supplied by Dr. M.A. Saghai Maroof, UC Davis). The rDNA fragment patterns identified B. napus as the natural hybrid between B. oleracea and B. campestris as a perfect match was observed between the bands of B. napus and those corresponding to the two parental diploid species. After testing several accessions for these three species, (supplied by D. Cohen, UC Davis); we detected differential band intensities for B. campestris and for B. napus reflecting variability for sequence copy number. For B. oleracea, the variability was limited to the presence in some cabbages of a weak band corresponding to a fragment of 4.2 kb. This

seems to represent the non-transcribed spacer sequence, since it did not show after hybridizing with a wheat rDNA probe carrying only transcribed sequences (pTA250.2).

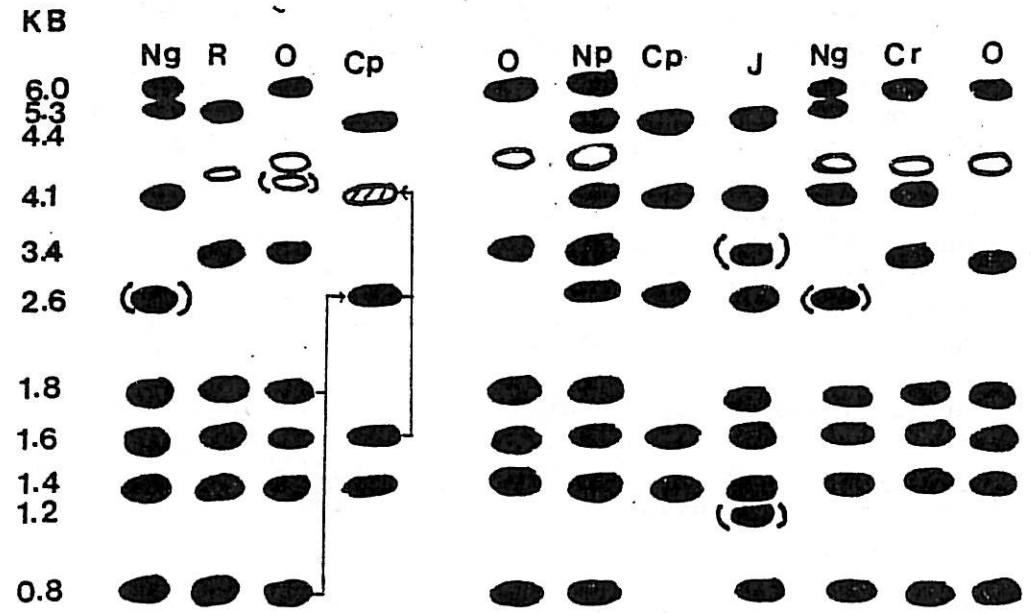
The rDNA banding pattern of B. carinata and B. juncea also had the bands observed in their diploid parental species, although we also observed for some cases a few novel bands. A limited survey of various accessions for the parental and hybrid species accounted for most of these deviations. For B. nigra, and B. carinata we observed variability for sequence copy number and possibly for restriction sites. For example, some accessions of both species lacked a fragment of 2.6kb always found in B. campestris but always absent in B. oleracea and presumably always lacking in radish also (8). For B. juncea the most prominent variability observed was in the presence of a unique band of 1.2 kb in a single plant, indicating restriction site variability. This was not found in any other plant of the species tested. Based on the radish rDNA information (8), whose restriction fragment pattern is very close to B. oleracea, and a hybridization experiment using the probe pTA250.2 it is possible to infer that this variability is due to restriction site changes in transcribed sequences. As these sequences highly conserved in plants, reflecting the fact that wheat rDNA probes hybridize with Brassica DNA, the restriction site changes observed are expected to provide information relevant to Brassica evolution.

From our preliminary rDNA survey we conclude that B. napus and B. carinata are allotetraploid species of recent origin, reflected by the similarity of their bands to those of their parental species. On the other hand, B. juncea might be an older allotetraploid showing divergency for some restriction sites reflected by the presence of unique fragments not observed in its parental diploid species. In contrast, the chloroplast DNA data indicates little divergence B. carinata and B. juncea with its diploid species, and larger divergency of B. napus from its diploid parents. This contradiction has been explained by Palmer et al (5) by introgressive hybridization of B. napus with one of its diploid parental species, most likely B. oleracea (6).

With respect to interspecific differences among the diploid species, the lack of the fragments 1.8 kb and 0.8 kb in B. campestris can be accounted by the presence of a 2.6 kb fragment (formed by the addition of 1.8 + 0.8 kb fragments). Interestingly enough, B. nigra has these three fragments, although some accessions lack the 2.6 kb fragment accompanied by gain of intensity in the 1.8 and 0.8 fragments further supporting this origin of the 2.6 kb fragment. Thus, B. campestris may have evolved from B. nigra progenitors which were segregating for the lack of the restriction site responsible for splitting the 2.6 kb fragment into 1.8 and 0.8 fragments. Furthermore, the lack or presence of this fragment in some B. carinata accessions indicates that this hybrid had multiple origins, in which different lines of B. nigra and B. oleracea were involved. B. oleracea as well as radish have a fragment of 3.4 kb not observed in the other two diploids. This could be due to a loss of another restriction site. This fragment, as expected, was also found in B. napus and B. carinata. All, except one B. juncea plant lacked this fragment, indicating restriction site loss in some of the rDNA sequences. B. adpressa (x=7) has a very complex restriction pattern which might account for all the fragment sizes found in the rest of the diploids tested.

Our work demonstrates that it is possible to mark the chromosomes of each genome with these two types of molecular markers: isozymes and DNA restriction sites. This could be exemplified better on the tri-genomic hybrid we obtained by crossing B. carinata with B. campestris, where several rDNA fragments specific for each of the three diploid species could be accounted for. These markers will be very effective for studying genome evolution in Brassica.

Our goal is to create chromosome addition lines for each diploid species, by crossing and backcrossing each allotetraploid to one of its parental diploid parents. The progressive loss of chromosomes of the second parental diploid species not involved in the cross can be monitored by chromosome counts and electrophoretic/restriction site patterns in the resulting progenies. In this way, it will be possible to dissect each chromosome for each genome determining its possible origin on the basis of linkage relationships and duplicated loci. Also, we will be able to determine in this fashion if the marker loci are present in monosomic or multisomic condition. Other species included in this study are those with x=7 chromosomes, such as B. adpressa and Diplotaxis erucoides. The wild species of the oleracea cytodeme are also being studied. At this point we have developed cytogenetic stocks of B. campestris, B. oleracea and B. nigra with four to six extra alien chromosomes. We hope to obtain monosomic addition lines from these in the next backcross.




( ) lacking in some plants
non-transcribed fragment
faint for some accessions

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# SOME EXPERIMENTAL HYBRIDS ON DIPLOTAXIS HARRA (FORSK.) BOISS. COMPLEX

E. Sobrino Vesperinas

Diplotaxis harra (Forsk.) Boiss. (Mediterranean and Irano-Turanian distribution), and D. crassifolia (Rafin.) DC. (Mediterranean) have identical chromosome numbers (n = 13) and strong morphological similarities to D. glauca (J.A. Schmidt) O.E. Schulz and D. hirta (Chev.) Rustan et Borgen (endemic to the Cape Verde Islands). Accordingly the possibility of obtaining interspecific hybrids as a measure of phylogenetic proximities was investigated.

In the experimental hybrids obtained, observations were carried out on germination, morphology, fertility of the pollen and seed production (Table 1). Additionally, in three hybrids, the $F_2$ generation was obtained by open pollination in order to study the possibilities of perpetuation of the combinations, if these species were not geographically isolated.

There were no difficulties in obtaining hybrids and, except for one case, a high number of viable seeds were obtained per crossed flower. There were no important differences in the ease of obtaining hybrid seed from crossing D. harra (Forsk.) Boiss. and D. crassifolia (Rafin.) DC. together or with the two species from Cape Verde.

Pollen fertility was average or high in $F_1$ hybrids whilst it dropped in the $F_2$ (Table 1). In one of the experimental hybrids, D. harra (Forsk.) Boiss. x D. crassifolia (Rafin.) DC. var. lagascana (DC.) O.E. Schulz, observations on a few cells revealed regular formation of 13 bivalents.

Table 1. Experimental hybrids obtained: viable seeds per crossed flower (A), percentage of germination (B), and pollen fertility (C).

| Experimental hybrids | A | B | C | |
|---|---|---|---|---|
| | | | F1 | F2(*) |
| D. harra x D. crassifolia var. lagascana | 69 | 90 | 85 | 42 |
| D. hirta x D. harra | 25 | 86 | 80 | 67 |
| D. crassifolia x D. hirta | 3 | 20 | 84 | 34 |
| D. glauca x D. harra | 59 | 73 | 85 | - |
| D. crassifolia x D. glauca | 30 | 34 | 68 | - |

(*) obtained by open pollination

The pollen fertility of the $F_1$ was so little affected that it was possible to obtain viable seed and plants of two triple hybrids:

*D. glauca* x (*D. harra* x *D. crassifolia*)

*D. glauca* x (*D. harra* x *D. hirta*)

The high interfertility shown, as well as the morphological similarities and identity in the chromosome number previously mentioned, suggest the presence of a strong affinity between the two groups of species. This finding is interpreted as a clear case of geographic disjunction, with microdifferentiation, which has not yet reached specific level. Probably, the correct taxonomic treatment would be to consider the taxa studied as subspecies of *Diplotaxis harra* (Forsk.) Boiss. Especially bearing in mind that the ecological conditions are similar for both groups, since the Cape Verde islands have a very arid climate (Aquino, personal communication) like the Mediterranean.

## BRASSICA NAPOLERACEA

M.S. Chiang

*Brassica napoleracea* is a colchicine doubled triploid $F_1$ hybrid ($2n=28$, genomes $a_1c_1c$) between *B. napus* ($2n=38$, $a_1a_1c_1c_1$) and 2x-cabbage (*B. oleracea* ssp. *capitata*, $2n=18$, cc). The somatic chromosome number of *B. napoleracea* is 56.

Under the field conditions at St-Jean, Quebec, the average plant height and width are 63 cm and 101 cm, respectively. Plants of *B. napoleracea* have very good root system and highly resistant to clubroot pathogen *Plasmodiophora brassicae* races 2 and 6 (ECD 16/02/31 and 16/02/30). It does not form fleshy root. There are 26 expanded leaves in average per plant, the expanded leaf has a long (15 to 23 cm) petiole with a very large terminal lob (25 cm x 31 cm) and several pairs of small ones. Leaf surface is crimpy with a distinct white mid-rib. The margin of the terminal lob is rather wavy. Leaves are susceptible to powdery mildew. The yellow flower has normal filaments and anthers, produces good quantity of pollen grains and self compatible. Seed set ranged from 3 to 15 with an average of 9.8 seeds per pollination.

# THE USE OF SDS-PAGE FOR SPECIES AND CULTIVAR IDENTIFICATION IN BRASSICA AND RELATED GENERA.

E.H. Oost and J.P.M. Relou

The use of the SDS-PAGE* technique for identification purposes has been evaluated for a number of cruciferous species and cultivars. SDS-PAGE was carried out as described by Laemmli (1970), but with slight modifications. Proteins were extracted from single seeds. Seeds were obtained from various Variety Research Institutes, Genetic Resources Centres and breeding firms. As far as possible, all accessions used for electrophoresis have been grown out to verify their identity.

The electrophoretic profiles of 6 wild Brassica species, Sinapis alba 'Emergo' and S. arvensis L. proved to be a clear and reliable basis for identification. The profiles of both genomic forms of x Brassicoraphanus Sageret (AARR and CCRR, see Oost, 1984) looked very similar, but both showed several intermediate bands in comparison to the profiles of their parental species.

Variation between electropherograms of the species of the Brassica triangle was rather subtle but fairly consistent.

Differences were mainly present in the region of protein bands with molecular weights varying from ± 65 k Dalton to 85 k Dalton.

The electrophoretically most variable cultivated species, B. rapa L.s.l. (including Pe Tsai, Pak Choi etc.), was studied most extensively.

Some within-cultivar-variation of electrophoretic profiles was found in certain openpollinated cultivars of B. rapa.

Unfortunately, this might hamper reliable cultivar identification of these cultivars. On the other hand, profiles of F1 hybrids of B-rapa generally were very uniform.

Further study is aimed to allocate intermediate bands of the interspecific hybrids of the Brassica triangle (Oost and Relou, 1986) and to try and link specific bands to genetic traits (half-seed method, backcrossing, etc.).

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* Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis.

# ROOT TUMOURS IN INTERSPECIFIC CROSSES OF BRASSICA

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

Rapeseed and mustard form an important group of oilseed crops grown in India. Brassica campestris var. toria, brown sarson and yellow sarson, and Eruca sativa are also grown but Indian mustard (Brassica juncea) is the predominating species cultivated. This species has a long history of cultivation in India and is tolerant to several stresses like drought, insects and diseases. It is highly tolerant to strong winds which cause breakage of stems and shattering of grains. Recently Brassica napus, which is popularly known as 'Gobhi sarson' by the farmers, has been observed in several fields. It has a good yield potential with a higher degree of photosensitivity, stem breakage and grain shattering. It has longer pods and pedicels, higher pod intensity and smaller grain size than Brassica juncea.

In view of these defects interspecific hybridization between Brassica juncea and B. napus was started at the Regional Research Station, Faridkot during 1981-82 for transferring economic characters from one species to the other. Good seed setting was obtained when B. juncea was used as female, however, no seed set could be obtained with B. napus as female. The $F_1$s were taller and had more branches and pods than either of the parents. The fertility was very low with only 1-3 seeds in some of the pods only. Huge variation was observed for all traits. Surprisingly large root tumours were observed in the $F_1$ and tumour formation was highly variable in $F_2$ and $F_3$. The roots of both parents were quite free of tumours. Possibly this character might have come from either of the diploid donors of these two amphiploid species. This will be thoroughly observed. Tumorisation seemed to be associated with seed setting, i.e. the lower the seed setting the larger were the tumours. This character could thus be used as a supplement in selecting fertile genotypes from this interspecific cross. A detailed study of tumorisation will be reported subsequently. The materials would be grown in $F_4$ generation and useful recombinants have been isolated. Progenies looking like B. juncea with longer pods, higher pod intensity, short stature, and longer pedicels and others resembling B. napus with upright pods, stiff stems and beaded pods have been selected for breeding fertile and stable types.

# CROSSABILITY BETWEEN ARTIFICIALLY SYNTHESIZED TRIGENOMIC HEXAPLOID AND CULTIVATED SPECIES IN GENUS BRASSICA

T. Takeda, O. Takahashi and Y. Takahata

No trigenomic species have been found in genus Brassica in nature. However, synthesized trigenomic species whose genome constitution is AABBCC is obtained in the cross between digenomic species (B. carinata, B. juncea and B. napus) and monogenomic species (B. nigra, B. oleracea and B. campestris) in three ways. Takeda (1983) carried out a chromosomal investigation on these hexaploid plants for over ten generations and suggested that (1) hexaploid nature could be kept in these generations, (2) the self fertility varied about 10 - 60% among individuals, (3) these plants could be utilized as a bridge plant to transfer genes from one species to another. The present experiment was carried out to learn the crossability of the artificially synthesized hexaploids with mono and digenomic species to use them as a bridge plant.

Two strains of trigenomic hexaploid such as CaC-334 (progeny of amphidiploid B. carinata X B. campestris) and JO-125 (progeny of amphidiploid B. juncea X B. oleracea) were used as maternal parents and 11 strains of six cultivated species (B. nigra, B. oleracea, B. campestris, B. carinata, B. juncea and B. napus) were paternal ones.

The results of hybridizations are shown in Table 1. Crossabilities were not different between two female strains, but were different between male species used. Fertility was always higher when digenomic species were pollen parents than monogenomic ones. In addition, it was better when the species containing A genome were used as a male, i.e., B. campestris (genome AA) in monogenomic species and B. juncea (AABB) and B. napus (AACC) in digenomic. The causes of these phenomena are under investigation.

Table 1. Results of hybridization between trigenomic hexaploid and cultivated species in Brassica

| Cross combination | Number of flowers pollinated | Number of seeds obtained | Number of seeds per pollination |
|---|---|---|---|
| CaC-334(AABBCC) X B.nigra(BB) | 291 | 49 | 0.17 |
| CaC-334(AABBCC) X B.oleracea(CC) | 296 | 8 | 0.03 |
| CaC-334(AABBCC) X B.campestris(AA) | 567 | 306 | 0.54 |
| CaC-334(AABBCC) X B.carinata(BBCC) | 65 | 110 | 1.69 |
| CaC-334(AABBCC) X B.juncea(AABB) | 91 | 429 | 4.71 |
| CaC-334(AABBCC) X B.napus(AACC) | 113 | 453 | 4.01 |
| JO-125(AABBCC) X B.nigra(BB) | 37 | 1 | 0.03 |
| JO-125(AABBCC) X B.oleracea(CC) | 126 | 5 | 0.04 |
| JO-125(AABBCC) X B.campestris(AA) | 124 | 44 | 0.35 |
| JO-125(AABBCC) X B.carinata(BBCC) | 77 | 220 | 2.86 |
| JO-125(AABBCC) X B.juncea(AABB) | 195 | 677 | 3.47 |
| JO-125(AABBCC) X B.napus(AACC) | 19 | 74 | 3.89 |

( ): Genome symbol

The relationships between the self-fertility of CaC-334 and the cross-fertility of CaC-334 with digenomic species were investigated. As shown in Fig. 1, significant positive correlations were present in all cases. The higher self-fertile individuals could be easily hybridized with Brassica digenomic species. They may be expected to increase the crossability with monogenomic species. The selection for higher self fertile plant may be necessary to effectively utilize the trigenomic hexaploid as a bridge plant between cultivated species.

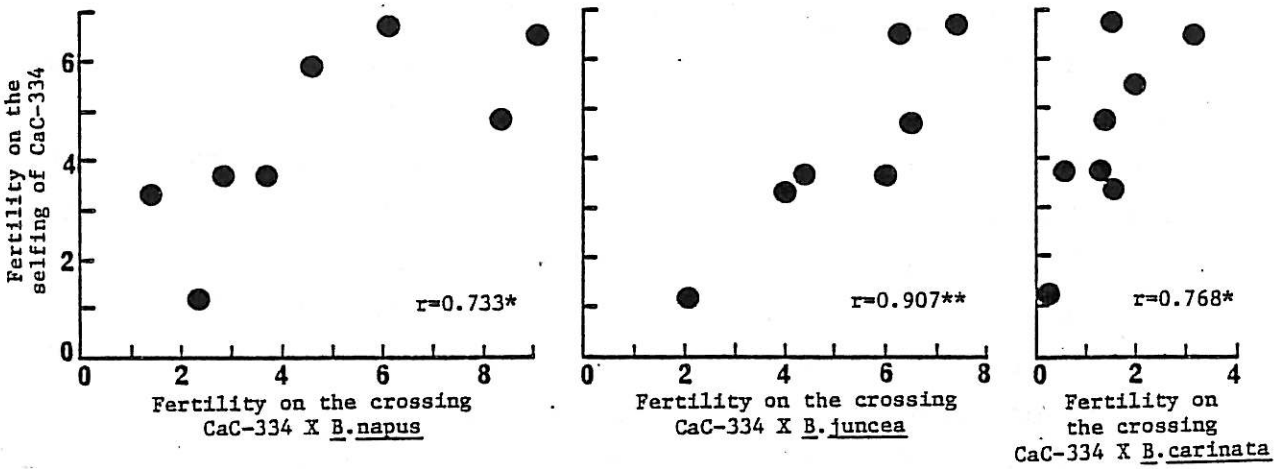


Fig. 1. Relationships between the self-fertility of CaC-334 and the cross-fertility of CaC-334 with three digenomic species. Fertility is expressed by the number of seeds per pollination. *, ** Significant at the 5 and 1% level, respectively.

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# CHROMOSOME STUDIES IN BRASSICA AND ERUCA

S.C. Verma and P.C. Chauhan

Two of the crucifer genera, Brassica and Eruca, include many important species for vegetable and oil crops. Several of the crop Brassicas are known since ancient times, and seeds of B. juncea have been found in the excavations of Chanhu-daro of Harappan Civilization (ca. 2300 - 1750 B.C.). Thus, considerable literature has accumulated on several aspects of their biology including particularly cytogenetics and self-incompatibility (Sikka and Sharma 1979, Tsunoda et al. 1980, Tanaka et al. 1981, Prakash and Tsunoda 1983). However, further studies on chromosome form and behaviour of the Brassicinae are still needed to reveal the extent and nature of variation within and between species, and to provide clues to the basic chromosome number/s of these genera.

The present day diploid species are regarded as secondarily balanced polyploids. Three proposals of basic chromosome number, x = 5, 6 or 8, have been debated, and all of them derive evidence from morphology of somatic and meiotic chromosomes, secondary bivalent associations, and chromosome pairing in haploids and hybrids (Alam 1936, Manton 1932, Catcheside 1937, Haga 1937; Sikka 1940, Mizushima 1950, Röbbelen 1960, Prakash 1973, 1974). There is not much support for x = 8, while the remaining two alternatives of x = 5 and x = 6 are being contested equally (Prakash and Tsunoda 1983).

Meiotic studies of nine taxa, all under cultivation, were made from aceto-carmine squashes of pollen mother cells fixed in acetic alcohol. In general, meiosis was normal and almost all the pollen were stainable. In Eruca sativa, a proportion of the PMCs revealed one or two quadrivalents at diakinesis and at metaphase-I, corroborating some earlier reports. Table 1 summarises the n-chromosome number (= No. of bivalents) and the means per cell of rod and ring types

of bivalents. Secondary associations between bivalents were observed in only three taxa, and the table also registers the mean number of secondary associations per cell.

Comparison of these data with some of the earlier reports reveals differences in the means of rod and ring bivalents which is not unexpected considering the wide diversity of the species. Such variation in the recombination potential, reflected by the number of chiasmata per cell, is related probably to the breeding of these crops.

Table 1

Meiotic Data in Nine Taxa of *Brassica* and *Eruca*

| Taxon | n-chr. number | bivalents+ Rods | Rings | sec. assoc. per cell |
|---|---|---|---|---|
| *Brassica campestris* | | | | |
| ssp. *oleifera* var. brown sarson | 10 | 1.5 | 8.5 | - |
| var. toria | 10 | 2.5 | 7.5 | - |
| ssp. *rapifera* | 10 | 10.0 | | - |
| *B. juncea* | 18 | 6.0 | 12.0 | - |
| *B. oleracea* | | | | |
| var. *botrytis* | 9 | 5.0 | 4.0 | - |
| var. *capitata* | 9 | 5.0 | 4.0 | 2.3 |
| var. *gongylodes* | 9 | 3.8 | 5.2 | - |
| *B. nigra* | 8 | 3.5 | 4.5 | 3.5 |
| *Eruca sativa* | 11 | 4.0 | 7.0 | 3.4 |

+ The figures represent overall means of a large number of PMCs, analysed in random samples.

Secondary associations of bivalents at prometaphase of meiosis-I have often been relied upon as evidence of relict homology or homoeology, and the frequent occurrence of this phenomenon in the tribe Brassiceae has been utilized in deducing the basic chromosome numbers of its genera, including *Brassica* and *Eruca*. These are observed presently in

only three taxa (Table 1), and from a detailed study of a very large number of PMCs it may be said that deriving basic chromosome number from secondary associations would be rather sceptical. But, large variation in these inter-bivalent "interactions" may possibly reflect cryptic structural alterations/rearrangements in their genome organization.

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# CHROMOCENTRES IN THE EUROPEAN RADISHES

Indira Panicker, U.C. Mehta and N. Dayal

Localized heterochromatic chromocentres have been considered as an adaptive character. A comparative study of the amount and distribution of pericentric constitutive heterochromatin, represented by chromocentres in the interphase nuclei of many plant species including radish (Raphanus sativus L.), may be of considerable evolutionary value (Stebbins, 1971). With this objective in mind, we studied the number and distribution of chromocentres in seven European varietal populations of radish and compared them with the Indian populations.

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

The European radishes, on the whole, had relatively a lower mean chromocentre frequency (11.3) than the Indian (13.6), but the CV (%) for the chromocentre frequency in the former was higher than that in the later. Among them the lowest mean chromocentre frequency was noted in the black rooted RRN (10.0) which was followed by DB (10.6) and the highest in ST (13.0). Other varietal populations had a mean value around 11.0. The distribution pattern of chromocentres in the nuclei also varied. Whereas RRN and VG showed an uniform pattern of distribution with majority of nuclei having 9-13 chromocentres, others showed a more varied pattern of distribution. ST was quite compact in this regard. There was a noticeable difference in this parameter between the European and the Indian radishes. Number of chromocentres per nucleus is presented in Table 1.

Our study demonstrates that the varietal populations of radish vary noticeably in the number and distribution of chromocentres, which are quite characteristic for a population. There are populations with both high and low number of chromocentres per nucleus. If chromocentres are any indication of visible heterochromatin, the population having a high mean chromocentre frequency may be regarded as more heterochromatinized than those having a low mean. It also indicates that the population having a higher number and wider distribution of chromocentres may be less heterozygous than those having a low number and narrower distribution. Thus the European radishes, in general, are less heterochromatnized, but more heterozygous than the Indian radishes.

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Table 1. Chromocentres per nucleus in European and Indian radishes

| Materials | Chromocentres/nucleus Mean ± SE | CV (%) | Source |
|---|---|---|---|
| Indian radishes | | | |
| Long White Green Top (GT) | 13.1 ± 0.12 | 8.9 | Dayal & Prasad,1983 |
| Japanese White (JW) | 14.2 ± 0.12 | 8.5 | " |
| Kalamikati Red (KR) | 13.4 ± 0.15 | 11.5 | " |
| Scarlet Globe (SG) | 12.6 ± 0.14 | 10.8 | " |
| Rainy Season Red (RR) | 12.9 ± 0.17 | 13.0 | " |
| Pusa Himani (PH) | 14.6 ± 0.14 | 9.3 | " |
| Chinese White (CW) | 15.2 ± 0.16 | 10.6 | " |
| Jaunpur Giant (JG) | 12.4 ± 0.10 | 8.3 | " |
| Contai Long (CL) | 14.1 ± 0.17 | 7.6 | " |
| Pusa Desi (PD) | 13.6 ± 0.13 | 2.9 | " |
| European radishes | | | |
| Rabano Ravanello (RRN) | 10.0 ± 0.18 | 18.7 | Present study |
| Remilong Ecarlet (DE) | 11.5 ± 0.14 | 12.2 | " |
| Riesenbutter Wez (RW) | 11.4 ± 0.18 | 10.8 | " |
| Saxa Trieb (ST) | 13.0 ± 0.17 | 13.3 | " |
| Doppelbock (DB) | 10.6 ± 0.14 | 13.2 | " |
| Violet de Gournay (VG) | 11.2 ± 0.16 | 14.8 | " |
| Round Rose (RRO) | 11.1 ± 0.12 | 10.8 | " |

# EFFECT OF VINCRISTINE ON CHROMOCENTRES IN RADISH

Indira Panicker and N. Dayal

Vincristine and vinblastine are two important alkaloids extracted from periwinkle (Vinca rosea L.) and are used in cancer-therapy. In recent years cytogenetic effects of these anti-cancer drugs have been studied on both plant and animal systems (Degraeve, 1978). However, cytogenetic effects of mutagens, both physical and chemical, on the heterochromatin fraction of genome are not known in plants. Here we report, therefore, the effect of an anti-cancer drug, vincristine, on the number and distribution of chromocentres, representing pericentric constitutive heterochromatin, in radish, Raphanus sativus L.

Only one varietal population of radish, Bombay Red, was used in the present investigation. 100-200 seeds were treated with different concentrations (25 µg/l, 50 µg/l and 100 µg/l) of vincristine (sold under the name of Neocristine and manufactured by ASSIA, Israel) for 6, 12 and 24 hours. Seeds were sown simultaneously in identical field condition and plants were raised. Methods for cytological analysis are the same as used earlier (Dayal,1975).

Vincristine affected both the number and the distribution of chromocentres at different concentrations and different duration of treatment. Mean chromocentre frequency was drastically reduced by the treatment. The distribution pattern of chromocentres in the nuclei was also noticeably affected. The most severe effect was noted at 100 µg/l at 24 hr and 12 hr treatment.

It is known that in plants vincristine produces, at the cellular level, such effects as accumulation of mitotic figures, arrested metaphases with highly coiled chromosomes, multipolar anaphases with lagging chromosomes in the interpolar region, tendency to polyploidy by doubling of the chromosomes but failure of chromatid separation and, at high dose, pycnosis. The induction of chromosome damage by vinblastine was studied in Vicia, Hordeum and Nigella and some chromosomal abnormalities have been noted (Degraeve,1978). Reduction of mean chromocentre frequency and variation in the distribution pattern of chromocentres on treatment with colchicine have earlier been reported in radish. Similar effect is noted here on treatment with vincristine. These variations possibly arise due to the fusion of heterochromatic chromocentres as a consequence of treatment with the drug. Whether they have anything to do with the meiotic chromosome behaviour is not known at present; only a future study will throw light on this problem.

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# THE CHANGE OF CHROMOSOME NUMBERS IN AUTOPOLYPLOID STRAINS IN RAPE

S. Tokumasu

The author already reported the mechanism of maintenance of polyploidy in artificially raised tetraploid strains in Raphanus sativus (Tokumasu, 1961), Brassica pekinensis (Tokumasu, 1982a) and B. japonica (Tokumasu, 1982b).

It is also a problem in rape (Brassica napus, 2n=38) whether or not chromosome numbers of plants can be constant and stable after tetraploid strains (2n=76) are newly induced. In the $C_4$ generation of a certain tetraploid strain, the author examined chromosome numbers of plants and found that they were distributed between 2n=46 and 2n=63, suggesting the decrease of chromosome numbers during four generations (Tokumasu, 1985b). Then, in order to elucidate the change of chromosme numbers of plants from one generation to another, chromosome counting was carried out with some aneuploids and their open-pollination progenies. The aneuploids used were five, whose chromosome numbers were 2n=76, 2n=67, 2n=58, 2n=52 and 2n=46. They all were obtained during the course of crossing experiments among different polyploids (Tokumasu, 1984a, b, c, 1985a). Table 1 shows the chromosome numbers of these aneuploids and the range of chromosome numbers of their progenies. Average chromosome numbers of the progenies are also presented. Chromosome numbers in each progeny were distributed widely. However, the average chromosome number of each progeny tended to decrease in comparison with the chromosome number of its parent. This tendency was stronger in the low chromosome-number plants (2n=52 and 2n=46) than in the high chromosome-number ones (2n=76, 2n=67 and 2n=58). Therefore, some difficulty is suggested with the maintenance of polyploidy in the tetraploid strain of rape. Chromosome numbers of tetraploids, after newly established, may continue to decrease through ensuing generations until they finally reach the diploid level. The degeneration of polyploidy may proceed more rapidly as constituent plants come nearer to the diploid in terms of chromosome numbers. In rape, there is no mechanism of maintaining polyploidy such as found in Brassica pekinensis and B. japonica. Details will be published (Tokumasu, 1985b, c, d).

Table 1. Relation of chromosome numbers between some aneuploids and their progenies

| Chromosome number | Parental aneuploids (a) | Progenies: Range (b) | Progenies: Average (c) | Difference (a - c) | % * (a - c)/a |
|---|---|---|---|---|---|
| | 2n=76 | 64 - 80 | 75.0 | 1.0 | 1.3 |
| | 2n=67 | 58 - 68 | 63.9 | 3.1 | 4.6 |
| | 2n=58 | 54 - 63 | 57.8 | 0.2 | 0.3 |
| | 2n=52 | 40 - 54 | 45.2 | 6.8 | 13.1 |
| | 2n=46 | 38 - 48 | 42.6 | 3.4 | 7.4 |

*Rate of decrease of chromosome numbers (%)

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# A PRELIMINARY GENETIC STUDY OF DIFFERENT MORPHOTYPES IN MUSTARD (Brassica juncea L.)

Shiru Chen and Nankui Tong

There are many different morphotypes of vegetable mustard (Brassica juncea L.) in China. Up to date, there were few reports about their genetic study.

In order to get some genetic information to characterize the different morphotypes for good use in breeding program, a comparative studies of peroxidase and acid phosphatase isozymes, karyotype analysis, chromosome behavior in miosis and inheritance of some important characters were studied by using fifteen cultivars and twelve crosses representing the different morphotypes of mustard (root, stem, and leaf mustard).

The polyacrylamide gel eletrophoresis was used for studying isozymes at three leaves seedling stage. The method of root tip cell suspending, flaming-drying and staining in Giemsa was used for karyotype analysis. The chromosome behavior in miosis of intervarietal $F_1$ hybrid was studied by smearing and staining in modified carbol fuchsin. The inheritance of leaf color (red vs green), flower color (yellow vs white), leaf shape (normal vs incision and terminal lobed vs non-terminal lobed), basal branching (basal branching vs high branching) and enlarged root (enlarged vs not enlarged) were studied by observation of parents, $F_1$ and $F_2$ generations.

The results are summerized as follows:

1. Both peroxidase and acid phosphatase isozymes showed some differences among cultivars, especially for the latter. In heterozygous conditions, there was no hybrid band appearance for peroxidase, but it was present for acid phosphatase isozyme. The results suggested that acid phosphatase isozyme would be better than peroxidase isozyme used as genetic marker in mustard germplasm research and breeding program.

2. The chromosome number of cultivars represented different morphotypes are all the same ($2n = 36$). Although there was no conspicuous difference in their karyotypes been observed, their karyotype symmetry had some variation. The chromosome behavior of intervarietal hybrids in miosis was normal. These facts suggested that all variations in different morphotypes of mustard were intraspecific variations.

3. The leaf color, leaf shape and basal branching habit of different cultivars examined were controled by one pair of genes, and flower color appeared to be controled by two pairs of interacted factors. The red leaf color, yellow flower color, basal branching, enlarged root, incision leaf, large foliage and large terminal lobed leaf were all dominant characters.

# A MUTABLE GENE FOR FLOWER COLOUR IN INDIAN MUSTARD

I.J. Anand and P.K. Mishra

The petal colour of the flowers of Indian mustard (Brassica juncea (L) Czern and Coss) normally ranges from light to pale yellow. However, strains with creamy-yellow to white petalled flowers are not uncommon. The differences in petal colours do not affect the floral morphology of the strains, either in size or structural composition, nor do they affect the functional aspects of the flower. Studies have suggested that the normal yellow colour of the flowers is dominant to white and that a pair of genes are interacting in the inheritance of flower colour.

In the course of breeding for yield improvement among diverse Indian types, the authors observed a new mutant with small white flowers possessing crumpled petals. The other interesting feature of this mutant was the protrusion of the stigmas of the flower buds at a very early stage of pod development, thus making it protogynous. The small flower buds that were likely to flower 8-10 days later threw their stigmas out from the buds, thus making it difficult to locate an unopened bud in the inflorescence. The observed mutant, designated as 'White Protruding' was selfed and crossed reciprocally with the normal yellow flowered plants to study the genetic behaviour of the observed mutated characters. The white protruding character

bred true on selfing, however, occassional branches with yellow non-protruding stigma flowers were observed on the white protruding plants. Further, a few plants with half the inflorescence possessing white protruding and the other half normal yellow were observed. Again, white protruding flowers with one, two, three or all petals normal yellow and even half white and half yellow petals in the same flower were observed. The frequency of the latter group of variation was also fairly large (24.53%), thus making it difficult to explain the observed results on the basis of simple normal mutation. The flower colour om the F1's of direct and reciprocal crosses revealed the dominance of normal yellow colour of petals and non-protrusion of the stigma in buds.

Genes as a rule are very stable, however, instances of mutable genes that mutate rather frequently in the course of development are not uncommon. The present finding can also be explained on a mutable gene basis.

Genetic investigation on the inheritance of these heterozygous ($\underline{Y}/\underline{w}^{m}$) variegated and the homozygous white protruding ($w^{m}/w^{m}$) flowers is underway. It is likely, that this new mutant with its frequent mutable effect will generate sufficient interest with the developmental geneticists, and the authors will be most willing to supply the same on request.

# INHERITANCE OF GLUCOSINOLATES IN CABBAGE

M.S. Chiang, C. Chong and R. Crête

A study of the inheritance of three specific glucosinolates and total glucosinolate content in cabbage is underway. Six genetic populations were used for this study including high and low glucosinolate content parental lines, $F_1$, $F_2$, $BC_1$ and $BC_2$ progenies. The three specific glucosinolates studied are thiocyanate ions, goitrin and volatile isothiocyanates.

Plants were planted in two fields: the soil of one field was infested with the clubroot pathogen Plasmodiophora brassicae race 2 (ECD 16/02/31) whereas the other was not. Methods of glucosinolate extraction and analysis has been described previously (Chong et al., 1985).

To date, only the analyses of the thiocyanate ions were completed. Since the thiocyanate ion content from the two fields were similar, the data obtained were pooled for genetic analyses.

The results indicate that low content of thiocyanate ion has a high degree of overdominance with a relatively low heritability (21%).

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# NEW SOURCES OF MALE STERILITY IN *BRASSICA*

I.J. Anand

Since Anand and Rawat (1978) and Rawat and Anand (1979) reported male sterility in Indian mustard (*B. juncea* (L) Czern and Coss), there has been considerable interest in screening the germplasm of crucifers for newer sources of male sterility at this Institute. A number of new sources of male sterility in different species of *Brassica* have been identified and variously reported. Attempts are also being made to synthesise male sterility through neclear substitution of one species to the cytoplasmic backgound of the other related or unrelated crucifers.

The occurrence of male sterility in *B. campestris* var. *dichotoma* (brown *sarson*) was reported by Katiyar (1983) and in *B. campestris* var. *trilocularis* (yellow *sarson*) by Singh and Saini (1983). The mode of inheritance is being investigated, and the preliminary results suggest that these spontaneously occurring male sterilities are caused by nuclear genes.

The author observed spontaneous male sterility in *B. napus* in one of the exotic collection of West German origin. The plant bore inflorescences and flowers like those of the parental line but it differed from the latter by having non-functional rudimentary anthers with no pollen. The inheritance pattern of this male sterility

(Anand and Rawat, 1984) suggested it to be due to a pair of genes (Ms ms) interacting at a single locus. No linked marker or pleiotropic effect of the male sterile gene has yet been found.

A new source of male sterility in B. juncea was observed recently by Anand et al. (1984) in the selected bulk population of the improved cultivar, 'Pusa Bold'. The population was morphologically uniform but a few plants were functionally male sterile. They bore relatively small flowers compared to the normal fertile flowers. The stamens were short with small anther lobes that did not dehisce at flowering. Anthersacs developed normally but the pollen formed was completely non-viable and was tightly enclosed by the epidermal layer of the anthersac. The inheritance of this male sterility is being investigated.

Another source of spontaneous male sterility was recently observed by Anand (1984) in B. carinata. The male sterile plant was dwarf with more flowering branches and bearing yellowish white flowers compared to the deep yellow flowers of the parental line. Only rudimentary anthers developed. Microscopic investigation revealed poorly developed anthersacs with few inviable pollen grains. The receptivity of the female gametophyte was normal, but pod setting under open pollinating conditions was very poor. The genetic behaviour of the male sterility is being studied.

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# MECHANISM OF MALE STERILITY IN BRASSICA JUNCEA.
## I. MANIFESTATION OF STERILITY AND FERTILITY RESTORATION.

I.J. Anand, P.K. Mishra and D.S. Rawat

Male sterility in brown or Indian mustard (B. juncea (L) Czern and Coss) was first observed by Anand and Rawat in 1976 in a line derived from a plant of alloplasmic origin. The initial studies of intra-specific hybridisation between the male sterile line and a number of indigenous and exotic B. juncea germplasm revealed the sterility to be cytoplasmic in origin (Anand and Rawat, 1978), as pollen fertility was not restored in the crosses. The male sterile plants under natural open pollinating conditions have been found to produce 95-98% pod and seed setting compared to normal fertile lines (Rawat and Anand, 1979). Further, intra-specific hybridisation resulted into three different forms of expression of male sterility:

i) Petaloid: where anthers were modified to narrow petals,

ii) Stigmoid: where anthers were modified to elongated stigmatic papillae, and

iii) Rudimentary: where anthers were modified to small white rudimentary structures.

The three forms are interchangeable under extreme temperatures, that is also proved by histological studies. The stigmoid type is often associated with multiple false ovaries with 3-6 ovules which remain non-functional. There

is also an undesirable linkage between the stigmoid type and bending of the siliqua. Studies by Mishra (1984) have clearly revealed substantial differences among the three forms of male sterility and the normal fertile in respect of morphological, agronomical, biochemical and histological traits. The effect of environment on the expression of male sterility was studied under 14 different environments (both under laboratory/field conditions) and it was observed that the male sterility was highly stable. Studies on the origin of this cytoplasm using the techniques of Fraction I protein and isozyme pattern are in progress.

The male sterile lines were crossed with number of other species of Brassica and related crucifers with a view to searching for fertility restoring genes. Among the various interspecific and inter-generic crosses, the following partially restored fertility.

i) MS B. juncea x B. nigra (only one line of Pakistan origin),

ii) MS " " x B. campestris (two lines of Indian & Canadian origin),

iii) MS " " x B. napus (only one line of Swedish origin),

iv) MS " " x B. carinata (two lines of African origin).

The partially fertile plants initially showed pollen viability from 30 to 46%. Further selection for pollen fertility restoration (i.e. well developed anther lobes with profuse pollen production has led to the development of restorer lines with 91-98% pollen viability Pollen fertility restorers were picked up from each of the sources, and constant selection for pollen production and the ability to restore fertility in crosses with male sterile sources has developed restorer (R) lines where restoration ranges from 60 to 70% for B. campestris (RC), B. nigra (RN) and B. napus (RNa) restorers. The preliminary data on the inheritance of pollen fertility restoration in each of the source RC and RN suggest that one to two gene loci govern the inheritance. The two restorers (RC & RN) were crossed reciprocally, and with irradiated pollen, to obtain complete pollen fertility restoration.

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# MECHANISM OF MALE STERILITY IN *BRASSICA JUNCEA*. II. HISTOLOGY OF FLORAL BUD DIFFERENTIATION

P.K. Mishra and I.J. Anand

Sections of 10 µ thickness of terminal floral buds of normal fertile (F), petaloid, rudimentary and stigmoid male sterile form (Ms), and partial restorer (Pf) of male fertility, revealed normal development of sepals and petals for all lines. Pistil development was normal for all lines except stigmoid, which showed three rows of ovules instead of two as observed in normal fertile.

Abnormality in stamen development and anther differentiation was observed for all lines except normal fertile. In the petaloid form, the anthers were modified to structures that resembled normal petals. Anther development of the rudimentary form was characterised by the development of a mass of parenchymatous filament cells with occasional initiation of single-sac anther lobes having a few aborative pollen microspore mother cells. Stigmoid was characterised by the modification of stamens to floral structures that were light green in colour, either fused with the ovary or free, and possessing capillary hair-like protrubences (stigmatic papillae) at the tip like the stigmatic surface, stigmoid stamens sometimes had one row of ovules.

Floral buds were observed with infrequent structural modifications of either petaloid + rudimentary, petaloid + stigmoid or rudimentary + stigmoid. No case of all the three types of modifications of anthers in the same flower bud was observed.

Anthers in the buds of partial male fertility restorer plants (from RN and RC) exhibited one anthersac in each lobe instead of two as in normal fertile. Partial restorers had fewer pollen grains per microscopic field and contained a higher percentage of sterile pollen grains than normal fertiles.

CONCLUSION:

It can be concluded from the absence in male sterile lines of anther loculi tapeta and pollen mother cells that inhibition of anther development occurred either at the stage of carpel and male archesporial cell differentiation or at the differentiation of pollen mother cells.

# MECHANISM OF MALE STERILITY IN *BRASSICA JUNCEA* III. PROTEIN CONTENT AND AMINO ACID PROFILE OF REPRODUCTIVE ORGANS

P.K. Mishra, I.J. Anand and S.R. Chatterjee

The protein content of the inflorescence and the amino acid profiles of stamens were analysed in the three forms of male sterile lines and their maintainer and restorer plants derived from *Brassica nigra* and *Brassica campestris* sources.

A comparison of crude protein content among the male fertile lines indicated that there was no significant difference between the normal fertile (nonrestoring) and restorer sources. The protein contents of male fertile lines (restoring and non-restoring) were significantly higher than all the male sterile lines. Among the male sterile lines, the petaloid form had a significantly higher protein content than the stigmoid, and the rudimentary form had an intermediate content.

There were significantly greater contents of acidic amino acids in the petaloid and stigmoid forms, and in the restorers derived from *B. nigra*, than in normal fertile forms. All the male sterile and restorer lines had significantly lower basic amino acid content than normal lines. The neutral amino acid contents were similar in all the materials.

The amino acid profile of male sterile lines revealed that petaloid (ms) contained more of glutamic acid (Glu), proline (Pro) and ammonia and less of asparatic acid (Asp), histidine (His), cysteine (Cys), methionine (Met), threonine (Thr), phenylalanine (Phe), and lysine (Lys) in comparison to maintainer line (normal fertile). For other amino acids, no definite trend was observed.

The rudimentary form of male sterility exhibited more Glu, Thr and ammonia and less Asp, His, Cys, Met and Phe than the normal fertile. There were no differences in contents of the other amino acids.

The stigmoid type had more of the acidic amino acids (Asp and Glu), Pro and Met, and less of His, Cys, Asn and Lys than the normal. No clear cut difference was observed for other amino acids. Apparently stigmoid (ms) contained more acidic proteins than the normal.

The amino acid composition of normal fertile and restorer (campestris source - RC) differed only for His, and Met. The restorer from the B. nigra source (RN) exhibited significant differences in the contents of Asp, Glu, Leu, Phe, Arg, Thr, valine (Val) and serine (Ser.).

Conclusion:

In general, it was observed that all the three forms of male sterile lines had less crude protein in the inflorescence. There were invariably less cysteine and histidine but more glutamic acid incorporated in the polypeptide chain and there was no consistent pattern for asparatic acid or methionine.

Table 1 : Protein content and amino acid profile of normal, male sterile and restorer lines in mustard (data partly reproduced)

| Line | Protein | Amino acid g/100 g. of protein composition | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % | Cys | His | Glu | Asp | Met | Pro | Lys |
| Normal (F)* | 30.66 | 2.05 | 3.33 | 12.11 | 9.82 | 1.18 | 4.75 | 8.33 |
| Petaloid (MS) | 26.46 | 0.86 | 2.55 | 14.18 | 9.09 | 0.38 | 5.42 | 7.31 |
| Rudimentary (MS) | 25.09 | 1.64 | 2.08 | 13.37 | 9.18 | 0.84 | 4.77 | 7.92 |
| Stigmoid (MS) | 24.19 | 1.71 | 1.30 | 13.13 | 10.26 | 1.60 | 5.18 | 7.61 |
| RC (PF) | 31.64 | 1.95 | 2.67 | 12.64 | 9.43 | 1.71 | 4.41 | 8.12 |
| RN (PF) | 30.07 | 1.95 | 2.20 | 13.91 | 10.68 | 0.91 | 4.94 | 7.92 |

* F, MS and PF refers to Fertile, Male sterile and Partial Fertile respectively.

# MECHANISM OF MALE STERILITY IN *BRASSICA JUNCEA*. IV. COMMERCIAL EXPLOITATION OF HYBRID VIGOUR

I.J. Anand, D.S. Rawat and P.K. Mishra

A methodology for exploiting cytoplasmic male sterility for heterosis breeding of seed yield was suggested previously by Anand and Rawat (1978) and has been further improved for crop plants where pollination is predominantly anemo-or entemo-philous in certain domesticated *Brassica* species and sunflower. The breeding method (Fig. 1) involves the following steps:

1. Transfer of male sterility in recommended improved varieties by repeated backcrossing (4-5 backcross will do).
2. Test combining ability and heterotic performance of the variety/varieties being developed for male sterility with that of other productive varieties (in any mating design). Select pollinator lines that have good combining ability and heterotic effects with the variety converted into male sterile background.
3. Suitably identify pollen requirement of the male sterile variety/varieties developed from step 1 by growing it alternately with the selected pollinator line (from step 2) in a fixed ratio of male sterile lines to pollinator line.

4. Harvest separately the seeds from both the male sterile variety/varieties (the hybrid seed) and the fertile pollen variety (the varietal seed).

5. Mechanically mix a known proportion of the harvested hybrid seed with that of the pollinator line (from step 4) in some suitable ratio (i.e. MS 60: F 40; MS 70: F 30; MS 80: F 20 and so on) and release it for commercial cultivation (through seed production agencies) if found promising in yield trials. In the mechanically mixed hybrid population, the plants of the pollinator line will provide sufficient pollen to fertilise the male sterile flowers of the hybrid plants, thus enhancing seed production. The hybrid plants will express heterosis for related yield traits such as for greater number of fruit bearing branches with more flowers and ovules per ovary etc.

Until complete fertility restorer lines are made available, the methodology suggested has the edge over the pure line varietal approach normally followed, in self fertilising crops. The seed harvested from growers' plots, if resown, will not suffer much inbreeding depression, as continuous heterozygosity in the population will be maintained by the crossing of male sterile hybrid derivatives with mixed pollen producing plants of varietal seed.

Using this methodology, the pollen requirements of the male sterile lines have been worked out. The data suggest that a ratio of MS 80: F 20 i.e. four parts of a male sterile and one part of a fertile variety of B. juncea in a mixture can provide enough pollen to produce the hybrid seed. Hybrids using this methodology have been obtained in isolation and will be tested in trials.

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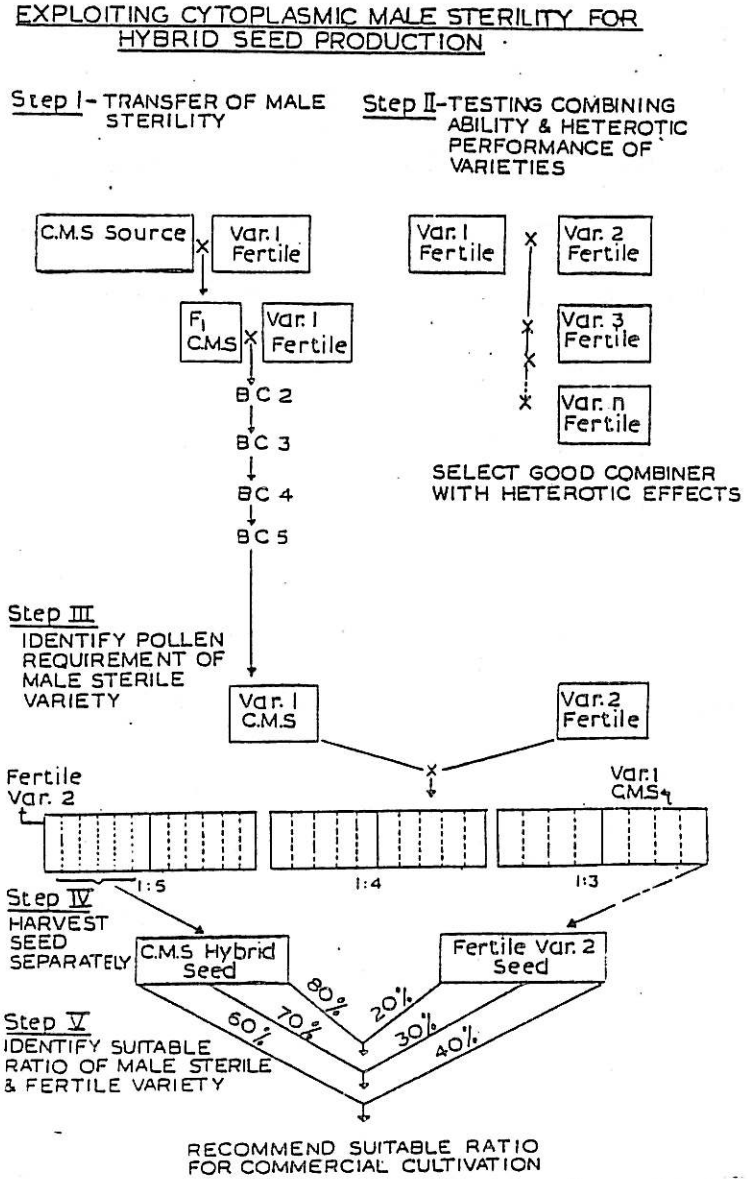

# MECHANISM OF MALE STERILITY IN *BRASSICA JUNCEA*

## V. ORIGIN OF MALE STERILITY

S. Swarup and I.J. Anand

In order to investigate the probable progenitors of the observed male sterility, studies were carried out on a number of morphological, physiological, agronomical and biochemical traits using both statistical (Canonical and $D^2$ techniques) and biochemical (seed protein and B-esterase isoenzyme electrophoretic banding patterns) analysis.

At morphological level, 30 representatives comprising different species and sub-species of 12 different taxa of the genus *Brassica* along with the male sterile line of *B. juncea* were included to elucidate the genetic relationships among different taxa through a study of genetic divergence. The clustering of different taxa, in general, were in accordance with the accepted pattern of classification. For example, the various leafy types of Chinese origin (ssp. *narinosa*, *chinensis*, *japonica* and *nipposinica*), the two diverse *nigra* lines of Indian and European origin, the ssp. *oleifera* (viz. var. *toria*, yellow and brown *sarson*) and the various synthesised amphidiploids of *B. juncea* were clustered in respective groups indicating closer genetic affinity among themselves.

The $D^2$ statistic was sensitive enough to differentiate not only at the taxonomic species level but was also found potent in determining the donor parents of synthesised amphidiploids. The three taxa found genetically closer to MS - B. juncea included the amphidiploids JN (japonica x nigra), RN (rapa x nigra) and YN (yellow sarson x nigra) and the monogenomic (n=10) sub-species yellow sarson.

The biochemical studies of polyacrylamide gel electrophoresis (PAGE) and the esterase isozyme on the seed protein profile of the various taxa suggested quantitative differences in the banding pattern and that certain bands were characteristic of the genus as a whole. The banding pattern of B. nigra and B. fru ticulosa, the two monogenomic species with gametic chromosome number 8, was highly divergent. B. oleracea var. alboglabra (n=9) was characterised with a band which could not be detected in any other taxa. The subspecific variation in the banding pattern of n=10 species was clearly marked out. B. tournefortii diverged considerably from the rest of the monogenomic species.

The banding pattern of digenomic Brassica species (n=17, 18 and 19) revealed a great deal of homogeneity in the protein profile. The various B. juncea lines exhibited remarkable homogeneity irrespective of their wide geographically diverse origins.

While comparing the different synthesised amphidiploids against their parents, it was found that all the bands of the amphidiploids may not necessarily be present in their parental species and vice-versa. The esterase isozyme banding pattern and the seed protein electrophoretic profile of the two amphidiploids JN and YN was almost similar and compared favourably with MS B. juncea and the subspecies japonica. The average similarity index (a.s.i.) values of the mentioned taxa further supported a close affinity among these lines. The various investigations carried out thus suggested that the probable progenitors of the MS B. juncea appeared to have involved as one parent in yellow sarson (B. campestris ssp. oleifera var. yellow sarson) either as n=10 elemental species or in combination with B. nigra as an amphidiploid (YN), and the other derived from japonica x nigra (JN) or rapa x nigra (RN) amphidiploid.

# MALE STERILITY IN COLE CROPS- A SERIAL STORY

Q.P. van der Meer

Several types of male sterility are known, having different backgrounds:
- Based on one pair of recessive genes: ms ms = male sterile (Cole, 1959, Nieuwhof, 1961).
- Temperature dependent; some genotypes are only male sterile at either heigh or low temperatures (Nieuwhof, 1968; Williams, 1974).
- Based on the species cross Brassica nigra x B. oleracea (Pearson, 1972).
- Based on the species cross male sterile Raphanus sativus (radish) x B. oleracea (Bannerot, 1974).
- Based on the species cross B. napus x B. oleracea (Chiang, 1979).
- As a result of GA 4+7-treatment (Van der Meer and Van Dam, 1979).

Both at the IVT and elsewhere it appeared that all the above-mentioned types have one or more handicaps regarding their use for hybrid breeding. After investigating several other male sterility types the IVT is now looking for maintainers of the first mentioned (ms ms-) type.
The approach consists of two phases. In the first place the ms-frequency is determined in a number of varieties. Subsequently a screening for maintainers (= N ms ms) of male sterility (= S ms ms) is done in varieties showing the highest ms-frequencies.
A start was made, in 1983, in crossing male sterile ms ms plants with plants of a number of autumn cauliflower varieties.
The offspring were repeatedly investigated with respect to their sex expression in the open (in 1984). The following remarkable results were obtained:

Percentages of male sterile plants in offspring of crosses between, on the one hand, male sterile plants, and on the other hand, plants of autumn cauliflower varieties.

| Variety | Male sterile plants x variety | Number of plants per offspring | % male sterile plants | |
|---|---|---|---|---|
| | | | var. | m.st.pl. x var. |
| Balanza | | 39 | 18 | |
| | m.st.pl. x Balanza | 19 | | 47 |
| Lukra | | 17 | 13 | |
| | " x Lukra | 46 | | 37 |
| Nimba Meda | | 25 | 0 | |
| | " x N. Meda | 41 | | 27 |
| Iglo ($I_2$) | | 31 | 0 | |
| | " x Iglo ($I_2$) | 27 | | 19 |
| Alpha B ($I_2$) | | 30 | 0 | |
| | " x Alpha B ($I_2$) | 46 | | 24 |
| Flora Blanca ($I_1$) | | 20 | 0 | |
| | " x Fl. Blanca ($I_1$) | 42 | | 26 |
| Oze White Top ($I_1$) | | 34 | 0 | |
| | " x O.Wh. Top ($I_1$) | 29 | | 24 |

It is obvious that high frequencies of ms genes occur in the varieties investigated. This is in disagreement with earlier investigations in which extremely low frequencies were found (Jensma, 1957; Nieuwhof, 1961). Moreover the last five varieties seem to possess N cytoplasm, because otherwise the percentages of male sterile plants in their offspring would be higher than 0.

## DISCUSSION

From the data obtained so far no definite conclusions can be drawn regarding the genetic background involved. For elucidation of the inheritance and for tracing (possible) maintainers a number of pair crosses have been made in 1985.

Notwithstanding the incomplete knowledge of the inheritance it seems very probable that backcrosses of male sterile plants to plants of the varieties mentioned will result in high percentages of male sterile plants in the following generation. So it seems easy to obtain male sterile inbred plants which can be used for experimental hybrids. Subsequently the male sterile parent of superior hybrids must be propagated vegetatively for the production of commercial seed.

The disadvantage of the high cost of vegetative propagation is, at least partly compensated by a better stability of such hybrids in comparison with hybrids produce on the basis of two generatively propagated parents. If maintainers are found (also) the utilization of generative propagation of the male sterile hybrid parent must be investigated.

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# INTERRELATIONSHIP AMONG PLANT CHARACTERISTICS IN RUTABAGA (*BRASSICA NAPUS* L. SSP. *RAPIFERA*).

V.I. Shattuck

Nine cultivars of rutabaga (*Brassica napus* L. ssp. *rapifera*) were grown at 2 locations in Ontario, Canada to explore the interrelationships of several plant characteristics. The traits evaluated included foliage dry weight, root dry weight, biological yield, root diameter, root length, root dry matter percentage and the soluble sugar level of the storage root. The entries included 'Altrasweet', 'American Purple Top', 'Bangholm', 'Danestone', 'Laurentian', 'Niko', 'Vige', 'Wilhelmsburger' and 'Wye'. Data collected was analyzed using ANOVA. Dry root weight, root diameter, root length, biological yield and leaf dry weight were subjected to logarithmic transformation prior to the analysis. Genotypic correlations (rg) were calculated using the following equation:

$$rg = \frac{MP_{xy} - \text{residual } MP_{xy}}{\sqrt{(MS_x - \text{residual } MS_x)(MS_y - \text{residual } MS_y)}}$$

where MP = mean cross products

MS = mean square of the characters x and y being compared and residual $MS_x$ and $MS_y$ are the mean square error terms for each appropriate character.

The location influenced all traits with the exception of percentage dry matter and root diameter. In addition, genotype x environment interaction was detected for root soluble sugar concentration. These findings suggest caution in selecting rutabaga research sites for use in performance trials. In the case of percentage soluble sugars it would be advisable to screen cultivars at more than one location.

The genotypic correlation coefficients between the pairs of seven characters are presented in Table One. Root yield although positively associated with all components analyzed was most highly correlated with the size of the plant (biological yield) and foliage weight. The accumulation of root dry matter per unit area was highly correlated with the soluble sugar concentration of the root juice (rg=0.77). Attempts to increase root dry matter levels might therefore lead to a corresponding increase in root soluble sugar content. This relationship was linear at both sites. The regression of dry matter percentage (DMP) on soluble sugars (SS) yielded the following equation:

$$DMP = 1.17\ (SS) + 1.97$$

A low association was recorded for foliage weight and root soluble sugar levels. Dry matter percentage of roots was similarily poorly correlated with the extent of foliage at harvest. These weak associations suggest the percentage of soluble sugar and dry matter in rutabaga roots is not totally determined by the plant capability to produce photosynthates. The low correlation between bulb diameter and length (0.26) should not impede the efforts of plant breeders attempting to modify root shape.

It was determined by multiple regression analysis that percent dry matter, foliage weight, root diameter and root length accounted for 86 percent of the variability in dry root weight.

**Table 1.** Genotypic correlation coefficients among plant characters in rutabaga.

| | Soluble sugars | Root diameter | Root length | Root dry weight | Percent dry matter | Foliage weight |
|---|---|---|---|---|---|---|
| Biological yield | 0.54 | 0.87 | 0.71 | 0.96 | 0.32 | 0.97 |
| Foliage weight | 0.31 | 0.90 | 0.66 | 0.91 | 0.11 | |
| Percent dry matter | 0.77 | 0.33 | 0.19 | 0.53 | | |
| Root dry weight | 0.85 | 0.71 | 0.76 | | | |
| Root length | 0.42 | 0.26 | | | | |
| Root diameter | 0.22 | | | | | |

# GENETIC VARIABILITY AND SELECTION INDICES IN BROWN SARSON

Naresh Yadava[1], P.R.Kumar[2], and R.K.Behl[3]

Brown sarson (Brassica campestris var. brown sarson) is an important oilseed crop and commands premium in the Indian market due to its higher oil content. Despite this fact, the pace of progress of developing high yielding genotypes to ensure high oil recovery per unit area, has not been encouraging (Kumar,1981). Moreover, seed yield and its components being quantitative characters are largely influenced by environmental fluctuations and direct selection for seed yield alone, many a times, has proved to be a vague criterion. Therefore, to ensure appreciable gain, breeding programme in brown sarson has to be preceded by enlargement and assessment of genetic variability, computation of a selection criterion to enable breeders to decide about the magnitude and direction of selection pressure during selection phase. The present study,therefore, deals with such an attempt.

Thirty nine genotypes of brown sarson developed recently at the Dry Farming Research Centre, Bawal of the Haryana Agricultural University(Kumar, 1979), were grown in a randomized block design with three replications following the recommended package of practices. Observations on randomly selected 10 plants from each genotype in each replication were recorded for ten important characters. Phenotypic (PCV) and genotypic coefficient of variation, heritability (broad sense), expected genetic advance and selection indices were computed using standard procedures. Relative efficiency of various selection indices and expected genetic advance thereof was expressed as per cent of genetic advance expected from selection on the basis of yield alone.

The analysis of variance revealed the existence of significant variability among genotypes for all characters. This is substantiated by wide range of variation for each character particularly for plant height, total number of siliqua per plant, number of siliqua on the main shoot, number of secondary branches per plant and seed yield(Table 1). For oil content and 1000 seed weight characters, however, the range of variation was relatively low. In general, there was good a agreement between genotypic and phenotypic coefficient of variation for all the characters, both being the highest for the number of secondary branches per plant and the lowest for the oil content. Heritability estimates, indicative of heritable genetic variation, invariably ranged from moderate to high, except for the oil content. The highest and the lowest heritability estimates were recorded for the total number of siliqua per plant and the oil content, respectively. Heritability estimates when studied in conjunction with genetic advance gives a better picture for predicting the resultant effect of selection (Johanson et al., 1985). Since the expected geneticadvance and

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heritability estimates were considerably high for the total number of siliqua per plant, an effective improvement in seed yield can be brought about by selection based upon this character. For all other characters, the expected genetic advance was low, indicating thereby that straight selection for these characters would not be effective. These results are in accordance with the reports of Lodhi et al. (1979).

Component characters like number of primary branches($X_2$), number of secondary branches ($X_3$), total number of siliqua per plant($X_4$), number of siliqua on main shoot($X_5$) showing positive association (data not given) with seed yield ($Y_1$) were considered for the synthesis of selection indices using all possible character combination. As expected, the highest selection gain was envisaged by selection indices based on all the five characters (Table 2). In general, all the higher order indices gave higher expected genetic gain when the yield alongwith one or two component character was considered. However, it was interesting to note that as and when the character like total number of siliqua per plant came in any combination, the expected genetic gain was considerably high.

Keeping in view the constraints over time and space, it would be desirable to base selection on few simply definable characters. In this context, a combination of total number of siliqua per plant and seed yield appeared to be an effective selection indices since it gave sufficiently high genetic gain (only 8 per cent less than the best combination based upon all the five characters). For further improving the productivity of this allogamous crop, inclusion of genotypes, BS-2, BS-27, BS-36, BS-37 and BS-43 in hybridization programme followed by selection using judicious selection indices would be rewarding.

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Table 1. Parameters of variability in brown sarson

| Characters | Range | | Genotypic coefficient of variability | Phenotypic coefficient of variability | Heritability (%) | Genetic advance (%) |
|---|---|---|---|---|---|---|
| | Min | Max | | | | |
| 1. Days to first flowering | 40.56 (BS-27) | 54.30 (BSH-1) | 6.15 | 8.97 | 46.97 | 3.98 |
| 2. Plant height(cm) | 84.06 (BS-1) | 131.96 (BS-44) | 8.53 | 13.79 | 38.25 | 11.06 |
| 3. Primary Brahces/ plant | 4.00 (BS-10) | 10.00 (BS-5) | 13.66 | 28.58 | 26.42 | 0.89 |
| 4. Secondary branches/plant | 2.46 (BS-19-1) | 12.80 (BS-36) | 35.52 | 55.63 | 40.76 | 3.21 |
| 5. Height of the main shoot(cm) | 40.06 (BS-1) | 51.66 (BS-43) | 7.57 | 12.44 | 37.01 | 4.47 |
| 6. Total number of siliqua/plant | 59.40 (BS-10) | 198.60 (BS-43) | 28.76 | 41.32 | 48.46 | 51.37 |
| 7. Siliqua on the main shoot | 16.56 (BS-4) | 34.53 (BS-43) | 11.79 | 19.66 | 35.99 | 3.74 |
| 8. 1000 seed weight(g) | 3.46 | 5.40 | 7.62 | 12.39 | 37.79 | 0.43 |
| 9. Yield/plant(g) | 3.16 | 11.13 | 19.91 | 44.34 | 20.16 | 1.15 |
| 10. Oil content(%) | 43.38 (BS-4) | 47.68 (BS-2) | 1.38 | 3.47 | 15.90 | 0.52 |

Table 2. Selection index and expected genetic gain in brown sarson.

| Combinations | Expected genetic gain | Selection index |
|---|---|---|
| $Y_1 + X_2$ | 1.925 | 3.2569 |
| $Y_1 + X_3$ | 5.0104 | 4.6731 |
| $Y_1 + X_4$ | 53.9738 | 60.3993 |
| $Y_1 + X_5$ | 4.8600 | 11.8060 |
| $X_2 + X_3$ | 4.1375 | 2.4037 |
| $X_2 + X_4$ | 52.2675 | 57.5944 |
| $X_2 + X_5$ | 4.6129 | 12.7600 |
| $X_3 + X_4$ | 55.3817 | 63.356 |
| $X_3 + X_5$ | 5.7100 | 11.985 |
| $X_4 + X_5$ | 53.71 | 68.5689 |
| $Y_1 + X_2 + X_3$ | 5.6905 | 5.008 |
| $Y_1 + X_2 + X_4$ | 54.7524 | 64.008 |
| $Y_1 + X_2 + X_5$ | 6.2865 | 15.6706 |
| $Y_1 + X_3 + X_5$ | 7.4460 | 15.1409 |
| $Y_1 + X_4 + X_5$ | 56.4221 | 48.7443 |
| $X_2 + X_3 + X_4$ | 24.0725 | 67.668 |
| $X_2 + X_3 + X_5$ | 12.1426 | 13.5873 |
| $X_2 + X_4 + X_5$ | 54.67 | 64.1966 |
| $X_3 + X_4 + X_5$ | 57.8831 | 72.65 |
| $Y_1 + X_2 + X_3 + X_4$ | 43.7146 | 57.74 |
| $Y_1 + X_2 + X_3 + X_5$ | 6.8629 | 27.8848 |
| $Y_1 + X_2 + X_4 + X_5$ | 57.16 | 72.18 |
| $X_1 + X_3 + X_4 + X_5$ | 60.5309 | 76.1479 |
| $Y_1 + X_2 + X_3 + X_4 + X_5$ | 61.00 | 78.07 |

$Y_1$ = Yield

$X_2$ = Primary branches
$X_3$ = Secondary branches
$X_4$ = Total number of siliqua
$X_5$ = Siliqua on main shoot.

# BREEDING IMPROVED GREEN-CURDED CAULIFLOWERS

Andrew Gray and Peter Crisp

## Introduction

All major forms of cauliflower (Brassica oleracea var. botrytis) have white or cream curds. In Italy, the centre of the broccoli/cauliflower gene pool, two relatively primitive forms with green curds are cultivated. Green and white curds differ by virtue of two genes (1).

In several southern and eastern regions of southern Italy smooth green-curded cauliflowers occur. They do not apparently have a common name and we refer to them collectively as 'Cavolfiore di verde Macerata' or simply 'Macerata' after one regional type. Characteristically they have large, smooth, stiffly held or slightly reflexing leaves on short stems. In southern Italy the Macerata appears to overlap genetically with the purple heading broccoli of Sicily; ricey curds of the Macerata (i.e. in which the curd surface has differentiated into flower buds) may be indistinguishable from the Sicilian broccoli.

Around Rome the very distinct 'Cavolo broccolo Romanesco' is grown. Like the Macerata the curd is green, but has a highly whorled pyramidal conformation chacteristic of the north eastern Italian type known as 'Jesi'; this curd conformation is highly heritable (2). The plant habit of the Romanesco is tall with some tendency to lodge. The leaves are undulating, indented and petiolate and do not usually envelop the curd. Although referred to as 'Cavolo broccolo' Romanesco is a true cauliflower in the sense that the edible part is a curd, as distinct from being composed of flower buds (3).

When grown under British conditions both types perform well from May sowings, the Macerata typically matures in late August/September and the Romanesco in September to December.

Existing Italian commercial seedstocks are highly variable and large proportions of the curds may be of poor quality and small size. Nevertheless there is increasing interest in Britain in these crops firstly because of the attractive appearance and culinary qualities of the Romanesco and secondly because green curds per se are more frost tolerant than white curds (4).

We report here on preliminary attempts to improve stocks of the late maturing Romanesco.

## Materials and Methods

Our breeding programme started in 1982 and is based on recurrent selection from several Italian seedstocks, mostly obtained as part of the European Community crucifer genetic conservation programme (5,6). Two cycles of selection have now been completed and subjective assessments show improvements in uniformity and quality.

In 1984 a replicated trial was conducted of three parental Romanesco stocks and mass-pollinated progenies of four, five and six selected plants from each of these stocks. The parental plants had been selected as being of good quality and size, and their harvest dates had been recorded.

## Results

The trial procedure and detailed results are available from the authors on request. Here we present a summary (Table 1). The overall means of the progenies gave, in effect, the results of one cycle of mass selection: they showed improvements in curd weight and several quality characters. Significant differences between the progeny means indicated residual genetic variation in the improved population: again, weight and quality of the curd showed differences.

Table 1 Performance of parental stocks and first generation progenies selected from them

| Character of curd | Parental Mean | Progeny means | | |
|---|---|---|---|---|
| | | Overall mean | Range | SED |
| Weight (g) | 408 | 449 | 363 - 571 | 34.0 |
| Diameter (cm) | 14.0 | 14.2 | 13.5 - 15.5 | ns |
| Scores (in all cases, the lower, the better) | | | | |
| Depth | 2.6 | 2.3 | 2.0 - 2.6 | 0.21 |
| Riceyness | 0.4 | 0.3 | 0.0 - 1.1 | 0.24 |
| Bractedness | 1.4 | 1.2 | 0.9 - 1.5 | 0.18 |
| Looseness | 1.2 | 0.6 | 0.3 - 0.8 | 0.16 |
| Maturity (days from sowing) | | | | |
| Mean | 144 | 148 | 136 - 158 | 3.4 |
| Variance | 257 | 161 | 62 - 243 | ns |

SED is calculated from the genotype x replicate interaction; ns indicates that genotypic means did not differ significantly when compared with this interaction.

Considerable segregation had occurred for mean maturity time, giving a 22 day spread of the progeny means. As is usual in cauliflowers, maturity time proved to be highly heritable: the pooled parent/offspring correlation was 0.72 (with 10 degrees of freedom).

## Conclusions

Green-curded cauliflowers appear to be suited to cultivation in parts of Britain, and there are clear prospects of improving the Romanesco cauliflower (and perhaps other green types) to give uniform, good quality cultivars, maturing in succession, and, thereby encouraging their integration into British horticulture. Although we have restricted our efforts to population improvement by recurrent selection, it appears that the Romanesco possesses several highly effective self-incompatibility alleles and future breeding could exploit the breeding system to develop $F_1$ and other hybrid cultivars.

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# FACTOR ANALYSIS OF YIELD COMPONENTS IN CAULIFLOWER (BRASSICA OLERACEA VAR. BOTRYTIS)

S.C.PANDEY AND G.NAIK

In most of the crops, significant, positive or negative association between the yield and its components have been reported but little is known about the mechanism underlying such associations (Walton 1971). Factor analysis explains such associations in terms of a few causative influences (factors) in a multiple cause-and-effect system. Such an approach would be helpful in revealing and evaluating the unidentified sources of variation of a set of characters which would not have been otherwise suspected. The present attempt in cauliflower to explain the significance of this biometrical tool.

## MATERIALS AND METHODS:

Thirty three cauliflower lines of diverse origin were grown in Randomised Block Design with three replications in 1984. Ten competitive plants were selected for observations from each replication. The observations were recorded on days to initiation of curd, days to maturity of curd, plant height, number of leaves, width of leaves, length of leaves, diameter of curd, plant weight, curd weight and leaf weight. The average of all the characters across replications were used for statistical analysis. The factor analysis method was used that of Burt and Banks (1947). This method was used by Seal (1966). The communalities and factor loading were fixed by taking the highest correlation in each row or column of the C-matrix.

## RESULTS AND DISCUSSION:

The centroid factor loading obtained from the correlation matrix are given in Fig.1. It was possible to extract only two common causative influences (factors) to explain the intercorrelations of the total plant weight and other yield components since the coefficients in residual matrix were too low to allow for the extraction of more factors. The number of factors to be extracted would depend on the rule taken for investigation, that $p + k$ should be less than $(p - k)^2$, where p and k are the number of characters and factors respectively, appears useful Cattell (1965).

The percentage contribution of factors 1 and 2 was 52 and 48. Length of leaves, plant height and diameter, weight of leaves and weight of curds had maximum contribution for plant weight and ultimately to economic yield. The communalities indicates the amount of variance of variables for the factors taken together. The characters, days to initiation of curd, curd weight, leaf weight, plant height and width of leaves had maximum values indicates the maximum contribution of these characters towards yield. Factors loading also indicates the contribution of the variables. Factor 1 and 2 has different degrees of positive loading on all the character but with varying magnitude. The highest factors loading was observed for weight of curd, weight of leaves, days to initiation of curds, plant height and width of leaves. All the characters studied were expression for plant weight can be divided into two groups such as factor 1 and 2.

Fig.1 - Arrow Diagram showing the effects of Factors on Nine Variables in Cauliflower.

| Variables: | Factors loading: | Factors: |
|---|---|---|
| Diameter of curd | 0.55 | |
| Width of leaves | 0.88 | 1 |
| Weight of leaves | 0.97 | |
| Weight of curds | 0.97 | |
| Days to maturity of curds | 0.47 | |
| Number of leaves | 0.47 | |
| Length of leaves | 0.78 | 2 |
| Plant height | 0.93 | |
| Days to initiation of curds | 0.99 | |

The high estimates of heritability accompanied by high genetic advance of the quantitative characters enables the plant breeders to base the selection programme on the phenotypic performance. Johnson et al. 1955, Pandey and Naik (1984) found in cauliflower that days to initiation of curd, days to maturity of curd, curd weight, leaf weight had high heritability (80 - 99%) and genetic advance percent of mean

(22 - 79.54) while plant weight, number of leaves and length of leaves had low (31.61) to moderate (64.27 to 77.08%) heritability low to high genetic advance. Thus it is possible to make improvement by following simple selection procedures in curd weight.

Factor analysis showed which yield components were associated with which characters. The plant breeder would thus have available information to determine the characters for which selection should be made to maximixe the yield. In the present investigation weight of curd, weight of leaves, curd diameter and width of leaves were the main yield contributing factors needs exploitation.

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# INBREEDING STUDIES IN GARDEN CRESS

N. Dayal, S.N.Singh and B.B.Lal

Garden cress, Lepidium sativum L., is an annual, semicultivated crop plant grown in India and the world over for a variety of economic reasons, viz. vegetables, salad, medicines etc. It is moderately cross-pollinated species with a sporophytic self-incompatibility system ( Sampson,1962 ). Although being an important vegetable crop in India, it has not attracted the attention of cytogeneticists and plant breeders. In the present report we deal with the study on the effect of inbreeding on morpho-physiological and fertility characters in this valuable crop plant in order to understand the genetic structure of its various populations.

Materials used in the present study comprised of four cultivars of garden cress collected from diverse geographical regions of India and arbitrarily named as cultivars I, II, III and IV. Methods for selfing are the same as used for radish earlier (Dayal, 1974-75). Characters studied include seed germination, plant height, number of branches, days to flower, seed and silicula set in the first two inbreeding generations ($I_1$ and $I_2$).

Our study showed that forced inbreeding drastically reduce percent seed germination. Young seedlings were sluggish and lazy in growth; many of them soon turned yellow and died. The effect of inbreeding was more drastic in $I_1$. A significant between population variations were noted in these parameters. It also reduced the average plant height and the number of branches, particularly in $I_2$. Plants of the inbred families in general needed more days to flower than those of the control. Seed and silicula set was noticeably reduced. Inbred seeds were in general shrievelled and smaller in size and many of them did not germinate. Interestingly, the four cultivars reacted differently to inbreeding; cultivars I and II being more vulnerable than cultivarsIII and IV. Plants within a population also showed varying response to inbreeding, indicating that there existed both self- and cross-compatible plants in the populations. Several genotypes differing in these characters have been isolated and are being studied.

It is well known that inbreeding as a method of genetic analysis helps to reveal the genetic potentiality of an allogamous and heterozygous population. It breaks up the genetic balance of such population into a number of reproductively independent lines with increased homozygosity. Here it has been shown that forced inbreeding affects several morpho-physiological and fertility characters in garden cress. Inbreeding is also known to affect the chromosome characters in this plant. In contrast to the control, their inbred families ($I_1$ and $I_2$) have a significantly lower mean chiasma frequency but a higher frequency of chromosome abnormalities at metaphase I and anaphase I (Dayal & Singh, 1985).

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# SEED PRODUCTION OF RADISH (RAPHANUS SATIVUS L.) AFTER SELFING

M. Nieuwhof

At the IVT (Wageningen) a research project was started to investigate the possibilities of breeding radish cultivars that have a shorter growth period in winter than the existing cultivars. As part of this project a selection programme was started to obtain information on the response of radish to inbreeding. This short paper reviews experiences with seed production of radish after repeated selfing.

For selfing early plants were selected from cultivars and families of the type Early Round Red, as commonly grown in the Netherlands. Selfing was carried out in spring and summer, in cages 1.2 m high and 0.6 m dia., placed in a coldhouse. The cages were isolated by means of cheese cloth. Pollination was carried out by flies.

Table 1. Seed yield (g per plant) after selfing of early radish plants selected from different cultivars.

| Cultivar | Number of parent plants | Average seed yield | Frequency distribution (%) 0 g | < 1 g | 1-10 g | >10 g |
|---|---|---|---|---|---|---|
| Robijn | 193 | 11.4 | 14 | 9 | 40 | 36 |
| Rota | 38 | 5.8 | 29 | 5 | 43 | 21 |
| Triplo | 68 | 10.8 | 15 | 16 | 30 | 40 |
| Other cultivars[1)] | 31 | 16.6 | 13 | 3 | 45 | 33 |

1) Katra (4n), Minitas, Novired, Saxa, Radar, Revosa, Robino, Verano

Table 2. Generation effect on seed yield (g per plant).

| Generation parent plants | Number of parent plants | Average seed yield | Frequency distribution (%) 0 g | <1 g | 1-10 g | >10 g |
|---|---|---|---|---|---|---|
| I0 | 49 | 9.3 | | 24 | 51 | 24 |
| I1 | 70 | 16.0 | 10 | 1 | 32 | 56 |
| I2 | 41 | 8.8 | 39 | 5 | 29 | 27 |
| I3 | 14 | 11.5 | 21 | 7 | 28 | 42 |
| I4 | 19 | 5.2 | 11 | 5 | 68 | 16 |

As shown in Tables 1 and 2, most plants produced seeds after selfing by means of flies as pollen vectors. Many plants produced even more than 10 g of seeds. This was not only the case with the early types of Early Round Red, but also with later types such as Saxa and Revosa and with the tetraploid cultivar Katra. This demonstrates that only a weak incompatibility mechanism is operative in the radish material involved. Yet differences may occur between the cultivars, but comparisons cannot be made as seed production of the plants of the different cultivars was not always practised in the same year. When radish is propagated by selfing growth vigour decreases. This may also be accompanied with smaller seed yields. From Table 2 it can be seen, however, that also after 4 generations of selfing most seed plants may give sufficiently high seed yields.

# HETEROSIS IN INTERSPECIFIC HYBRIDS OF BRASSICA

S.C.PANDEY AND G.NAIK

Among the cole crops the importance of cauliflower (Brassica oleracea var. botrytis L. sub. var. cauliflora D.C.) follows cabbage with regard to area and production in the world. In India, cauliflower is grown either in hills or in plains throughout the year. However, no systematic breeding work has been done for the southern part of India. The present attempt has been made to find out the heterotic effects of hybrids at seedling stage in interspecific and intervarietal crosses of cauliflower and Broccoli under Bangalore condition.

## MATERIAL AND METHODS:

One broccoli line 137 obtained from Brazil having high tolerance to black rot (Xanthomonas campestris) crossed with Indian cauliflower varieties Poosi, Early Kunwari, Superfine Maghi, Early Dawn, Autumn King, Shalimar Moti, Super Snow Ball, Early Cauliflower, Poosa and Jawahar Moti along with cauliflower line 138 and 149 obtained from U.S.A. Twenty hybrids along with thirteen parents were grown in nursery bed in 1985. Keeping row to row 15 cm and plant to plant 5 cm. Twenty one days' old ten hybrid seedlings and their parents were randomly selected to record the observations on length of seedling (cm), number of leaves, leaf area index (sq.cm.) and fresh seedling weight (g). Average data over three replications of all the hybrids and parents were used to work out heterosis as percentage increase or decrease of $F_1$ performance over standard variety (Early Kunwari), Mid Parent (M.P.) and better parent (B.P.).

## RESULTS AND DISCUSSION:

Twelve hybrids exhibited positive heterosis over standard variety, seventeen hybrids over mid parent and fourteen hybrids over better parent for seedling length. Healthy and tall hybrids were obtained from Superfine Maghi x Broccoli 137 (24.55**, 14.28** and 2.9) followed by Early Dawn x Broccoli 137 (21.56**, 27.27** and 25.31**) and 138 x Autumn King (10.78**, 22.93** and 14.93**). Cauliflower lines crossed with broccoli produced taller seedling than broccoli as female parent, indicating the presence of reciprocal differences between the two parents.

Number of leaves and leaf area index are the prime factors for seedling vigour and ultimately leads to source sink and produced heterotic hybrids. All the hybrids except Superfine Maghi x Broccoli 137 gave positive heterosis for number of leaves. Maximum heterosis was observed from Jawahar Moti x Early Kunwari (50 percent each), followed by Broccoli 137 x Early cauliflower (60,00, 50.00 and 48.94%). Seventeen crosses maifested heterosis for leaf area index over economic heterosis (S.V.), thirteen crosses over mid parent and eleven crosses over better parent. Highly heterotic hybrids were obtained from Superfine Maghi x Broccoli 137 (88.57, 43.98 and 41.98%) followed by Early Dawn x 138 (62.32, 42.30 and 25.79%). Heterosis in number of leaves and leaf area index indicate that it is a function of multiplicative effects of genetic divergence of the characters in the germplasm used for making hybrids. Thirteen hybrids gave positive heterotic effect for seedling weight over standard variety, eleven hybrids over mid parent and six hybrids over better parent. Highly heterotic hybrids involve cauliflower line Early Dawn x Broccoli 137 and intervarietal hybrids of Early Dawn x 138. Seedling weight is indicative of seedling vigour which ultimaely contributes to the establishment of good plant and high yield.

It is concluded from the present findings that highly heterotic hybrids can be selected from the seedling stage to save the costly inputs, space and time in handling the large number of hybrids. The hybrids obtained from Superfine Maghi x Broccoli 137, Early Dawn x Broccoli 137, Early Dawn x 138 seems to be useful.

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# B. CARINATA A POTENTIAL OILSEED CROP FOR RAINFED AGRICULTURE

I.J. Anand, J.P. Singh and R.S. Malik

Among the oilseed crops grown in India, turnip rape and mustard occupy second place to groundnut both in area and production. These crops are annually grown on an area of 4.5 million hectares producing 2.4 million tonnes of oilseeds. Indian or brown mustard (B. juncea), however, accounts for the bulk of the area and production due to its having comparatively better tolerance to drought, diseases and pest than the turnip rapes (B. campestris vars. sarson and toria). 92% of the total area is under semi-arid climate where seed yields are drastically low reducing the national average to 6.0 Q/ha.

While screening for aphid resistance in Brassica and related genera (Malik, 1978 and Anand 1984), it was observed that B. carinata, Ethiopian mustard, had the potential to yield more than the domesticated Brassicas under naturally occurring aphid infestations. This prompted the first author to introduce B. carinata strains on a large scale and to test their yield potential under Indian agroclimatic conditions. Trials comprising the three best cultivars in each of the four different Brassica species (carinata, juncea, napus and campestris) were grown during the crop seasons of 1982-83 and 1983-84 under both rainfed and irrigated conditions at the farm of the Indian Agricultural Research Institute, New Delhi.

The results showed that B. carinata cultivars not only exceeded cultivars of the other Brassica species in seed yield in both years under rainfed conditions (Table 1), but also produced the maximum oilyield. Under irrigation conditions the seed and oil yields of B. carinata were greater than B. campestris ssp. oleifera and B. napus but less than those of the B. juncea cultivars.

The higher oil yield of B. carinata under rainfed condition results from this species having a well-developed, deep tap root system enabling the plant with numerous lax branches and more pods per plant. B. carinata cultivars were late in flowering (105-115) and maturity (160-175 days), but because of their superior oil yields they can compete better against the domesticated species in rainfed agriculture.

Studies have also revealed that B. carinata is the most tolerant species in respect of aphid infestation and is completely resistant to all the major prevalent diseases including Alternaria blight, white rust and downy mildew. Lines with non-shattering siliquae bearing yellow seeds and high oil content (47.9%) have been identified. Tremendous variability also exists for other traits, including seed weight (maximum 5.14g for 1000-seeds), siliquae per unit area, seeds per siliqua, siliqua length and seed colour. The artificial synthesis of B. carinata from its

putative parents and the discovery of male sterility (Anand, 1984) is likely to accelerate the pace of breeding work in this species. Single plant yields of up to 380g and small plot yields of selected lines exceeding 40Q/ha have been observed. The composition of the oil and meal cake of this species is not likely to pose a problem of acceptability by the consumer as sinigrin is the major glucosinolate (Anand and Malik, 1978), imparting a characteristic pungent odour which is highly acceptable in India.

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Table 1 : Performance of different Brassica cultivars for oil-yield traits under rainfed conditions (1982-84)

| CULTIVAR | YIELD (Q/HA) | | | OIL CONTENT % | | | OIL YIELD (Q/HA) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1982-83 | 83-84 | Mean | 82-83 | 83-84 | Mean | 82-83 | 83-84 | Mean |
| HC -2 (BCR)* | 26.8 | 31.8 | 29.3 | 39.6 | 40.8 | 40.2 | 10.6 | 12.9 | 11.8 |
| DIC-26 (BCR) | 18.2 | 24.8 | 21.5 | 41.4 | 42.5 | 41.9 | 07.5 | 10.5 | 09.0 |
| DIC-3 (BCR) | 23.1 | 28.0 | 25.5 | 39.4 | 38.5 | 38.9 | 09.1 | 10.8 | 09.9 |
| VARUNA (BJ) | 14.2 | 20.0 | 17.1 | 40.2 | 40.9 | 40.5 | 05.7 | 08.2 | 06.9 |
| PUSA BOLD (BJ) | 16.4 | 22.9 | 19.6 | 42.3 | 43.3 | 42.8 | 06.9 | 09.9 | 08.4 |
| RLM - 514 (BJ) | 12.5 | 18.3 | 15.4 | 41.6 | 42.8 | 42.2 | 05.2 | 07.8 | 06.5 |
| BO - 54 (BN) | 06.4 | 05.4 | 05.9 | 40.6 | 41.1 | 40.9 | 02.6 | 02.2 | 02.4 |
| BO - 15 (BN) | 08.3 | 10.6 | 09.5 | 38.7 | 39.5 | 39.1 | 03.2 | 04.2 | 03.7 |
| AG - 29 376 (BN) | 07.6 | 06.9 | 07.2 | 38.4 | 39.5 | 38.9 | 02.9 | 02.7 | 02.8 |
| PUSA KALYANI (BC) | 09.6 | 11.5 | 10.5 | 47.1 | 50.0 | 48.5 | 04.5 | 05.7 | 05.1 |
| DBS - 1 (BC) | 07.6 | 08.0 | 07.8 | 45.4 | 46.2 | 45.8 | 03.4 | 03.7 | 03.5 |
| DYS - 1 (BC) | 09.1 | 10.1 | 09.6 | 44.9 | 46.4 | 45.6 | 04.1 | 04.7 | 04.4 |

BCR = Brassica carinata; BJ = B. juncea; BN = B. napus; BC = B. campestris

# A METHOD FOR THE ASSESSMENT OF THE SELFING RATIO IN ZERO ERUCIC WINTERRAPE (BRASSICA NAPUS L.)

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The inheritance of the erucic acid content is a good means for studying the ratio of selfing to outcrossing in winter rape (Brassica napus L.). The character high erucic acid content is dominant to the zero erucic one and the phenotypic expression of this character occurs already in the seeds growing on the mother plant (ANAND and DOWNEY 1981). HÜHN and RAKOW (1981) analyzed the erucic acid content of each 80 single seeds from zero erucic plants neighbouring high erucic stands and detected the outcrossing ratio of the zero erucic plants from the percentage of single seeds containing erucic acid. But single seed analyzes are very time consuming and expensive. Therefore we recognized the relationship between the erucic acid content of a sample and the percentage of zero erucic seeds this sample, which originated from selfing.

In both 1979 and 1981 we drilled a stand of the high erucic variety "Sollux" (50 percent erucic acid). Into that we seeded single seeds of some zero erucic lines in a distance of 2,5 x 3 metres of each other to avoid crossing between them. The single plants flowered together with the pollinator and were harvested separately. In three series we analyzed the erucic acid content by GC of each 100 single seeds of 11, 10 and 19 plants, respectively. For calculating the correlation and regression between the erucic acid content of a sample and the percentage of zero erucic seeds of this sample we assumed, that the former corresponds to the arithmetic mean of the 100 single seeds' erucic acid content. Therefore we calculated this mean (variable x) and estimated the percentage of zero erucic seeds (variable y). We found a very close negative correlation between both characters, which were in the three series -0,97; -0,97 and -0,98, respectively, with no significant differences. The total correlation over all the 40 plants was -0,98. The three regression equations for the respective series did not differ significantly, too, and the general regression equation was $y = 105,5 - 3,83\ x$. For practical purposes read for x erucic acid content of the sample. Repeated investigations in the past years confirm this results.

Our method consists of the following steps:

1. Drill a stand of a high erucic variety and seed into it single seeds of the lines to be tested in the above mentioned way.
2. Analyze the erucic acid content of a sample from the harvested zero erucic plant (the sample should be at least 1 gram, the greater the better).
3. Calculate the selfing ratio by means of the above given regression equation.

Instead of analyzing a great number of single seeds per plant it is sufficient to analyze one sample. Thus this method allowes to search the selfing ratio of a very great number of plants in the relatively short time between seed harvesting and seeding, a prerequisite for effective breeding programs. It may be helpful for the study of many problems regarding the breeding system of winter rape. That may be the evaluation of male - sterile or self - incompatible lines or the selection for increased outcrossing ratio as a foundation for breeding synthetic varieties. Also it may be suitable for investigations of the efficiency of insect pollination or the necessary isolation distance between varieties in seed multiplication.

In 1980 and 1984 we studied some twenty breeding lines and found a great intra - line variability and significant differences in the selfing ratio between lines, indicating genetical differences. For more details see RUDLOFF and SCHWEIGER (1984).

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# DIALLEL MATING MATRIX IN A BUD-SELFED FAMILY OF ERUCA SATIVA WITH REFERENCE TO MULTIGENIC CONTROL OF SELF-INCOMPATIBILITY IN CRUCIFERAE

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The oleiferous crucifer Eruca sativa is known to be highly self-incompatible, although self-compatibility is reported (Alam 1936). Contrary to Singh's (1958) contention of a gametophytic system, Verma et al. (1977) demonstrated in it a sporophytic type of SI, like other crucifer genera (Bateman 1955). However, their complex data necessitated invoking a system of at least 3 genes with cooperative action, but four genes were not excluded in view of the secondarily balanced polyploid nature of the species (Verma and Lewis 1977). Theoretical aspects of sporophytic incompatibility with 2 and 3 genes, reference to Eruca, were elaborated by Lewis (1977). Subsequently, Verma and Hinata (1983) analysed two second inbred generation families which supported principally the concept of multigenic system of incompatibility. It may be said that despite the oft repeated generalization of a single S gene with multiple alleles the crucifer system is indeed complex (Lewis 1979, Sharma & Murty 1979, Wallace 1979a) and it still requires investigations on its genetics.

Inbred families of bud-selfing origin are superior to those derived from crosses, particularly when involvement of more than one gene is suspected, as is the case in Eruca (Verma and Lewis 1977), Brassica (Zuberi et al. 1981, Hinata et al. 1983) and Raphanus (Putrament 1960, Lewis 1979, Okazaki and Hinata 1984). Wallace (1979b) has emphasized the requirement of a minmum of 13 sibs for 95 per cent probability of recovering all the three possible genotypes (aa, bb, ab) from selfing a S-allele heterozygote (ab).

In crucifers, the recognition and subsequent rejection of unacceptable pollen occur at the surface of stigma cell (Roberts and Dickinson 1981). Based on pollen tube penetration of stigma papillae, the mating matrix within an $I_2$-generation family of Eruca sativa (Fig. 1) reveals five notable features. Firstly, 3 of the 13 sibs are fully self compatible. Secondly, none of the SC are compatible with all the sibs, either as pollen or as pistil parent. Thirdly, two of the remaining 10 plants are male sterile (see also Verma 1984). Fourthly, excluding the three SC sibs, there are at least five (or even six) phenotypic incompatibility groups which exceed the maximum of three expected on one S gene system. More than one gene control is therefore evidenced. Fifthly, the mating matrix of 10 plants (excl. the SC) shows 25 out of the 72 crosses to be compatible (+,O,●, Fig. 1) which proportion seemingly approximates to an inbred family derived from S-allele heterozygote with matched codominance of the pair of S-alleles ($\dot{a}$ $\dot{b}$, a dot over an allele shows activity in pollen, and an underline shows activity in stigma).

The last mentioned situation prompted us to accommodate these data within one S-locus, with the assumption that the mutual relations of alleles in heterozygotes are subject to interaction by factors segregating independently of the S-locus to produce the resultant incompatibility phenotype ( Fig. 1). The probability of 'residual genetic background' affecting the allele relations in S-allele heterozygotes has been raised also in Brassica campestris var. brown sarson (Verma et al. 1985)(cf. Nasrallah and Wallace 1968, Richards and Thurling 1973a,b, Wallace 1979a, Sharma and Murty 1979). The present $I_2$-family of Eruca sativa shows good agreement with the assumed S-allele relations, barring few crosses (e.g. 8 x 1, 9 x 1, 7 x 13). The assumptions made here, and in Brassica (Verma et al. 1985), in a way reflect the emerging possibility of multigenic type incompatibility system in the Cruciferae. Operationally,

all those factors (aside from polygenic modifiers) which determine the activity of the S-alleles, or the incompatibility phenotype, may constitute the components of the incompatibility system.

There are a number of reports in literature which call upon the involvement of oligogenic factors independent of the S-locus, either affecting the mutual relation of the pairs of S-alleles or suppressing the activity of S-alleles partially or completely in only stigma or both stigma and pollen (Murakami 1965, Thompson and Taylor 1971, Nasrallah 1974, Hinata et al. 1983, Hinata and Okazaki 1985). The alleles at loci other than the S-gene may act sporophytically or gametophytically. These hypotheses accounted for the occurrence of partial or full self-compatibility within a family of sibs, particularly when the assumption of changes or interaction of S-alleles for causing self-compatibility were excluded by the data (cf. Bateman 1954, Thompson and Taylor 1966, Zuberi et al. 1981).

The recent postulate of Hinata et al. (1983) that at least two factors should be satisfied for normal expression of incompatibility (in Brassica campestris), is indeed relevant, and a step forward, toward further understanding of the multigenic control in Cruciferae. Hinata et al. (1983), and Hinata and Okazaki (1985), invoked two genes, namely the S-gene and the M-gene. The latter is thought to be concerned with the disturbance of pollen tube intrusion, and the callosic rejection response is an indication of the recognition reaction by the S-gene. It should be endeavoured to determine whether the various loci proposed by different authors are allelic, but at least the M gene of Hinata's school appears to be different from the Su locus (Nasrallah 1974) and the F locus (Murakami 1965).

Functionally, SI is expected to be an integrated metabolic system comprising a few sub-systems, and a defect in either may lead to its breakdown, causing full or partial compatibility. Biochemically, SI is believed to involve

the S-specific glycoproteins of stigma papillae which presumably interact with specific lectins of the pollen, and SI occurs through complementation between pollen lectin and specific sugar moiety of the stigma cells (Shivanna 1985). Recently, Sharma et al. (1985) were able to overcome SI in Eruca sativa by treating the stigma with lectin before making pollination.

Fig. 1 Mating matrix in diallel of $I_2$ generation family of Eruca sativa. Plants 2 and 7 are male sterile, and 3,4 & 11 are fully self-compatible. Assumed genotypes with the probable allele relations in the heterozygous class are shown in pollen (bottom most row, by a dot over the allele/s) and stigma (left-hand column, by underline of allele/s), except the self-compatibles. Pollen of plant 5 exhibits variable allele relations, and to some extent the stigma of plant 13. There are other alternatives, requiring more than one gene. This explanation also requires the influence/interaction of additional genes.

MALE

| FEMALE Geno. | | 6 | 2 | 1 | 10 | 8 | 9 | 12 | 13 | 5 | 7 | 3 | 4 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aa | 6 | | | ± | | ● | ● | | o | | | ● | | |
| | 2 | | | ± | | + | + | | + | | | + | | |
| ab | 1 | | | | | o | o | | o | ● | | ● | o | ● |
| | 10 | | | | | ● | ● | | o | ● | | ● | o | ● |
| bb | 8 | ● | | | ● | | | | | | | | o | ● |
| | 9 | ● | | | ● | | | | | | | | o | o |
| ab | 12 | | | | | | | | | | | ± | ± | ● |
| ab~ | 13 | ± | | | | ± | | | | ± | | | ● | |
| ab | 5 | o | | ● | ● | | | | | | | | | |
| | 7 | + | | + | + | | | | + | | | | + | + |
| SC | 3 | ● | | ● | ● | | o | | | o | | ■ | ● | ● |
| | 4 | | | | | | | o | ● | | | ● | ■ | ● |
| | 11 | o | | ● | ● | ● | | ● | | | | ● | ● | ■ |
| Geno. | | aa | | ab | | bb | | ab | ab | ab | | SC | | |

Legends: Blank - Incompatible. (●)- Reciprocally compatible. (o)- Non-reciprocally compatible. For male sterile, compatible crosses, when used as female, shown as (+). The sign (±) denotes partial compatibility (variable). The sign (■) denotes full self-compatibility (plants 3,4 & 11).

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# THE PRODUCTION OF ATRAZINE-RESISTANT BRASSICA NAPUS X B. OLERACEA HYBRIDS

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Brassica breeders have shown interest in transferring genetically-determined traits from Brassica napus to B. oleracea and vice-versa, but generally have not had much success because these species are extremely difficult to hybridize (McNaughton and Ross, 1978). Disease resistance, S-alleles, stem tenderness and herbicide tolerance are some traits commonly mentioned. Chiang et al (1980) did transfer resistance to race 2 of Plasmodiophora brassica from rutabaga (B. napus) to cabbage (B. oleracea), but only obtained a very low rate of hybridization. The objective of our study at Guelph was to find a way of improving the rate of hybridization of the two species to a level where it would be feasible to attempt an inter-specific transfer within the framework of a conventional breeding/selection programme. We chose to attempt to transfer atrazine resistance from B. napus to B. oleracea.

Only about half the ovules in a B. napus ovary start to develop when a flower is pollinated with B. oleracea pollen. By the 12th day after pollination, these ovules start collapsing and most are shriveled and discoloured by the 18th day. Our approach was to let the ovules develop on the mother plant for as long as possible and then to rescue the dying embryos in aseptic culture. We now have developed a reasonably successful rescue and regeneration protocol for recovering B. napus - B. oleracea hybrids. The rate of hybridization varies from nil to 3 hybrids per pollination, depending on the parental lines and varieties. The hybrids themselves grow into large and very vigorous plants, generally morphologically intermediate to the parent species. Their fertility level (as measured by pollen viability) varies from 0 to 40%. Cytological characterization of these hybrids is under way. All the hybrids tested so far show resistance to atrazine. The interspecific hybrids do not set seed when backcrossed to B. oleracea, but backcrosses can be obtained from some of them by the same embryo rescue technique used in the first phase of hybridization. The hybrids also set some self-seed.

The next phase of this research will consist of further characterizing the hybrids, backcrossed and $F_2$ plants and in determining which method will be the most efficient in transferring the desirable trait from the hybrid to the recurrent parent.

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# SYNTHESIS OF ATRAZINE RESISTANT *BRASSICA NAPUS* THROUGH PROTOPLAST FUSION

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Protoplast fusion can be an important tool in plant breeding because it allows novel combinations of organelle-encoded traits. The goal of the work reported here was to use protoplast fusion to transfer *Brassica* chloroplasts into the Ogura (2) cytoplasmic male sterile (cms) lines of *Brassica* that currently contain *Raphanus* chloroplasts and *Raphanus* cms-containing mitochondria. Pelletier et al (3) have shown that replacement of *Raphanus* chloroplasts with *Brassica* chloroplasts in this cytoplasm corrects the chlorophyll deficiency at low temperatures, a trait that has prevented the commercial use of this cytoplasm for hybrid seed production. They used protoplast fusion to accomplish this goal and analyzed regenerated plants for the presence of novel combinations of organelle-encoded traits. The use of *in vitro* selection procedures for the desired organellar traits can be valuable in reducing the number of regenerated plants which require analysis. Atrazine resistance, which is carried by some *Brassica* chloroplasts, provides a selectable marker that distinguishes between *Brassica* and *Raphanus* chloroplasts. This communication reports an *in vitro* selection procedure for atrazine resistant *Brassica* somatic hybrids, the successful regeneration of one such plant and the use of tetrazolium blue as an assay for verification of the atrazine resistance trait.

The basic strategy for somatic hybridization paralleled the experiments of Schenck and Robbelen (7), who fused protoplasts from *B. campestris* and *B. oleracea* to recreate the amphidiploid species, *B. napus*, and Pelletier *et al* (3), who fused protoplasts from atrazine resistant lines of *B. napus* with a cms line of *B. napus* to create a cytoplasm containing both of these traits. *B. campestris* was used as the source of atrazine resistant chloroplasts and *B. oleracea* as the source of cms-encoding mitochondria. The selection procedure relied on the facts that *B. campestris* does not regenerate from protoplasts in the medium used and that atrazine inhibits the growth of protoplast-derived calli from *B. oleracea*.

Leaf protoplasts from *B. oleracea* var *botrytis* and etiolated hypocotyl protoplasts from *B. campestris* were used in the fusion experiments. *B. oleracea* (derived from Green Comet broccoli) contained the Ogura cms and was developed by Dr. M. Dickson, New York Agricultural Experimental Station, Geneva, NY. *B. campestris* cv Candle contained atrazine resistant chloroplasts and was developed by Dr. W. Beversdorf, University of Guelph, Guelph, Ontario. The procedures for protoplast isolation and culture previously described (5,6) were used without modification except that etiolated hypocotyl protoplasts were centrifuged in 0.18M $CaCl_2$, pH 5.8 while leaf protoplasts were centrifuged in 0.5M sorbitol, 10mM $CaCl_2$ and 5mM MES, pH 5.8. Fusion occurred in 15ml conical tubes using PEG 6,000 with a high calcium, high pH dilution (1). Protoplasts were cultured at a concentration of $5 \times 10^4$/ml in the series of media, B-G, developed by Pelletier et al (3). After 4 weeks 1300 protoplast-derived calli were transferred to solidified medium E with and without 50μM atrazine. The frequency of shoot formation was very low but one shoot was regenerated on medium E containing 50μM atrazine. After transfer to medium F and G without atrazine, this shoot developed into a plant with trichomes. Because *B. oleracea* lacks trichomes and because *B. campestris* (which does contain trichomes) does not regenerate from protoplasts using these procedures, this plant was tentatively identified as a somatic hybrid.

The morphology of the somatic hybrid is unlike either of the parents. The leaves are abnormally rugose and contain numerous trichomes. The stem is thicker than that of B. campestris and almost completely lacks trichomes. Several clones from axillary buds have been derived from the original hybrid; all of these are slow growing. The hybrid and clones were difficult to root; best root formation occurred in Magenta boxes containing 1/2 MS salts, 1% sucrose, 0.1mg/l NAA and 1% agar (Jourdan, unpublished).

The tetrazolium blue assay (4) was used to verify the presence of atrazine resistant chloroplasts in the somatic hybrid. Nitro-blue tetrazolium is reduced by photosystem II electron transport to a blue-black diformazan salt. Because atrazine inhibits photosystem II electron transport, it prevents reduction of this stain in atrazine sensitive, but not atrazine resistant protoplasts, with a threshold of between 1 and 10μM. Leaf protoplasts from the putative hybrid were purified by flotation and resuspended in buffer (0.6M sorbitol, 50mM Hepes, 10mM $NaHCO_3$, pH 7.6) with and without 50μM atrazine. Tetrazolium blue was added to a concentration of 0.01% and the protoplasts incubated at a light intensity of 150μE/m2/sec for 30 mins. The percentage of protoplasts stained with tetrazolium blue was 86 in the control and 84 in buffer containing atrazine, indicating that atrazine resistant chloroplasts were present. Analysis of the cms trait will be made in the somatic hybrid and in clones derived from the hybrid by following microspore development when the plants flower.

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# IN-VITRO SELECTION OF HERBICIDE TOLERANT RAPESEED

H. Schenck and G. Röbbelen

Weed control in rapeseed is still not solved satisfactory. Field applications of herbicides are mainly prophylactic and only rarely determined by weed development. Herbicide tolerant rapeseed, therefore, would reduce herbicide applications. In addition volunteer control would become possible, if the new double low rapeseed cultivars (without erucic acid and glucosinolates) were herbicide tolerant.

In order to widen the genetic basis beyond the first described atrazine-resistant material (cf. BEVERSDORF et al. 1980), mutation induction and in-vitro selection were applied to somatic stem embryo systems of rapeseed, Brassica napus. These were generated by culturing immature embryos of diploid rapeseed (SCHENCK 1983) and of haploid embryos derived from crosses of B.oleracea x B.campestris (GLAND 1982) as well as by anther culture (THOMAS et al. 1976, LICHTER 1982). The culture medium used in the first two cases for stem embryo induction was a modified LS-medium (SCHENCK 1983). For later propagations $S_1$-medium lacking mannitol and hormones (SCHENCK and RÖBBELEN 1982) was used. X-rays (5,000 R at 1,000 rad/min) were used for mutagenesis. For selection, the water soluble herbicides Bentazon (3-isopropyl-2,1,3-benzothiodiazine-4-on-2,2-dioxide) and Mecoprop (CMCP: 2-(4-chloro-2-methylphenoxy)-propionic acid) were used by mixing their filter sterilized solutions with adequate amounts of autoclaved nutrient S-agar-medium.

The first selection step occurred directly after the X-ray treatment with 200 mg/l for each herbicide. Surviving green sectors were collected and transferred onto herbicide free medium for recovery and further propagation. Depending on the growth rate of the explants after several transfers (every 3 weeks) a second selection was conducted with the same herbicide concentrations. After a second recovering and propagation cycle on normal S-agar the explants were planted in 300 ml Erlenmeyer flasks containing 100 ml S-agar to induce plant formation. Plants derived from the different experiments were transferred to the greenhouse; but reserve material was always retained in in-vitro culture.

After adaptation to greenhouse conditions (at least for 4 weeks) the selected plants were sprayed with herbicide solutions in concentrations as recommended for field applications, i.e. similar to 4 l/ha. The effects were scored 9 and 22 days after the treatment. The surviving plants were grown to seed maturity, the haploid genotypes following a 0.05% colchicine treatment for 12 hrs (GLAND 1981).

The results of the in-vitro selections are summarized in Table 1. After 2 selections 26 plants survived on the Bentazon, but only 4 on the Mecoprop medium.

Table 1: Surviving explants after two in-vitro selections on herbicide agar

| Material | Bentazon | | Mecoprop | |
|---|---|---|---|---|
| | 1st select. | 2nd select. | 1st select. | 2nd select. |
| Source material | 2617 | 51 | 2167 | 22 |
| Selected (total) | 99 | 26 | 90 | 4 |
| Surviving (total) | 51 | 26 | 31 | 4 |
| of these diploid | 30 | 9 | 14 | 4 |
| tetraploid | 21 | 17 | 8 | - |

After transfer into soil and greenhouse conditions the spraying revealed one clone, No. 11, which tolerated the used Bentazon concentrations (Table 2). This clone had been derived from an interspecific cross of B.oleracea x B.campestris. Seeds were received from the surviving plants for further genetic studies, which are now underway.

Table 2: Plant death (%) after Bentazon spraying of greenhouse plants in two successive experiments, scored after 9 and 22 days

| | Clone No. | Experiment 1 (March 1984) | | | Experiment 2 (July 1984) | | |
|---|---|---|---|---|---|---|---|
| | | Number of plants | % killed after 9 d | 22 d | Number of plants | % killed after 9 d | 22 d |
| Haploid lines | 11 | 15 | 24 | 53 | 53 | 30 | 30 |
| | 88 | 4 | 27 | 80 | 24 | 70 | 100 |
| | 33 | 6 | 39 | 100 | 48 | 40 | 100 |
| | 59 | 9 | 73 | 100 | 104 | 60 | 95 |
| | 70 | 9 | 75 | 100 | 24 | 60 | 100 |
| | 31 | 3 | 76 | 100 | 40 | 40 | 100 |
| | 44 | 12 | 82 | 100 | 56 | 80 | 100 |
| | 60 | 11 | 82 | 100 | 56 | 100 | 100 |
| | 66 | 18 | 84 | 100 | 32 | 80 | 100 |
| | and others | | | | | | |
| | Untreated controls | | | | | | |
| | L 16 | 5 | 68 | 100 | 47 | 95 | 100 |
| | K 38 | 8 | 98 | 100 | 24 | 100 | 100 |
| Diploid lines | 127 | 4 | 50 | 100 | 16 | 90 | 100 |
| | 129 | 2 | 50 | 100 | 24 | 90 | 100 |
| | 114 | 7 | 51 | 100 | 16 | 90 | 100 |
| | 15 | 3 | 51 | 100 | 4 | 100 | 100 |
| | 113 | 12 | 97 | 100 | 24 | 100 | 100 |
| | and others | | | | | | |
| | Untreated controls | | | | | | |
| | Oro 1247 | 11 | 72 | 100 | 37 | 100 | 100 |

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

Nobumichi INOMATA

By recent year, it was very difficult to produce the hybrids between Brassica campestris and B. oleracea (Hosoda et al. 1969). In recent studies, however, the hybrids between them were produced easily by ovary culture in vitro (Inomata 1977, 1978a, 1978b, 1979, 1983b, 1984; Matsuzawa 1978; Takeshita et al. 1980). Cytogenetical studies on the $F_1$ hybrids were reported (Inomata 1980), and crossing ability of the $F_1$ hybrids and their progenies were also described (Inomata 1982, 1983a, 1985). In the cytogenetical studies, the $F_1$ hybrids had almost no pollen fertility. But when the $F_1$ hybrids used as a female plant and they crossed B. napus, many hybrids were obtained, and they had good pollen fertility and showed 38 chromosomes (Inomata 1982, 1983a). It is supposed that B. campestris and B. oleracea are important gene sources of the breeding of B. napus.

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

The materials used in the present experiment were B. campestris ssp. chinensis cv. Seppaku-taina, ssp. dichotoma cv. Brown Sarson DS-2 and ssp. pekinensis cv. Nozaki-hakusai No. 2, and B. cretica ssp. cretica 35, which was collected in Greece N. Sporades; the island of Skiaathos (Snogerup, personal communication). The seed was provided by Dr. Snogerup. The culture methods were the same as a previous paper (Inomata 1978b). The culture medium used in the present experiment was Nitsch and Nitsch's (1969) minerals added with 300mg/l of casein hydrolysate, 50g/l of sucrose and 9g/l of agar.

The results are shown in Table 1. Number of ovaries explanted in the medium was from 40 to 45 in each cross combination. Many ovaries were infected with bacteria in the cross of ssp. chinensis x ssp. cretica, but many full-grown embryos were obtained and the almost embryos grew into mature plants. Although many hybrids were obtained in the cross of ssp. dichotoma x ssp. cretica, almost hybrids died in passing through hot summer and cold winter in Japan. Production rate of interspecific hybrids was very high and it was better than that in the previous papers. Morphological characteristics of leaf was intermediate between B. campestris and B. cretica. Pollen fertility was examined in 32 $F_1$ hybrids. In thirteen out of 32 hybrids in the cross of ssp. chinensis x ssp. cretica, mean pollen fertility was 3.1%. In two out of 32 hybrids in the cross of ssp. dichotoma x ssp. cretica, pollen fertility was 0% and 90%. In the rest of the hybrids in the cross of ssp. pekinensis x ssp. cretica, mean pollen fertility was 5.4%.

The first meiotic division was examined in 12 $F_1$ hybrids having 19 chromosomes. The results are shown in Table 2. Five hybrids were examined in each cross of ssp. chinensis x ssp. cretica and ssp. pekinensis x ssp. cretica. Mode of the chromosome configuration at PMCs was $9_{II}+1_{I}$ (32.5%). Chromosome pairing of the PMCs was resemble to the previous papers in the hybrids between B. campestris and B. oleracea. These $F_1$ hybrids may be useful for the breeding of B. napus.

Acknowledgment

I thank Dr. S. Snogerup at the Botanical Museum, the University, Lund for providing the seed of B. cretica.

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

| Cross combination (B. campestris x B. cretica) | No. of capsules examined (A) | No. of embryos further cultured: Late torpedo | Walking-stick | Full-grown embryo | No. of hybrids obtained (B) | B/A x 100 |
|---|---|---|---|---|---|---|
| ssp. chinensis[1] x ssp. cretica | 16 | 0 | 5 | 45 | 47 | 293.8 |
| ssp. dichotoma[2] x ssp. cretica | 44 | 24 | 12 | 20 | 34 | 77.3 |
| ssp. pekinensis[3] x ssp. cretica | 29 | 41 | 22 | 21 | 54 | 186.2 |
| Total or mean | 89 | 65 | 39 | 86 | 135 | 151.7 |

1: cv. Seppaku-taina x ssp. cretica 35. 2: cv. Brown Sarson DS-2 x ssp. cretica 35. 3: cv. Nozaki-hakusai No. 2 x ssp. cretica 35.

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

| Cross combination (B. campestris x B. cretica) | First meiotic division: No. of PMCs observed (%) | $1_{III}+8_{II}$ | $1_{III}+7_{II}+2_{I}$ | $9_{II}+1_{I}$ | $8_{II}+3_{I}$ | Other types |
|---|---|---|---|---|---|---|
| ssp. chinensis[1] x ssp. cretica | 128 | 45 (35.2) | 6 (4.7) | 40 (31.2) | 9 (7.0) | 28 (21.9) |
| ssp. dichotoma[2] x ssp. cretica | 60 | 4 (6.7) | 10 (16.7) | 16 (26.7) | 20 (33.3) | 10 (16.7) |
| ssp. pekinensis[3] x ssp. cretica | 141 | 31 (22.0) | 17 (12.1) | 51 (36.2) | 15 (10.6) | 27 (19.1) |
| Total or mean | 329 | 80 (24.3) | 33 (10.0) | 107 (32.5) | 44 (13.4) | 65 (19.8) |

1: cv. Seppaku-taina x ssp. cretica 35. 2: cv. Brown Sarson DS-2 x ssp. cretica 35. 3: cv. Nozaki-hakusai No. 2 x ssp. cretica 35.

# EFFICIENT PLANT REGENERATION FROM MESOPHYLL PROTOPLASTS OF FERTILE AND CMS CAULIFLOWER (Brassica oleracea cv botrytis)

Pablo S. Jourdan, Elizabeth D. Earle, and Martha A. Mutschler

Our laboratory is involved in the development of Brassica oleracea lines having altered cytoplasms generated via protoplast fusion or mutagenesis. We are particularly interested in correcting a chloroplast-associated, low temperature-induced chlorosis occurring in ogu cytoplasmic male sterile lines. A prerequisite in such work is the efficient and routine culture of protoplasts as well as regeneration of plants. Recently, we reported a procedure for the regeneration of plants from mesophyll protoplasts of a commercial hybrid variety of broccoli (Green Comet; Harris Seed Co., Rochester, NY, USA) (1). There are, however, two limitations to using this material for analysis of potential variability in regenerated plants. First, genetic studies would be complicated by the hybrid nature of the starting material. Second, the shoot regeneration frequency from protoplast-derived calli of Green Comet carrying the ogu cms cytoplasm is rather low; this low frequency would reduce the overall efficiency of somatic hybrid production in protoplast fusion experiments. To overcome these limitations we looked for inbred lines of B. oleracea that carry the ogu cytoplasm and that exhibit high regeneration capacity from mesophyll protoplasts. We report here on a cauliflower line that meets such requirements and which we are currently using in somatic hybridization experiments.

Cauliflower #7642 is an advanced breeding line developed by Dr. M. Dickson of the New York State Agricultural Experiment Station at Geneva, NY. The line is available with a normal fertile cytoplasm as well as with the ogu cytoplasmic male sterility derived from Raphanus sativus. Seeds of 7642 were surface-sterilized with 0.5% sodium hypochlorite and germinated in vitro on 1% agar-solidified hormone-free medium containing MS salts and vitamins (2) and 1% sucrose. We found it important not to seal containers with Parafilm because of a significant build-up of ethylene which is apparently associated with an adverse effect on the plants (Lentini and Earle, unpublished); instead we use a porous tape (Filter Tape, #19-9708 Carolina Biological, Burlington, NC, USA) that permits free gas exchange. The main advantage in growing seedlings axenically is that surface sterilization is not required prior to protoplast isolation. Alternatively, seedlings could be germinated directly in pots with vermiculite and fertilized weekly with MS salts and vitamins. The seedlings were kept in a culture room maintained at 22°C, 16 h photoperiod, 50-70% relative humidity and at a light intensity of 80$\mu E/m^2/sec$. Young, fully-expanded leaves of one- to two-month old plants were used as source of protoplasts.

Protoplasts were isolated and cultured essentially as described for Green Comet broccoli using the culture media sequence developed by Pelletier et al (3). Under these conditions, protoplasts of both the cms and fertile line began to divide after 4-6 days and by the fifteenth day up to 20% of all protoplasts (60% of those that had formed walls) had divided at least once. After 30 to 40 days, small colonies were transferred to solid media and typically 300-400 calli were obtained from an initial culture of approximately 25,000 protoplasts (overall plating efficiency of 1-2%). There appear to be no significant differences between the sterile and fertile cytoplasms in initial division frequency and subsequent plating efficiency. However, whereas

shoot regeneration occurred in about 85% of the fertile calli, only about 45% of the cms calli formed shoots, indicating that the cytoplasm may influence the ability of calli to regenerate. Regeneration most commonly occurred by multiple shoot formation although embryo-like structures also formed in some calli. Regenerated shoots could be rooted and hardened in the same medium used to germinate seeds; about 95% of the plants survived the hardening process. Finally, plants could be transferred to soil and grown to maturity in a greenhouse or in the field.

Over 500 plants of both the fertile and cms lines have been regenerated. A striking feature of the plants was their morphological uniformity, abnormal types accounting for less that 10% of the plants. One hundred cms plants were exposed to cold temperatures (10°C) for 2 weeks to induce the typical yellowing associated with this cytoplasm and select for possible variants. None were found. Similarly, there was no change to fertile flowers in all 195 cms plants examined, indicating that this cytoplasmic male sterility is stable through at least one tissue culture cycle. A wide range of floral abnormalities commonly associated with this cytoplasm was also noted in the regenerated plants: absence of anthers or reduced number of anthers; absence of petals; fused sepals and ovaries; fused multiple ovaries. At present, organelle DNA is being analyzed for possible culture-induced changes in restriction patterns. One hundred regenerated plants of the fertile line are being selfed for future analysis of potential somaclonal variants that may be of interest to breeders.

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# STUDY ON IN VITRO PROPAGATION OF $F_1$ HYBRID, SELF-INCOMPATIBLE LINE AND MALE STERILE LINE OF CABBAGE (Brassica oleracea L. var. capitata)

Shiru Chen and Xiaojia Wang

An in vitro technique was developed for rapid propagation of cabbage $F_1$ hybrid and its parental lines (self-incompatible and cytoplasmic male sterile line) in the study.

The $F_1$ hybrid "Jingfeng-1", self-incompatible (SI) line "Huang miao-7880-1-5-2-15-9" and the cytoplasmic male sterile (CMS) line "m8240-1-7-2" (the original CMS stock obtained from Dr. P. H.Williams, The University of Wisconsin-Madison, U.S.A.) were used as experimental materials in research.

The primary explants were all from seeds originally. Hypocotyls with apical meristem and cotyledons were inoculated aseptically on MS media containing different combinations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA). All cultures were incubated in a culture room at 25±1° C and were illuminated by cold white fluoresent lamp for 24h. or 16h. per day at light intensity of 3000 Lux.

After four succissive cultures, a 34032 shoots per 99 days rate of multiplication for $F_1$ hybrid materials and a 25344 shoots per 108 days for CMS materials were obtained on the medium with no NAA and 2mg/l BA; and a 12267 shoots per 90 days for SI materials on the medium with no NAA and 4mg/l BA. For root induction the best results were obtained on MS medium without any supplement of auxin and cytokinin for all the experimental materials.

Once complete plants were regenerated, the rooted plants were removed from the culture vessels, and were potted in small plastic containers and subsequently moved into enclosure in which the humidity could be maintained above ambient. After ten to fourteen days acclimatization, the regenerated plants were planted in field.

The morphological evaluation and cytological examination indicated that there was a highly genetic uniformity between the regenerated plants and their original varietis or lines. There was no significant difference between the yield of regenerated hybrid and the yield of standard hybrid. The in vitro propagated hybrid maintained its heterosis obviously. The regenerated SI and CMS plants maintained the self-incompatibility and the male sterility repectively as their original lines.

The results from research suggested that the in vitro propagation system provided by this experiment might be suitable to rapid clonal propagation of $F_1$ hybrid cabbage and its parental SI or CMS lines. It is likely to provid a promising means for multiple use of $F_1$ hybrid and rapid propagation of cabbage hybrids and their parental lines on a large scale.

# IN VITRO SELECTION FOR RESISTANCE TO ALTERNARIA BRASSICICOLA IN BRASSICA NAPUS SSP. OLEIFERA (WINTER OILSEED RAPE) USING PARTIALLY PURIFIED CULTURE FILTRATES.

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Alternaria brassicicola (Schw.) Wilts., the cause of grey leaf spot of brassicas, has been reported in seed stocks of winter oilseed rape (Prasanna & Lennard 1982; Humpherson-Jones 1985). There is little known resistance to Alternaria spp. in the oilseed rape crop, although chemical controls prove effective. This paper reports on the use of partially purified culture filtrates from A. brassicicola to determine i) whether toxic compounds are produced in culture; ii) whether tissues carrying resistance to such compounds may be generated and selected in culture; and iii) whether plants regenerated from such resistant tissues show increased levels of resistance to the pathogen.

Secondary embryoids from secondary embryogenic culture lines of Brassica napus ssp. oleifera cv. Primor were used in all experiments. These lines all originated from a single anther embryoid which had been diploidised with colchicine. All the lines were maintained on M&S medium (Murashige & Skoog, 1962) with 0.8% Difco agar and 2% sucrose added, but without plant growth substances. When regenerants were required, secondary embryoids were transferred for 3-7 days to M&S medium supplemented with kinetin (Ingram et al 1984; MacDonald & Ingram 1984) and then transferred to fresh medium without growth substances for root development. Plantlets were potted in peat balls and placed in high humidity in the greenhouse until they were established.

Single spore isolates of Alternaria brassicicola were established on potato dextrose agar (PDA). The pathogen sporulated well on this medium. Inoculated bottles of potato dextrose broth were incubated at 25$^{o}$C in low light for 3 weeks. The culture liquid was filtered, dried, extracted in methanol, redried, and taken up in distilled water.

A detached leaf bioassay and preliminary experiments showed that only the methanol fraction of the culture filtrate was toxic to secondary embryoids, resulting in a 90% kill, compared with a maximum of 20% in the controls. The toxicity of this fraction was destroyed by autoclaving. The methanol fraction was added, filter sterile, to M&S medium to give a predetermined concentration of 12.5mg $ml^{-1}$ and was used as a selection medium. Secondary embryoids were incubated on the selection medium for 4 weeks before scoring for survival. Of 391 embryoids screened, 64 (16%) survived. These were numbered and transferred to the standard culture medium to allow the development of further secondary embryoids and to ensure a lapse of at least three months before further exposure to the selection medium. Lines from individual selected embryoids were then retested on the selection medium and compared with a single unselected line (Table 1.)

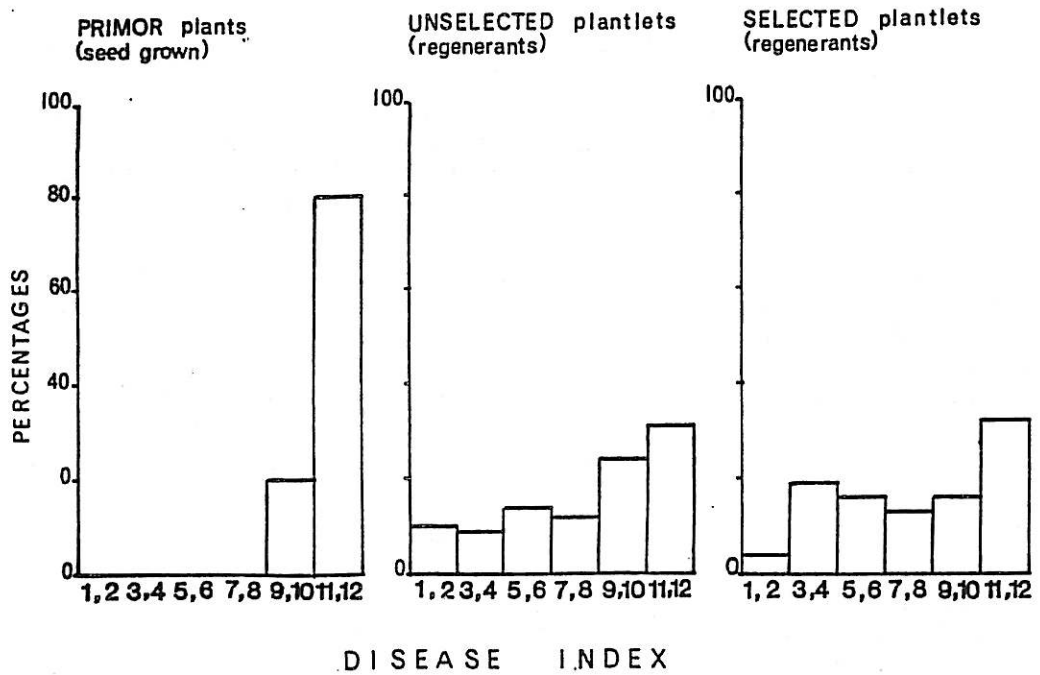
**Table 1.** Relative survival on the selection medium, in percentages, of reselected lines compared with an unselected control.

| Tissues | Better than control | Equal to control | Worse than control | Total number |
|---|---|---|---|---|
| 1st reselection | 52 | 8 | 40 | 25 |
| 2nd reselection | 65 | 6 | 29 | 17 |
| 3rd reselection | 77 | 9 | 14 | 35 |

Despite a period of several months on M&S medium without the toxic fraction, a number of the selected lines retained a greater level of resistance to the toxic medium. Some showed an improvement of up to 5 times the survival of the unselected lines. Plants have been regenerated from one of these lines and used to assess their resistance to the pathogen.

The regenerants were maintained for one month in the greenhouse following regeneration. Detached leaves from these, and from 6 week old seedlings of Primor, were inoculated with A. brassicicola, and scored after 7 days, (Fig.1.) All the Primor plants tested were very susceptible to the pathogen, whereas the regenerants from secondary embryoids exhibited a wider range of reactions, and included a number of resistant plants.

**Fig.1.** Reaction types of seed grown Primor and regenerants to Alternaria brassicicola, scored after 7 days using the index 0 = fully resistant, to 12= fully susceptible.



In some cases leaves from different stems of multistemmed regenerants gave widely differing reaction types. In the seed grown-plants of Primor which only produced single stems, the maximum difference between reaction types of individual leaves on each plant was 3, and this was only recorded in 22% of the plants. In the 19 unselected regenerant plants which were multistemmed, 70% showed differences of 3 or more, with a maximum difference of 5 in 30% of the plants. Among the 32 multistemmed selected regenerants, 59% showed differences of 3 or more, and a maximum difference of 7 was observed in 3% of the plants. Close examination of these multistemmed regenerants suggested that the stems originated from secondary embryoids which had not been supressed during regeneration (MacDonald & Ingram, 1984). For this reason, in compiling the scores for Fig.1, each stem was scored as a single "plantlet". Individual stem cuttings established from these plants and are being assessed further.

## Discussion

When grown in culture *A. brassicicola* produces a substance(s) toxic to secondary embryoids. The active compound(s) has not yet been identified, and have yet to be isolated satisfactorily from infected leaves, but preliminary experiments have yielded encouraging results. At present there is insufficient evidence to determine whether the toxic substance(s) is a pathotoxin(s). Symptoms similar to those produced by the pathogen on host plants, necrosis and chlorosis, were observed in detached leaf bioassays, but this has been shown to be a non-host-specific response.

Reselection of secondary embryoids on medium supplemented with toxin(s) has shown that selection for resistance to this toxin(s) is stable, but exhibits a population response. This could suggest that the selected character may be an adaptation to the culture environment, or possibly a dose response phenomenon. However, as the level of resistance was maintained during periods away from the selection pressure prior to reselection, it is likely that the character may be, at least in part, genetic.

Some regenerants showing good levels of resistance were detected, but a population response was evident, with a full range of reaction types represented among the total population of regenerants. Variation in reaction types from multistemmed plants may account for this. The fact that the proportion of resistant plants obtained following screening with the toxic medium is only slightly higher than in the unselected line emphasises that *in vitro* selection for stable resistance to toxic culture filtrates may not necessarily result in resistance to the pathogen. It is not known yet whether the resistance observed is heritable. Seed has been obtained from regenerated plants which have shown increased resistance to the pathogen, and will be used to test this.

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Juvenile Black Rot Resistance in cabbage.

M. H. Dickson and J, E. Hunter

Cabbage inbreds with black rot , Xanthomonas campestris resistance from Early Fuji have become quite well developed. However, none express resistance at the seedling stage. This can result in contamination of seedlings grown for transplants in the proximity of sources of infection.

We screened the US cabbage Plant Introduction collection and identified PI 436606 from China sas having juvenile resistance. The PI is similar to Early Fuji being flat and having a fairly soft head. The original PI was not uniformly resistant but fol inlowing two generations of selection plants innnoculated at the first to 4th true leaf stage did not develop black rot. Susceptible lines such as Round up , LAWI-3, BI-16 and BI-20 will develop black ro in t and under severe conditions even LAWI-3 plants will die following innoculation at the first leaf stage. Plants of lines such as LAWI-3 with mature plant resistance with 5 or more leaves will exhibit resistance. PI 436606 also exhibits mature plant resistance and crosses with LAWI-3 also express mature plant resistance, but hybrids made with a susceptible are susceptible or intermediate indicating the PI has similar genes for mature plant resistance to LAWI-3, but additional genes for juvenile plant resistance. Resistance in crosses to LAWI-3 appears to be due to 1 recessive gene with possibly a modifying gene.

Willliams P. H., T. Staub, and J. C. Sutton. 1972 Inheritance of black rot resistance in cabbage. Phytopathology 62:247-252.

# Vascular Rot in Rutabagas

K. G. Proudfoot and L. A. McFadden

Rutabagas (Swedes) Brassica napus var. napobrassica are an important vegetable crop in Newfoundland and other provinces of Eastern Canada (Proudfoot 1982). Some years ago roots displaying a darkening of the vascular tissue were found after some months in storage. The problem was suspected as being due to infection by the Black Rot organism Xanthomonas campestris. However, attempts to infect seedling cabbage plants with the bacteria isolated from the diseased roots were not successful. Symptoms of X. campestris infection have not been observed in the field on crops of rutabagas or other cruciferous crops.

Further isolations from typically diseased roots have been undertaken and we have not identified X. campestris using selective media as described by Schaad (1983). However, using a Pseudomonas specific medium we have consistently isolated species of Pseudomonas from such infected roots. Most isolates identified to date are P. fluorescens marginalis, although one isolate is a non-fluorescent Pseudomonas species.

Infection of rutabaga roots appears to occur during trimming as discoloration spreads from the neck and true root areas. Infections may also start at site of injuries resulting from insect damage. Generally, the discoloration is dark chocolate brown rather than black, with some spread into the tissue surrounding the vascular tissue. Development of the discoloration is apparently related to temperature conditions, as only very slight discoloration can be seen when the roots are removed from storage, but extensive blackening occurs within a few days of removal.

Pseudomonas fluorescens has been reported as a bacterial disease of waxed rutabagas in Ontario (Bradbury 1966). Infection of vascular tissue was noted by him although the most common symptom was dark lesions on the surface of the turnip.

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# CLUBROOT DISEASE SEVERITY IN OIL RAPE AND GLUCOSINOLATE CONTENT

J. Rod and J. Voškeruša

Clubroot caused by Plasmodiophora brassicae is the most serious disease of crucifers in Czechoslovakia. That is why the Research Institute for Vegetable Growing and Breeding in Olomouc - in cooperation with other institutions - has begun to investigate this problem in more detail, especially with a view to resistance breeding. This task, however, has to be preceded by an investigation of pathogen distribution and occurrence of pathotypes as well as by a search for resistance sources.

In our previous work (unpublished) there were significant differences in resistance to race 6 among the 98 oil rape varieties examined. For example, variety "Jet Neuf" showed extreme susceptibility whereas var. "Tandem" (="Jet Neuf 404") was quite resistant. Otherwise these two varieties differ from one another only in their glucosinolate content. On the basis of this results the response to clubroot infection of 10 lines of oil rape (coming from the Research Station for Oil Plants in Opava) characterized by graded glucosinolate contents was examined under glasshouse and field conditions.

Even though there were substantial differences in disease severity between individual lines (table 1), significant correlations between disease severity and glucosinolate content were not noted ($r = 0.37$ in glasshouse tests and 0.12 in field tests). These results correspond to the findings of Rouxel et al. (1983).

Nevertheless, Chong et al. (1981 and 1983) found a correlation between clubroot resistance and glucosinolate content in cabbage. Therefore, it is necessary to verify in more detail whether this correlation really exists in only some crops in order to confirm or disprove the hypothesis of glucosinolate effect upon clubroot resistance in crucifers (Fenwick 1982).

Table 1

| Line number | Glucosinolate content (%)[1)] | Disease severity in: glasshouse | field |
|---|---|---|---|
| 409/2 | 0.18 | 50 ab | 13 b |
| 1/6 | 0.27 | 43 a | 2 a |
| 403/1 | 0.36 | 41 a | 3 a |
| 271/9 | 0.36 | 66 abc | 31 bc |
| 488/2 | 0.61 | 69 bc | 23 bc |
| 461/1 | 1.13 | 79 c | 27 bc |
| 272/2 | 1.18 | 65 abc | 43 c |
| 414/4 | 2.31 | 44 ab | 14 b |
| 967/1 K | 2.81 | 74 bc | 17 b |
| 580/3 | 3.62 | 69 bc | 17 b |

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

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# Effects of Resistant Plants as a Catch Crop on the Reduction of Clubroot Resting Spores

H. Yamagishi, H. Yoshikawa, M. Ashizawa,
S. Yui and K. Hida

Since the resting spores of clubroot can survive in soil for more than several years, it is very difficult to control the disease by fungicides. So breeding works have been done globally for the resistance to clubroot. In Japan, up to now, several Chinese cabbage, one turnip and one cabbage varieties resistant to clubroot have been released.

Besides the breeding work, we tested the effects of cultivation of resistant plants on the reduction of clubroot resting spores in soil.

In 1980 autumn, resting spores of clubroot were inoculated to the soil of field plots with the density of $5 \times 10^6$/1 ml fresh soil. Every year from 1981 to 1984, several kinds of crops including resistant strains of kale and turnip were cultivated in spring season and susceptible Chinese cabbage (1981) and susceptible turnip(1982 to 1984) were cultivated in autumn season. In Table 1 the kinds of crops cultivated in spring season are shown. Roots of spring crops were left in soil and those of autumn susceptible strains were carried out. The effects of spring crops on the density of resting spores were estimated by the disease incidence of susceptible turnip in autumn season.

The effects of spring crops on the disease incidence of autumn susceptible turnips are shown in Table 2. By the cultivation of spinach and sorghum, the disease of susceptible turnip was decreased only a little in comparison with the cultivation of susceptible strains in both seasons.

In contrast, by the cultivation of the resistant kale and turnip, disease incidences were suppressed drastically to the level of no practical problems. Of these two, resistant turnip showed higher effect. These effects of resistant strains became conspicuous from the third year of cultivation.

After four years cultivation, the soil of each plot was sampled. Besides, we prepared the artificially infested soil with known five densities( from $5 \times 10^2$ to $5 \times 10^6$ ). The density of resting spores in sampled soil was estimated by comparing the disease incidences of the index plant( susceptible Chinese cabbage ) cultivated in the pots containing sampled soil with those of artificially infested soil.

The spore density was estimated as about $5 \times 10^5$/ml soil in the sorghum plot, $5 \times 10^3$ in the kale plot and less than $5 \times 10^3$ in the resistant turnip plot respectively. On the other hand, the spore density in the continuous cultivation plot of susceptible strains was estimated as more than $5 \times 10^6$/ml.

From these, resistant strains of Cruciferous crops are considered to induce the germination of clubroot spores and be invaded, but suppress the development of spores in roots. And as a cosequence, the cultivation of resistant strains gives great effects to reduce the density of clubroot spores in soil.

( The details of this experiment are now in press for J. Japan Soc. Hort. Sci. in Japanese with English summary.)

Table 1 Kinds of crops cultivated in spring season to reduce the resting spores of clubroot in soil[a]

| Plot number | Crops cultivated in spring season |
|---|---|
| 1 | Susceptible Chinese cabbage(1981) and turnip(1982 to 1984) |
| 2 | Resistant kale 'Winter Nideriger Gruner Feinstgekyauster' |
| 3 | Resistant turnip '77b(selected from AAbbCC of ECD)' |
| 4 | Sorghum |
| 5 | No cultivation(1981) and spinach(1982 to 1984) |

a In autumn season susceptible Chinese cabbage(1981) and susceptible turnip(1982 to 1984) were cultivated in all plots.

Table 2 Incidence of clubroot disease in susceptible turnips in autumn season[a]

| Plot No. | 1982 | | 1983 | | 1984 | |
|---|---|---|---|---|---|---|
| | % of diseased plants | Disease index | % of diseased plants | Disease index | % of diseased plants | Disease index |
| 1 | 70 | 1,86 | 51 | 1.28 | 99 | 2.85 |
| 2 | 28 | 0.51 | 13 | 0.18 | 17 | 0.32 |
| 3 | 11 | 0.23 | 3 | 0.05 | 5 | 0.07 |
| 4 | 78 | 1.94 | 33 | 0.68 | 71 | 1.24 |
| 5 | 57 | 1.26 | 50 | 1.17 | 81 | 1.79 |

a Disease incidence of each plant was classified into indices from 0(No disease) to 3(very severely infected) and disease index of the plot was caluculated as follows;

$$\text{Disease index} = \frac{0 \times n\text{-}0 + 1 \times n\text{-}1 + 2 \times n\text{-}2 + 3 \times n\text{-}3}{\text{Total number of plants}}$$

(n-0, n-1, n-2 and n-3 are the numbers in each class)

# New soil born disease of Chinese cabbage caused by *Verticillium dahliae* Klebahn, and the resistance in *Brassica campestris* L.

S. Yui, M. Ashizawa and H. Yamagishi

New soil born disease 'Ōka-byō (it means yellow leaf disease) has appeared at Nagano prefecture in 1966, where is the largest highland summer sowing Chinese cabbage production area in Japan. Then, it has been spreading larger, especially in consecutive cultivating area. The pathogen was identified as *Verticillium dahliae* Klebahn in 1973.

The progress of this disease is rather slow that it is very difficult to confirm the diseased plant by its appearance before heading stage. The symptom is as follows. Outer leaves become yellow and unfold, then heading leaves become unheaded. Consequently, seriously infected field looks white-yellow in color. Vascular bundle of tap root of diseased plant shows distinct browning before heading stage.

*V. dahliae* has very wide host range. In Japan, it is reported that more than 10 different crops are infected by *V. dahliae*. Some major crops, for example, eggplant and tomato (*Solanaceae*), strawberry (*Rosaceae*) and chrysanthemum (*Compositae*), are susceptible. So, it is somewhat difficult to control this disease by rotation of crops. Furthermore, as it is soil born disease, fungicide doesn't affect sufficiently. In seriously infected area, cultivation is somehow continued by the combination of fungicide and disease delayed type varieties ('Chi-fu group Chinese cabbage). At present, the infected area has become wider and the degree of outbreak of the disease has become more intensive. It is strongly demanded to breed *Verticillium* resistant variety.

We inoculated *V. dahliae* to about 250 varieties or lines of *Brassica campestris* L. and *B. napus* L., and investigated the varietal differences of resistance. Severity of the disease was assessed on an arbitrary scale disease index at vertically cut surface of tap root; from 0 = no apparent browning to 3 = strong browning.

A part of these results are shown in Table 1. In *pekinensis* group, (it includes Chinese cabbage), most of the varieties or lines revealed intensive symptom, most of the disease index means were 2 to 3. In this group, only the variety 'Gokuwase Chi-fu' showed slightly lower index, that was 1.8. In *chinensis*, *narinosa*, *japonica* and *campestris* groups, there were some varietal differences. But most of the varieties or lines were infected seriously. In *rapifera* group (turnip), there existed a wide range of varietal differences. Particularly, some varieties that belong to ' Kanamachi' varietal group (small white turnip) showed nearly no symptom. Varieties or lines of *B. napus* showed slight symptom, but they seemed not so tolerant as 'Kanamachi' group.

We will cross the Chinese cabbage and 'Kanamachi' group turnips and analyze the inheritance of resistance. Because it takes about 50 to 60 days to complete an inoculation experiment, it is also important to establish more rapid seedling test.

Table 1. Varietal difference of resistance to *Verticillium dahliae* in *Brassica campestris* and *B. napus*

| name of variety or line | name of group or species | disease index * |
|---|---|---|
| Shirakuki santousai | *pekinensis* *** | 2.5 |
| Bekana | | 2.8 |
| Matsushima shin 2 gou ** | | 2.5 |
| Nozaki 2 gou ** | | 2.8 |
| Gokuwase Chi-fu ** | | 1.8 |
| Seppaku taisai | *chinensis* *** | 2.5 |
| Tokinashi taisai | | 2.5 |
| Sangatsuman | | 2.5 |
| Sendai yukina | *narinosa* *** | 2.8 |
| Aburana | *campestris* *** | 3.0 |
| Akihodane | | 3.0 |
| Hatana 3 | | 2.8 |
| Okute mibuna | *japonica* *** | 2.0 |
| Sensuji kyouna | | 3.0 |
| Komatsuna | *rapifera* *** | 2.3 |
| Kairyouhakata | | 1.3 |
| Hagikoroge | | 0.8 |
| Kanamachi tokinashi | | 0.5 |
| Shiramine | | 1.0 |
| Norabouna | *B. napus* | 2.0 |
| Shinkirina | | 1.5 |
| Kaburena | | 1.5 |
| Gifu green hakuran | | 1.3 |

* : mean of 4 to 5 plants
- 0 : no apparent browning
- 1 : slight browning
- 2 : apparent browning
- 3 : strong browning

** : heading Chinese cabbage

*** : all the groups belong to *B. campestris*

# OUTBREAK OF PERONOSPORA PARASITICA ON RADISH PODS IN INDIA

L.S. Suhag and J.C. Duhan

Sharma and Sohi (1982) recorded the occurrence of Peronospora parasitica (Pers.) ex. Fr. on radish (Raphanus sativus L.) from India. However, the effect of the disease on the pods of radish from this part of the world has not been reported. This paper reports the symptoms and yield losses due to P. parasitica on radish seed crops from Haryana (India).

P. parasitica was first observed during March 1985 on seed crops of the radish cv. Hissar-I at Haryana Agricultural University vegetable seed farms Hissar and Karnal. The infected pods were conspicuous by their dirty brown appearance. Dark brown, depressed and irregular lesions were also seen scattered on the floral branches. Such lesions developed dirty whitish growth of fungus frutification. Severely infected inflorescences were killed before pod formation. Intercellular mycelia with intracellular and bulbous haustoria were seen on the host. The conidia measured 24.45-33.50 x 21.30-27.25 µm (average of 50 measurements, 28.98 x 24.22 µm).

Five thousand pods (infected as well as healthy) were collected randomly from a P. parasitica infected crop on April 15, 1985 (harvesting time) and categorised into 0-4 scale (Table 1) on the basis of per cent pod area diseased. Yield related characters were recorded for the different infection levels. P. parasitica infection affected length of pods, number of seeds/pod and 1000 seed weight, leading to reduction in seed yield to the tune of 52.32%. As the area of infection increased, a corresponding adverse effect on all the yield parameters was observed. Viability of seeds was also reduced drastically.

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Table 1. Effect of Peronospora parasitica infection on seed yield and germination in the radish cv. Hissar-I

| Pod infection (%) | Length of pods (cm)* | Average number of seeds/pod* | Seed germination (%) | Weight of 1000 seeds (gm)** | % reduction in seed yield over seed obtained from healthy pods |
|---|---|---|---|---|---|
| 0 | 3.81 | 6.94 | 92 | 8.20 | 0.00 |
| 1-10 | 3.72 | 6.74 | 88 | 7.75 | 5.61 |
| 11-25 | 3.61 | 6.15 | 71 | 6.14 | 25.12 |
| 26-50 | 3.12 | 6.02 | 59 | 4.84 | 40.97 |
| 51-100 | 2.71 | 5.22 | 47 | 3.91 | 52.32 |

* Average of 50 pods
** Average of 3 replications

C.D. at 5% w.r.t. weight of grain = 0.43

# WHITE STEM ROT DISEASE OF BRASSICA JUNCEA

G.S. Saharan, J.C. Kaushik and C.D. Kaushik

This disease was observed for the first time at Haryana Agricultural University Farm, Hisar and Samrala substation farm of Punjab Agricultural University, Ludhiana in the first week of March, 1982 on 2% of the Brassica juncea plants. There is no report on the occurrence of this disease in epidemic form except from Uttar Pradesh, Bihar and Assam (Rai et al., 1974; Roya and Saikia, 1976).

The initial symptoms can be seen on the stem just above the ground level. In early stages of infection small, elliptical, water-soaked lesions appear on the infected plant parts. Later such lesions become white covered with cottony mycelial growth. In advanced stages the cottony fungal growth enlarges considerably along the stem and often entirely encircles it. The leaves and siliquae may also exhibit such symptoms. The infected stems, side-shoots and other plant parts become weak and collapse. At later stages of infection conspicuous black sclerotia are visible on all affected plant parts among mycelial growth. If the affected stem is split open, the sclerotia can be seen in the pith cavity.

The pathogen causing these white stem rot symptoms in Brassica juncea was identified as Sclerotinia sclerotiorum (Lib.) De. Bary. The disease has appeared in severe form because of the low temperature and unusual high humidity due to frequent rains in this season. This disease needs immediate attention from oilseed pathologists in Northern India because of changing weather towards frequent rains during winter months for the last two years.

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# SEEDLING DISEASES OF RAPESEED AND MUSTARD IN INDIA

R.U. KHAN AND S.J. KOLTE

Rapeseed (B. campestris var. toria, B. campestris var. yellow sarson) and mustard (B. juncea) crops have been found to be affected by seedling diseases in the range of 6-15% under the field conditions in the state of Uttar Pradesh in India. Affected seedlings of rapeseed and mustard showed distinct symptoms of collar rot, wilting, root and stem rots. Isolations from the diseased seedlings yielded most frequently the growth of fungus, Sclerotium rolfsii followed by the growth of fungi Rhizoctonia solani and Fusarium oxyporum. Two other fungi, that is, F. moniliforme and Penicillium spiculisporum were also obtained in isolations from some diseased seedlings.

In the pathogenicity test both in unsterilized and sterilized soil, S. rolfsii, R. solani and F. oxysporum were found to be highly destructive and caused collar rot, damping-off and wilt diseases respectively. F. moniliforme and P. spiculisporum were weakly pathogenic particularly in the post-emergence phase. S. rolfsii caused higher (53 to 84%) pre-emergence mortality of seedlings at all inoculum levels (2 to 25 g inoculum/kg of soil) as compared to R. solani and F. oxysporum under similar conditions. But R. solani caused higher (84 to 94%) post-emergence mortality than S. rolfsii and F. oxysporum at all levels of inoculum density. Plant age (7 to 42 days old) did not affect significantly seedling mortality caused by S. rolfsii, R. solani and F. oxysporum.

Seed treatment with mancozeb (0.2%) or thiram (0.2%) significantly reduced the pre-emergence mortality of seedling caused by S. rolfsii and F. oxysporum. Mancozeb (0.2%), metalaxyl (Apron @ 0.2%) and thiram (0.2%) gave 83 to 85% plant stand when the treated seeds were sown in soil infested with S. rolfsii (Table 1). Thiophanate methyl (0.2%) and carbendazim (0.2%) treated seeds gave significantly higher plant stand (33 to 35%) against R. solani in comparison with other fungicides and untreated seeds. Seed treatment with thiophanate methyl (0.2%) followed by captafol (0.2%), mancozeb (0.2%) and thiram (0.2%) showed the least (5 to 16%) pre and post-emergence mortality caused by F. oxysporum resulting in maximum plant stand of 78 to 81% in comparison with untreated seeds which showed only 16% plant stand (Table 1).

Table 1: Effect of fungicidal seed treatment on per cent plant stand of mustard plants in soil artificially infested with *S. rolfsii*, *R. solani* and *F. oxysporum*

| Fungicides | Plant stand (%)[a] | | |
|---|---|---|---|
| | *S. rolfsii* | *R. solani* | *F. oxysporum* |
| Captafol | 76.25 (61.03) | 4.50 (7.52) | 81.00 (64.50) |
| Mancozeb | 84.25 (67.10) | 4.00 (7.95) | 79.25 (63.19) |
| Thiophanate-methyl | 79.50 (63.62) | 35.25 (35.82) | 81.50 (64.56) |
| Thiram | 85.25 (67.55) | 17.50 (21.53) | 78.00 (62.18) |
| Carbendazim | 78.00 (62.17) | 33.50 (29.62) | 69.00 (56.27) |
| Metalaxyl (Apron 35 SD) | 83.75 (66.43) | 1.75 ( 6.55) | 58.25 (50.32) |
| Check (Untreated seeds) | 15.75 (23.29) | 12.75 (20.64) | 16.25 (22.93) |

Figures in parentheses are the Arcsin $\sqrt{\text{Percentage}}$ transformed values.

[a]35-40 days after sowing. Average percent plant stand is based on 400 viable seeds sown in four separate replications.

# ASSESSMENT OF LOSSES IN YIELD OF MUSTARD DUE TO POWDERY MILDEW DISEASE

G.S. Saharan and B.S. Sheoran

Powdery mildew of rapeseed-mustard caused by Erysiphe cruciferarum Opiz ex. Junell. has been reported to occur in epidemic form in Haryana causing considerable losses in yield (Saharan and Kaushik, 1981). There is a heavy reduction in yield when pods are heavily covered with powdery growth of the fungus in the young stage. The present study was carried out to measure the actual loss in yield and the effect on yield components of mustard infected with powdery mildew pathogen.

## Materials and Methods

A highly susceptible mustard variety viz, EC 126743 was sown on 26 October 1983 in replicated (thrice) plots of 3 m length having ten lines each. One set of the plots was sprayed with karathane (0.1%) and the other was kept unprotected to allow disease development. Repeated sprays were given at intervals of 10 days soon after the appearance of the first speck of mildew in the protected plots. To assess the losses in yield due to this disease yield components viz, number of pods per plant, pod length, seeds per pod, 1000 grain weight, total yield and per cent oil content were analysed from diseased and protected plots.

## Results and Discussion

It is evident from Table 1 that there was negative correlation of disease with all the yield components. The total yield was reduced by 17.5% in diseased plots in comparison to protected plots. Disease caused reduction in oil content up to 6.47%.

It is a common belief that the losses caused by powdery mildew are not as spectacular as in some other diseases. However, in the present study it was proved that powdery mildew of mustard caused reduction in number of pods/plant, pod length, seeds/pod, 1000 grain weight, total yield and per cent oil content. Powdery mildew of pea caused reduction in pod number from 21-31% with 24-46% reduction in pod weight of 100% infected crop (Munjal, et al., 1963).

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Table 1. Assessment of losses in yield of mustard infected with powdery mildew disease

| Yield components | Diseased | Protected* | Per cent reduction |
|---|---|---|---|
| Number of pods/plant | 653.5 | 693.5 | 5.76 |
| Pod length (cm) | 4.1 | 4.4 | 6.82 |
| Number of seeds/pod | 12.0 | 12.9 | 6.97 |
| 1000 grain weight (gm) | 2.171 | 2.189 | 0.82 |
| Total weight (q/ha) | 16.5 | 20.0 | 17.50 |
| Oil content (%) | 43.3 | 46.3 | 6.47 |

* The crop was protected by spraying karathane (0.1%)

## BOOK NOTICE

Diseases of Annual Edible Oilseed Crops Volume 2: Rapeseed-Mustard and Sesame Diseases by S.J. Kolte, Ph.D. 135 pp, Florida, CRC Press, Inc.

ISBN-0-8493-5381-5 Cat. No. 5381R Prepub US $58.00, Outside US $67.00

# PRODUCTION OF PECTOLYTIC AND CELLULOLYTIC ENZYMES BY ALTERNARIA BRASSICICOLA AND A.RAPHANI IN VITRO

A.K.Das, Rita Das and K.R.Samaddar*

Cruciferous vegetables and oil seed crops in West Bengal are severely affected by leaf spot disease caused by Alternaria brassicicola and A. raphani(Kundu and Samaddar, 1984). Qualitative presence of cellulase and protopectinase in the culture filtrates of the two organisms has been demonstrated(Das-Gupta and Verma, 1961). Quantitative estimation of pectinmethylesterase in the culture filtrates of these organisms has been made by the same authors(Das-Gupta and Verma, 1962). Systematic studies on different types of pectolytic and cellulolytic enzymes produced by these two pathogens are lacking. This paper reports a comparative study of pectolytic hydrolases and cellulolytic enzyme potentials of A. brassicicola and A. raphani in vitro using viscosimetric method.

The organisms were cultured on potato-dextrose(PD), czapek-Dox(CD), potato-sucrose(PS) and Richard broths with or without inducers. Presence of polygalacturonase(Endo-PG), polymethylgalacturonase(Endo-PMG), $C_x$ and $C_1$ cellulases in the culture filtrates was assayed. Sodium-polypectate(NaPP; Sigma,USA), pectin(Sunkist,USA) and carboxymethylcellulose(CMC;BDH,England) at a concentration of 0.5% (w/v) of the culture medium were used as inducers. Surgical grade absorbent cotton fibres or Whatman no.1 filter papers were used for induction of $C_1$ cellulase. Assay and determination of relative activity(RA) of enzymes were by the method of Bateman(1963).

Results (Table 1) showed that A. brassicicola did not produce Endo-PG in any of the culture media tested but produced Endo-PMG inducively in presence of pectin in Richard broth. In contrast, A. raphani produced Endo-PG and Endo-PMG constitutively in CD and Richard broths, but not in PD and PS broths. Addition of pectin to PD broth induced production of PMG by A. raphani but not in PS broth. Presence of NaPP in the medium did not increase the activity of Endo-PG with respect to CD broth, but caused enhancement of the activity with respect to Richard broth. Presence of pectin in CD or Richard broth caused 10 or 20% inhibition respectively of PMG activity as compared to media without the inducer.

It is evident from the results (Table 2) that A. brassicicola

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produced $C_x$ cellulase constitutively in Richard broth, but CMC when present in the medium completely inhibited the production. In contrast, A. raphani produced $C_x$ cellulase constitutively in CD and Richard broths and CMC caused significant inhibition of the production of the enzyme. It is also evident (Table 2) that both A. brassicicola and A. raphani produced significant amount of $C_1$ cellulase when grown on native celluloses such as cotton fibres or filter papers. Both the organisms degraded and grew well on media containing these native celluloses as sole source of carbon. The data suggested production of both $C_1$ and $C_x$ cellulases by these two organisms in presence of native celluloses in the medium.

Table 1. Production of Endo-PG and Endo-PMG by Alternaria brassicicola and A. raphani in vitro

| | | Relative activity of | | | |
|---|---|---|---|---|---|
| | | PG(Substrate:NaPP) | | PMG(Substrate:Pectin) | |
| Growth medium | Inducer | AB | AR | AB | AR |
| PD broth | | 0 | 0 | 0 | 0 |
| PD broth | + NaPP | 0 | 0 | - | - |
| PD broth | + Pectin | - | - | 0 | 71.4 |
| CD broth | | 0 | 83.3 | 0 | 100.9 |
| CD broth | + NaPP | 0 | 84.7 | - | - |
| CD broth | + Pectin | - | - | 0 | 90.2 |
| PS broth | | 0 | 0 | 0 | 0 |
| PS broth | + NaPP | 0 | 0 | - | - |
| PS broth | + Pectin | - | - | 0 | 0 |
| Richard broth | | 0 | 38.5 | 0 | 111.1 |
| Richard broth | + NaPP | 0 | 66.7 | - | - |
| Richard broth | + Pectin | - | - | 61.0 | 90.6 |

AB=A. brassicicola; AR= A. raphani
- sign indicates that the activity using the substrate was not tested.

It is apparent from the results that A. brassicicola is a poor producer of Endo-PG or Endo-PMG, but A. raphani produced significant amount of these enzymes in vitro with or without inducers. Both the pathogens produced $C_x$ and $C_1$ cellulases constitutively. Carboxymethylcellulose when present in the medium caused significant inhibition of the production of $C_x$ cellulase by both the pathogens.

Table 2. Production of $C_x$ and $C_1$ cellulase by Alternaria brassicicola and A. raphani in vitro

| Growth medium | Inducer | Relative activity of enzymes of A. brassicicola $C_x$ | A. brassicicola $C_1$ | A. raphani $C_x$ | A. raphani $C_1$ |
|---|---|---|---|---|---|
| PD broth | | 0 | - | 0 | - |
| PD broth | + CMC | 0 | - | 0 | - |
| CD broth | | 0 | - | 58.8 | - |
| CD broth | + CMC | 0 | - | 29.4 | - |
| Richard broth | | 55.6 | - | 66.7 | - |
| Richard broth | + CMC | 0 | - | 28.5 | - |
| Richard broth | + cotton fibre | - | 97.1 | - | 110.4 |
| Richard broth | + Filter paper | - | 96.5 | - | 80.2 |

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# RADISH ROOT EXTRACT AGAR, A SUITABLE MEDIUM FOR THE GROWTH AND SPORULATION OF ALTERNARIA BRASSICAE.

RAJAT THAKUR AND S.J. KOLTE

Alternaria brassicae can be easily isolated on culture medium from the infected host tissues at the first instance, but there are problems of maintaining its further growth in culture. The culture rapidly loses its vigour and sporulating ability on subsequent transfers. Just one or two transfers will make the fungus to grow poorly. This, therefore creates difficulty in handling the culture efficiently. It is in this view, the present studies were carried out with an overall objective of finding out the best possible medium for the growth of A. brassicae and to find out certain spore inducing factors which could maintain the good growth and sporulation of the fungus in culture.

Total eight media comprising of synthetic and non-synthetic nature were used to initially screen the media for better growth and sporulation of A. brassicae. Out of the above media, significantly higher size of the fungal colony (59.0 mm) was produced on radish root extract agar medium (pH 6.5-7.0) in comparison with V-8 juice agar (41-6 mm), carrot root extract agar (37.8), potato dextrose agar (35.8 mm), Asthana and Hawker's agar (20.6 mm), Elliott's agar (17.0 mm), Richard's agar (12.0 mm) and Czapek-Dox's agar (10.0 mm) that were tested.

Various spore-inducing factors were incorporated in the radish root extract agar medium, the results of which are presented in Table 1. The sporulation was best favoured by incorporation of sucrose and mannitol followed by ergosterol with 88-90 per cent spore germination. A moderate sporulation was also favoured by 10 minute exposure of 3-day old mycelial culture of the fungus to ultra-violet light, but the spores produced showed lower spore germination (62 per cent).

Thus it may be suggested that the radish root extract agar medium be used for culturing A. brassicae. The medium can be prepared by boiling grated radish roots (200 g) in distilled water (500 ml), till it is cooked and adding melted agar (20 g in 500 ml) to the radish root extract filtrate. In order to get the spores of the fungus in culture, the radish root extract may be amended with sucrose @ 1 per cent or with mannitol @ 1 per cent or with ergosterol @ 10 ppm concentration. It is hoped that the radish root extract agar with the above compounds could be useful for efficiently handling of the culture of A. brassicae.

Table 1 : Intensity of sporulation of A. brassicae and spore germination obtained from radish root extract agar amended with different substances and germination of spores from the culture exposed to ultra violet light.

| Spore inducing factors | Concentration | Sporulation/6mm disc ($x10^2$) | Germination (%) |
|---|---|---|---|
| Sucrose | 1% | 59.5 | 89.92(71.72) |
| Mannitol | 1% | 53.4 | 88.82(70.60) |
| Ergosterol | 10ppm | 51.4 | 88.36(70.28) |
| Cholesterol | 10 ppm | 19.6 | 82.82(65.54) |
| Ammonium nitrate | 0.2% | 12.3 | 80.04(65.38) |
| Magnesium sulphate | 0.05% | 4.3 | 61.96(51.94) |
| 3 day-old + Ultra violet (10 min) | - | 41.2 | 62.60(52.28) |
| 5day-old + Ultra violet (10 min) | - | 9.2 | 47.78(43.50) |
| C.D. at 5% | | 5.6 | ( 4.62) |

Figures in parentheses are the Arcsin $\sqrt{\text{percentage}}$ transformed values.

# TURNIP MOSAIC VIRUS IN RUTABAGA

V.I. Shattuck, V. Souza Machado and J.A. Tomlinson

Turnip Mosiac virus (TuMV) is a periodic problem for rutabaga growers and seed producers in Ontario, Canada. In recent years the incidence of this disease has reached alarming proportions around Huron county, the major rutabaga growing area in southern Ontario.

The economic loss attributed to this virus will depend on the growth stage that plants are infected. Young plants infected during the early part of the season may fail to develop a marketable root. Plants infected during later growth stages will usually develop roots of marketable size. Unfortunately, many of these roots may be left in the field since diseased plants are difficult to mechanically harvest. Furthermore, infected roots which are stored over the winter months may exhibit an increased susceptibility to root rotting pathogens.

The virus is transmitted solely by aphids. Attempts to control the spread of the virus after field establishment have met with disappointment. In most fields insecticides are not effective during July and August in controlling the movement of virus-infected aphids. If left unchecked these aphids will spread TuMV into adjacent fields. Therefore, virus control methods have been aimed at delaying the appearance of the disease until late August by eliminating immediate sources of infestation within and around fields. Late season infestation will usually lead to minimal production losses.

In 1984 seed for line 165 was obtained from Dr. J.A. Tomlinson at the National Vegetable Research Station in Wellesbourne, England. Line 165 has previously been shown to possess immunity to a TuMV isolate from Warwickshire, England.

In both laboratory and field evaluations conducted in 1985 around Guelph, Ontario line 165 exhibited high resistance to our native strain(s) of TuMV. Work is in progress to incorporate the resistant factor(s) from line 165 into Laurentian rutabaga, the predominant cultivar grown commercially in Ontario.

In view of the increased interest by growers around Huron county to greatly expand winter canola production,this rutabaga breeding project takes on added importance. Under controlled environmental conditions the senior author has demonstrated that TuMV-infected canola can serve as a source of infection for rutabaga seedlings. Further experiments will be conducted in the field to determine the ramifications of this finding.

# SEARCH FOR RESISTANCE TO OROBANCHE RAMOSA L. IN RAPESEED

E. Sobrino Vesperinas

Orobanche ramosa L. was reported by Romero-Munoz and Gonzalez-Torres (1983) and Sobrino (1983) as a parasite of rapeseed and one of the serious potential problems in extending the rapeseed crop in Spain. However, as rapeseed has been grown until recently only in areas free from the parasite, their inter-relations have not been studied.

To detect sources of resistance of potential value in the anticipated expansion of the crop, 33 cultivars and breeding lines from different sources (Germany, Spain, France and Sweden), all belonging to the zero or double zero type, were grown in soil having a high natural infestation.

The total number of floral shoots of O. ramosa L. per cultivar or line (3 replications, 24 $m^2$) was taken as the measure of susceptibility. Large differences between the entries were found (Fig. 1). No complete resistance was detected in any of the cultivars and lines, although three had a significant level of resistance (Z test, p=0.05) (Zar, 1984). The highest resistance was shown by the Spanish line CE-TO-32-2/82, with 3% infection compared with the most susceptible line (100% = 408 shoots/24 $m^2$).

In those with the greatest level of resistance, the number of shoots per plant of O. ramosa L. was low, varying from 1.6 to 2.3, compared with the maximum value of 6.7. The shortest floral shoots of the parasite were found on one of these resistant lines (6.8 cm compared with the maximum of 13.4 cm).

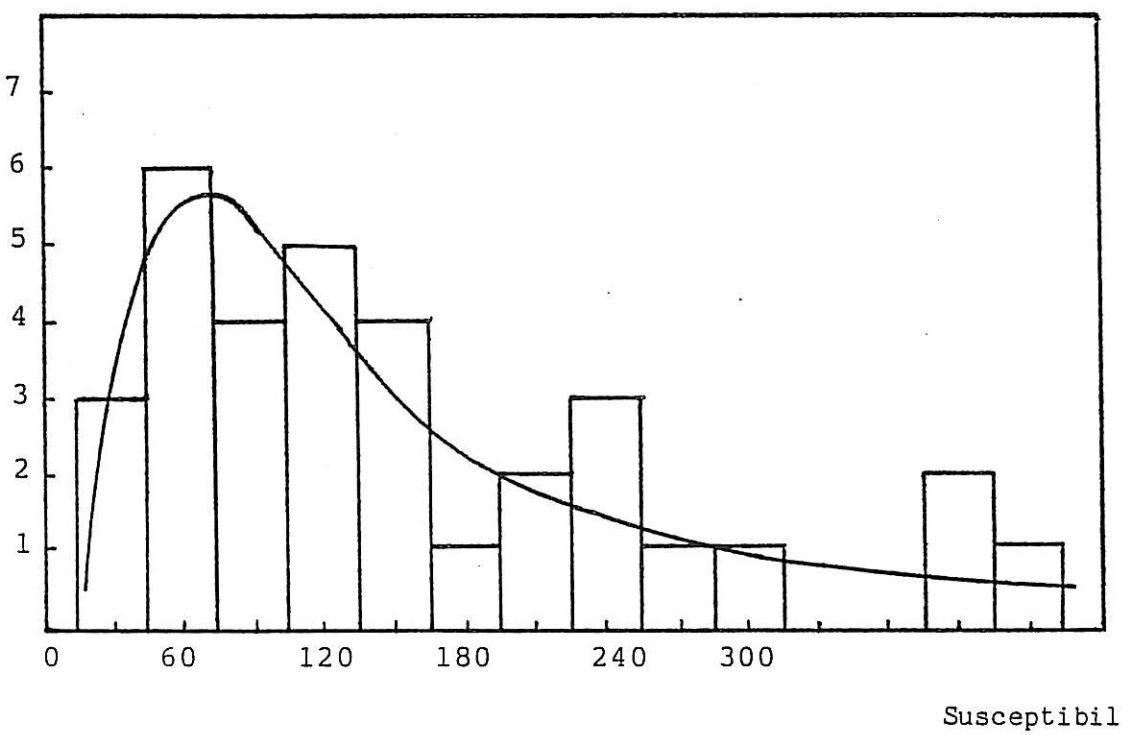


Fig. 1. Frequency distribution of susceptibility (floral shoots/24 $m^2$)

Resistance in rapeseed to the parasite, as for other crops attacked by species of Orobanche, resulted from mechanisms which prevent the effective union of the parasite haustorium with the roots of the host, thus depriving the parasite of adequate levels of nutrients.

Next season, a programme is scheduled with diallel crossing between the three most resistant cultivars and lines in order to obtain new material with improved resistance.

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# INFLUENCE OF CULTURAL PRACTICES ON THE ATTACK OF MUSTARD SAWFLY, ATHALIA LUGENS PROXIMA (KLUG.)*

V.K. Kalra **

The incidence of mustard sawfly, Athalia lugens proxima (Klug.) on Indian mustard, Brassica juncea (L.) Czern and Coss. is influenced by a number of factors of which cultural practices viz. date of planting, irrigation, fertilizer application, etc. are of much importance. In the present studies different levels of such factors have been evaluated for the incidence of this insect pest.

## Method and material

The experiment on Indian mustard, B. juncea was a split plot design with four different factors viz. date of planting [early (25th Sep.), normal (10th Oct.) and late (25th Oct.)]; irrigation (0, 1 and 2); nitrogen fertilization (0, 30 and 60 kg/ha); and row spacing (30, 45 and 60 cm). Since this pest occurs only in the vegetative stage of the crop and by that time only one irrigation is applied, the influence of the second irrigation is negligible. The dates of planting were randomized in main plots, irrigation in sub plots and combinations of nitrogen and spacings in sub-sub-plots of 16.2 sq.m. area. In the following season the irrigation levels were reduced to two (0 and 1) and row spacing was also deleted as one of the factors, since it showed no marked differences.

## Results and discussions

Mustard sawfly attacked the late sown crop in the month of December. No attack was recorded on the early and normal sown crops. The data recorded during the second and third weeks of December are presented in table 1. The average number of plants damaged by this insect in the second week of December (during first crop season), ranged from 7 to 20 per plot, but the variations were statistically non-significant among the plots sown with different combinations of cultural practice. However, during the 3rd week of December, variations in the number of plants damaged were significant. The maximum number of plants damaged by A. lugens proxima occurred in irrigated and fertilized (nitrogen fertilization) late sown plots with row spacings of 60 cm. The treatments having 20.2 to 24 damaged plants per plot were statistically similar. It is, therefore, evident that the irrigated, late-sown crop of mustard with an ample supply of nitrogen fertilizer harboured most sawfly larvae, irrespective of the spacings of the plants in the field.

In the succeeding season also, the late sown crop, supplied with the high level of nitrogen fertilizer, were damaged more by A. lugens proxima. However, the irrigation requirements in this crop for this insect could not be confirmed during this season.

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It could, therefore, be concluded that if the mustard crop is sown late (about a fortnight) in the season and is supplied with high amounts of nitrogen fertilizer, it will attract more sawfly, A. lugens proxima larvae.

Acknowledgements

The author is thankful to Drs N P Chopra, D S Gupta and T P Yadava for their useful guidance in conducting these studies.

Table 1. Number of mustard plants damaged by Athalia lugens proxima under different cultural practices.

| Treatments | | Number of plants damaged per plot (16.2 sq.m.) Season I Weeks of December II | III | Season II Weeks of December III |
|---|---|---|---|---|
| $D_3I_0N_0$ | $S_1$ | 11.00 | 9.66 | |
| | $S_2$ | 10.66 | 13.33 | |
| | $S_3$ | 9.66 | 12.33 | |
| $N_1$ | $S_1$ | 10.33 | 15.33 | |
| | $S_2$ | 8.00 | 15.00 | |
| | $S_3$ | 9.66 | 16.66 | |
| $N_2$ | $S_1$ | 11.66 | 15.33 | |
| | $S_2$ | 9.33 | 12.66 | |
| | $S_3$ | 9.00 | 7.33 | |
| $D_3I_1N_0$ | $S_1$ | 9.00 | 15.33 | |
| | $S_2$ | 14.33 | 11.33 | |
| | $S_3$ | 11.00 | 13.33 | |
| $N_1$ | $S_1$ | 11.66 | 14.33 | |
| | $S_2$ | 7.66 | 13.33 | |
| | $S_3$ | 10.66 | 20.33 | |
| $N_2$ | $S_1$ | 9.66 | 21.00 | |
| | $S_2$ | 9.66 | 16.00 | |
| | $S_3$ | 7.00 | 21.00 | |
| $D_3I_2N_0$ | $S_1$ | 16.33 | 16.00 | |
| | $S_2$ | 12.00 | 17.33 | |
| | $S_3$ | 11.66 | 24.00 | |
| $D_3I_2N_1$ | $S_1$ | 13.00 | 18.66 | |
| | $S_2$ | 12.00 | 22.33 | |
| | $S_3$ | 14.66 | 20.66 | |
| $N_2$ | $S_1$ | 11.33 | 21.33 | |
| | $S_2$ | 12.33 | 20.66 | |
| | $S_3$ | 13.66 | 21.33 | |
| C.D. (5%) | | N.S. | 3.69 | |

| Treatments | Season II Weeks of December III |
|---|---|
| $D_3I_0N_0$ | 11.17 |
| $N_1$ | 15.00 |
| $N_2$ | 15.83 |
| $I_1N_0$ | 11.17 |
| $N_1$ | 10.50 |
| $N_2$ | 13.17 |
| C.D. (5%) | 1.29 |

$D_3$= Late sown (25th Oct.)

I - Irrigation

$I_0$ - none

$I_1$ - one

$I_2$ - two

N - Nitrogen fertilization

$N_0$ - none

$N_1$ - 30 kg N/ha

$N_2$ - 60 kg N/ha

S - Row spacing

$S_1$ - 30 cm

$S_2$ - 45 cm

$S_3$ - 60 cm

# WATER STRESS AND PHYTOMYZA HORTICOLA TOLERANCE IN BRASSICA CAMPESTRIS VAR. BROWN SARSON

V.K. Kalra and S.S. Kharub*

Water deficit in plants induces tolerance to insect pests (Emden, 1966). According to Wearing (1972), intermittent water stress is largely beneficial and continuous water stress largely detrimental to the reproduction and survival of Myzus persicae and Brevicoryne brassicae feeding on Brussels sprouts. Kennedy et al. (1958) noted detrimental effects of water stress in various plants on Aphis fabae. However, no information is at hand about the influence of water stress on the incidence of pea leaf miner, Phytomyza horticola (Gour.) on Brassica campestris var. Brown sarson. Since this Brassica crop, in India, is grown mainly under rainfed conditions, an experiment, therefore, was conducted to study the tolerance induced by water stress in B. campestris against P. horticola.

The B. campestris var. Brown sarson (BSH-1) plants were grown in clay pots of 9" diameter and rogued at 4 leaf stage. One plant was left in each pot to which recommended doses of N, P and K fertilizers were applied. The plants were subjected to water stress at two stages of the crop growth i.e. bud initiation (I) and flower initiation (II). Seven different doses of water viz. 25, 50, 100, 150, 200, 250 and 300 ml per pot per day were tried. Two days before recording the observations on the number of leaf miners attacking per plant, the number of leaves present on the plant were also recorded.

Table 1. Average number of leaves and leaf miners (Phytomyza horticola) on Brassica campestris var. Brown sarson plants as influenced by various levels of water stress

| Treatments (ml of water per pot per day) | No. of leaves per plant | | Number of leaf miners per plant | | | |
|---|---|---|---|---|---|---|
| | Stage | | I - Stages - II | | | |
| | | | Days after sowing | | | |
| | I | II | 75 | 90 | 75 | 90 |
| 25 | 7.2 | 4.5 | 5.1 | 8.7 | 3.9 | 3.1 |
| 50 | 8.2 | 8.8 | 4.1 | 7.5 | 7.1 | 17.9 |
| 100 | 9.8 | 11.2 | 10.1 | 16.1 | 9.7 | 21.7 |
| 150 | 7.5 | 20.5 | 9.5 | 27.9 | 10.9 | 38.9 |
| 200 | 28.5 | 26.5 | 11.5 | 47.1 | 15.9 | 58.5 |
| 250 | 26.8 | 26.8 | 18.3 | 22.3 | 10.7 | 51.1 |
| 300 | 29.8 | 24.5 | 6.9 | 21.9 | 11.3 | 21.7 |
| C.D. (5%) | 5.5 | 4.9 | 1.9 | 3.6 | 2.0 | 3.4 |

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The data presented in Table 1 reveal that the number of leaves formed on the plants irrigated with 200 to 300 ml water per pot daily was statistically similar irrespective of the stage of the plants at which the stress was started. The foliage was significantly less on plants which got water less than 200 ml per day. In stage II, the number of leaves significantly increased as the amount of water applied was increased up to 200 ml/day, beyond which the number remained the same.

The incidence of leaf miner on 75 days old crop plants ranged from 3.9 to 18.3 per plant, the maximum being on plants getting 150 ml or more water per day. A fortnight later (90 days old plants), the attack by this pest increased to as high as 58.5 per plant (on plants getting 200 ml water per day) in stage II. In stage I, too, as many as 47.1 leaf miners/plant were recorded in the same treatment. As the amount of water applied was decreased or increased, the incidence of leaf miners declined significantly. However if it was reduced to 150 ml/day or less the number declined sharply.

It could, therefore, be inferred that water stress in B. campestris plants induced tolerance against P. horticola and if the water stress was given at the bud initiation stage, the number of leaves formed on the plant also declined, which in turn attracted less number of insects.

Acknowledgements

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# TURNIP ROOT-FLY RESISTANCE IN SWEDES - PRELIMINARY ENTOMOLOGICAL STUDIES

N. Birch

Two new swede (Brassica napus L. var. rapifera) cultivars Angus and Melfort bred at the Scottish Crop Research Institute (SCRI) have been shown to have consistently high resistance to damage by turnip root-fly (TRF, Delia floralis L.) (Shaw; 1982, 1984, 1985) although this resistance was not found to be closely correlated with high dry matter content as was first suspected (Gowers et al., 1984). Because of problems of screening for resistance due to large variations between sites and years, detailed field and laboratory based studies of TRF biology and mechanisms of resistance have been initiated at SCRI. The aim is to replace laborious and inconsistent field screening of new genotypes with a continuously available laboratory and/or chemical screen for resistance factors.

The aims of the entomological input, working closely with plant breeders and chemists, are to study:

(i) the biology of TRF in the field,
(ii) the behavioural responses of TRF in relation to physical/chemical changes in swedes, and
(iii) the mechanisms of host plant resistance with a view to developing rapid laboratory-based screens for resistance or associated factors.

Preliminary studies have so far concentrated on the following:

(i) field studies of female TRF oviposition periods and inter-cultivar preferences on susceptible and resistant swedes at two sites in Scotland,

(ii) egg inoculation experiments to study early stages of larval invasion and development,

(iii) laboratory and field assessments of damage in relation to pupal development and numbers,

(iv) mechanisms, relating detailed time-based plant sampling over the swede growing season with physical/chemical changes during key periods in TRF life cycle (i.e. peak oviposition, larval invasion, pupation) as well as possible induced changes in the host plant after insect challenge, and

(v) TRF can now be successfully cultured in the laboratory enabling continuous bioassay and behavioural studies to be carried out through the year.

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# PREFERENCE BY LIPAPHIS ERYSIMI (KALTENBACH) FOR TISSUES OF BRASSICA JUNCEA VAR. VARUNA

G.C. Sachan and Sumati

Various parts of Brassica campestris var. toria affect the growth and development of Lipaphis erysimi (Tripathi, 1982). In the present investigation the preference of L. erysimi for various parts of B. juncea var. varuna was studied.

This experiment was done in the laboratory at 25-27°C and 60-70% R.H. The tissues used were tender leaf, hard leaf, stalk, flower bud, open flower and pod. They were placed on moist filter papers on Petri dishes (20 cm dia.) and arranged equidistally in a circle near the periphery. The experiment was replicated 3 times. Nymphs were raised on Brassica campestris var. toria for 3 days from birth, starved for 4 hr and 150 were released in the centre of each Petri dish. The number of nymphs congregated on each test part was recorded at 4, 8 and 12 hrs after release.

More aphids were found on pods than on any other tissues, while the next highest numbers occurred on tender leaves (Table 1). The difference between the numbers on these parts was significant at 4 hr but thereafter numbers on tender leaves increased while those on pods remained more or less constant. Generally, higher numbers were recorded on open flowers and hard leaves than on flower bud and stalks but only in one comparison (hard leaf versus stalk at 8 hr) was the difference significant.

It is apparent that pods and tender leaves are most preferred by L. erysimi. This could be due to nutritional quality and tenderness of the parts. Tripathi (1982), in growth and developmental studies, also indicated that pods are more suitable for growth and development of this aphid on B. campestris var. toria.

Table 1. Mean numbers of L. erysimi nymphs on various parts of B. juncea var. varuna in preference test at 4 hr intervals

| Host part | Number of aphids recorded | | |
|---|---|---|---|
| | 4 hr | 8 hr | 12 hr |
| Tender leaf | 11.66 | 13.33 | 18.66 |
| Hard leaf | 8.00 | 9.33 | 8.00 |
| Stalk | 5.00 | 1.33 | 5.33 |
| Flower bud | 5.00 | 6.00 | 6.33 |
| Open flower | 10.33 | 8.00 | 6.33 |
| Pod (tender) | 20.66 | 17.66 | 22.33 |
| SEM ± | 2.17 | 2.33 | 1.54 |
| LSD at 5% | 6.69 | 7.18 | 4.74 |

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## INCIDENCE OF ATHALIA PROXIMA KLUG ON SPECIES AND VARIETIES OF BRASSICA

G.C. Sachan and Sumati

Mustard sawfly, Athalia proxima Klug, attacks the seedling stage of rapeseed mustard and other cruciferous crops in northern India. Sometimes damage is so severe that resowing becomes essential. In the present studies the incidence of this insect was observed on various species and varieties of Brassica. The experiment was sown on 15 November 1984 in a randomised block design with 3 replications. Each treatment had 2 rows per plot, 4 m long and 30 cm apart. Data were recorded on number of eggs laid, eggs hatched and the number of larvae on 10 randomly selected plants from each row at 7 day intervals from germination. For analysis of variance data were transformed to log (X+1) and comparisons were made among different treatments by using Duncan's multiple range test.

Most eggs were laid on cultivars of B. juncea (PR 18, 69.6; Varuna, 67.9; and Porbiraya, 57.6) followed by Sinapis alba (47.3), B. alba (38.3) and PR 15 (25.6). The varieties of B. napus had 5 or less eggs per 10 plants observed. Low number of eggs were also laid on the B. carinata lines and the two cultivars (Stoke and Lethbridge) of B. juncea. On all the plots slightly fewer eggs hatched than were laid. When plants were 3 weeks old, the number of larvae per 10 plants was highest on Porbiraya followed by B. alba. Numbers on varieties of B. napus, B. nigra, Pc1 and Pc2 were significantly lower than other test plants (Table 1).

From the foregoing observations it is apparent that B. juncea, B. alba, B. nigra and Sinapis alba are more preferred by A. proxima as compared to B. napus and B. carinata.

Table 1. Mean number of eggs, hatched eggs and larvae of *A. proxima* on different species and varieties of *Brassica*

| Species/ Varieties | Number of eggs per 10 plants | Number of eggs hatched per 10 plants | Number of larvae per 10 plants |
|---|---|---|---|
| *B. alba* | 38.3 d | 32.6 c | 10.0 ab |
| *B. carinata* | | | |
| Pc1 | 7.3 f | 4.6 e | 1.6 de |
| Pc2 | 7.3 f | 5.3 e | 1.0 e |
| *B. juncea* | | | |
| Blaze | 15.9 e | 11.9 d | 9.3 b |
| Lethbridge | 9.0 f | 8.6 de | 7.6 c |
| PR 15 | 25.6 e | 16.9 d | 7.3 c |
| PR 18 | 69.6 a | 51.6 a | 9.0 b |
| Porbiraya | 57.6 b | 43.6 b | 11.3 a |
| Stoke | 7.0 f | 6.3 e | 9.3 b |
| Varuna | 67.9 a | 55.6 a | 6.9 c |
| *B. napus* | | | |
| Altex | 4.0 f | 3.0 e | 1.0 e |
| Olivia | 4.6 f | 3.3 e | 1.0 e |
| Pant N1 | 4.3 f | 3.0 e | 0.6 e |
| Pant N2 | 4.6 f | 3.3 e | 1.3 e |
| Tilde | 4.3 f | 3.0 e | 1.0 e |
| Trawse | 4.6 f | 3.3 e | 1.0 e |
| WW 1313 | 5.0 f | 3.6 e | 1.3 e |
| *B. nigra* | 16.6 e | 13.3 d | 2.0 d |
| *Sinapis alba* | 47.3 c | 39.6 bc | 9.6 b |

Any two means having a common letter are not significantly different at 5% level

# THE OCCURRENCE OF NITRATE NITROGEN IN THE $F_5$ GENERATION OF BRASSICA NAPOCAMPESTRIS

B. Barcikowska and W. Brzezinski

The problem of nitrate ($NO_3$) accumulation in plants has become important recently, because of excessive levels of nitrogen fertilizing. The mechanism of nitrate toxicity is partially understood. After the reduction process in the rumen of animals, $NO_3$ ions give rise to nitrogen dioxide ($NO_2$). These ions incorporated into the circulation of blood cause injuries to the blood haemoglobin (2). There is also dependence between $NO_3$ content in feed and milk, which may be poisonous because of too high nitrate concentration (3). Nitrate is equally dangerous in water. Serious and occasionally fatal cases of infantile nitrate poisoning have occurred following ingestion of well water containing high nitrate levels (4).

Following these reports, we analysed some forms of Brassica napocampestris for nitrate content. Three progenies and two maternal varieties have been investigated. The summary of these data (Table 1) indicates that the amounts of $NO_3$ are rather high. This is certainly caused by the special ability of Brassica campestris to accumulate $NO_3$ ions. As is known, the nitrate content is conditioned not only by environmental factors like temperature, humidity, fertilisation etc, but also by the genus, species and even variety (1). According to French data from 1971, toxic action begins above a content of 0.35% N as $NO_3$ (equivalent to 1.54% $NO_3$ in the dry matter) (2). Unfortunately none of these forms investigated in this experiment is below this level of toxicity.

On the basis of these data it seems that there is a trend towards maternal inheritance of nitrate accumulation. Progenies of which the maternal form was Brassica napus c.v. Bishop with lower $NO_3$ content (5.01% in dry matter), were characterised by lower nitrate content (3.99% in DM). While those of maternal form B. napus c.v. Akela with higher $NO_3$ amount (6.17% in DM) showed higher nitrate-nitrogen levels (6.10 and 4.93% in DM).

Table 1. Nitrate content in the $F_5$ generation of Brassica napus x Brassica campestris ssp. pekinensis hybrids

| No. | Form - Variety | Number of plants investigated | $NO_3$ % in the DM (mean and border values) |
|---|---|---|---|
| 1. | B. napus c.v. Akela x B.c. ssp. pekinensis 'Chinese cabbage' | 15 | 6.10 (2.47-8.71) |
| 2. | B. napus c.v. Akela x B.c. ssp. pekinensis c.v. Granaat | 7 | 4.93 (1.46-6.47) |
| 3. | B. napus c.v. Bishop x B.c. ssp. pekinensis c.v. Granaat | 41 | 3.99 (1.62-7.39) |
| | Maternal forms: | | |
| 1. | B. napus c.v. Akela | 3 | 6.17 (5.63-6.86) |
| 2. | B. napus c.v. Bishop | 4 | 5.01 (3.65-6.34) |

Considering these results, one may expect that the tendency towards greater accumulation of nitrate ions by Brassica campestris may be overcome by interspecific crosses with B. napus with lower $NO_3$ accumulation tendency, which could lead to non-toxic Brassica napocampestris fodder forms.

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# DETERMINATION OF GLUCOSINOLATES IN BRASSICA SEED

Douglas I. McGregor

Separation of desulfo glucosinolates by high performance liquid chromatography (LC) has several advantages, most notably, it can be applied to both seed or vegetative samples, the eluate from the ion-exchange desulfation purification can be applied to the LC column directly without further derivatization, there is little or no interference on the LC column from non-glucosinolate substances, separation is effected without the use of buffers or ion-pairing reagents, and most of the major glucosinolates known to exist in Brassica species, including the four indoles, are well resolved so the relative amounts can be determined from one chromatogram (Sang, J. P. et al. 1983. Can. J. Plant Sci. 64,77-94). However, published response factors for LC of desulfo glucosinolates and methods for their determination (Sang, J. P. & Truscott, R. J. W. 1984. J. Assoc. Off. Anal. Chem. 67,829-833; Spinks, A. et al. 1984. Fette Seifen Anstrich. 86,228-231) are limited. Response factors can not be applied arbitrarily since flow rates and gradients for different makes of instruments must be adjusted for optimal resolution, and fixed filter instruments may have a restricted choice of wavelengths. Methods for determining response factors require isolation of at least milligram amounts of desulfo glucosinolates, sufficient to be weighed so that known weights may be reinjected into the LC.

A method has now been developed which facilitates calibrating LC of desulfo glucosinolates. The method requires collection of less than a micromole of each of the desulfo glucosinolate separated by LC on an anlytical column. The concentration of the individual desulfo glucosinolate is determined spectrophotometrically after reacting the glucose moiety with thymol (Brzezinski, W., & Mendelewski, P. 1984. Z. Pflanzenzuchtg. 93,177-183). The response relative to an internal standard is then determined by reinjection into the LC. The method has been combined with optimized conditions of myrosinase inactivation, glucosinolate extraction, ion-exchange desulfation purification, and LC separation for analysis of *Brassica campestris* L. and *B. napus* L. rapeseed, and *B. hirta* Moench and *B, juncea* L. Coss mustard seed.

## METHOD

### Apparatus

(a) Homogenizer.--A high frequency mechanical and ultrasonic homogenizer with a small (1 X 10 mm) shaft (Polytron homogenizer with PT10ST generator, Brinkman Instruments Inc., 50 Galaxy Blvd., Rexdale Ontario, Canada M9W 4Y5, or equiv.).

(b) Ion exchange columns.--100 X 8 mm ID columns (Bio-Rad polypropylene Econo-column, Bio-Rad Laboratories, 3140 Universal Drive, Mississauga Ontario, Canada L4X 2C8, or equiv.).

(c) A UV spectrophotometer.

(d) Liquid chromatograph.--A dual pump gradient instrument equipped with a variable volume injector and UV detector (Hewlett-Packard 1084B, Hewlett-Packard Co., Avondale, PA, equipped with a Hewlett-Packard 79841A variable volume injector, or equiv.).

(e) LC column.--A C18 reverse phase column 200 X 4.6 mm ID, 5 um particle size (Hewlett-Packard RP-18 or equiv.).

Reagents

(a) Sulfatase.--Type H-1 (Sigma Chemical Co., P. O. Box 14508, St. Louis MO 63178).

(b) DEAE-Sephadex acetate form.--Swell 10 g DEAE-Sephadex A-25 (Pharmacia Ltd., 2044 St. Regis Blvd., Dorval Quebec, Canada H9P 1H6) in water overnight. Slurry into a ca 15 X 200 mm column. Pass 50 ml sodium hydroxide (1 g dissolved and diluted to 50 mL with water) through column. Wash with ca 100 mL water to remove excess sodium hydroxide checking to ensure the pH has dropped to neutrality. Pass ca 100 mL acetic acid (2.9 mL glacial acetic acid diluted to 100 mL with water) through the column. Wash with ca 250 mL water.

(c) SP Sephadex C-25 sodium form.--Swell 1 g SP Sephadex C-25 (Pharmacia Ltd.) in water overnight. Slurry into a ca 15 X 200 mL column and wash with ca 250 mL water.

(d) Allyl glucosinolate.--Monohydrate, potassium salt(Aldrich Chemical Co., 1411 Fort St., Suite 1403, Montreal Quebec, Canada H3H 2N7).

(e) Petroleum ether.--Boiling range 30-60 C.

(f) Glucose solution.--Weigh 54.06 mg glucose into a 1000 mL volumetric flask, dissolve and dilute to volume with water.

(g) Sulfuric acid reagent.--Carefully add 690 mL conc. sulfuric acid to 190 mL water and cool.

(h) Thymol reagent.--Weigh 1 g thymol into a 100 mL volumetric flask, dissolve and dilute to volume with ethanol.

(i) Barium/lead acetate solution.--Weigh 15.3 g barium acetate, dissolve and dilute to 100 mL with water. Weigh 22.75 g lead acetate trihydrate, dissolve and dilute to 100 mL with water. Mix equal volumes.

(j) DEAE-Sephadex pyridine-acetate form.--Swell 10 g DEAE-Sephadex A-25 (Pharmacia, Ltd.) in water overnight. Slurry into a ca 20 X 400 mm column. Pass 500 ml sodium hydroxide (10 g dissolved and diluted to 500 mL with water) through column. Wash with ca 250 mL water to remove excess sodium hydroxide checking to ensure the pH has dropped to neutrality. Pass ca 400 mL pyridine-acetate (19.8 mL pyridine and 15 mL glacial acetic acid diluted to 500 mL with water). Wash with ca 250 mL water.

(k) Pyridine acetate solution.--Transfer 0.8 mL pyridine and 0.6 mL glacial acetic acid to a 100 mL volumetric and dilute to volume.

(l) Internal standard solution.--Accurately weigh 90.4 mg o-nitrophenyl-b-D-galactopyranoside (ONPGal) (Sigma Chemical Co.) into 50 mL volumetric flask, dissolve and dilute to volume with LC grade water.

(m) LC water.--Pass glass distilled water through a 0.45 um filter membrane (LC solvent A).

(n) Water/acetonitrile solvent.--Pass LC grade acetonitrile through a 0.45 um filter membrane. Add 3 volumes LC water to 1 volume LC acetonitrile (LC solvent B).

Purification and Assay of Sulfatase Activity

Weigh ca 70 mg sulfatase into a centrifuge tube. Add 3 mL water to dissolve, dilute with 3 mL ethanol and centrifuge. Decant off the supernatant and discard the precipitate. Add 9 mL ethanol to the supernatant and centrifuge. Discard the supernatant and dissolve the precipitate in 10 mL water. Add 1 mL DEAE-Sephadex A-25 acetate form to one Econo-column and 1.0 mL SP Sephadex C-25 sodium form to another. Pass the aqueous enzyme solution through the DEAE-Sephadex A-25 column then through the SP Sephadex C-25 column. Store the eluate at -20 C and thaw immediately before use.

Diluting 1 mL glacial acetic acid to 500 mL with water, and 1 mL ethylene diamine to 500 mL with water, and mixing 73 mL of the acetic acid solution with 40 mL of the ethylene diamine solution to prepare a pH 5.8 buffer. Weigh 7.8 mg allyl glucosinolate into a 100 mL volumetric flask, dissolve and dilute to volume with buffer. Add 2.0 mL of the buffered allyl glucosinolate and 0.25 ml of the aqueous enzyme solution and record the change in absorbance (1 cm pathlength at 226 nm. Calculate the activity of the sulfatase as the initial rate of change in absorbance per minute X 0.033. Minimum activity should be 30 X 10 -6 micromoles/minute.

Preparation of Oil-extracted Meal

If the seed is moist (> 7% moisture), dry ca 10 g in a forced-air oven overnight at 45 C.

Homogenize 2 g seed with sufficient petroleum ether (> 2 mL) to cover grinder head. Centrifuge, decant solvent, wash precipitated meal twice with additional petroleum ether, and air dry.

Determination of Relative Response Factors

To 1 mL of the glucose solution add 7 mL sulfuric acid reagent, 1 mL thymol reagent, mix thoroughly, heat in a boiling water bath 35 minutes and measure the absorbance against a reagent blank at 505 nm (1 cm pathlength). The absorption coefficient is calculated as the absorbance divided by 0.3.

Weigh 1 g meal into a tube and heat in a boiling water bath. When hot add 20 mL boiling water, thoroughly wet meal and continue heating for 3 minutes. Add 1 mL barium/lead acetate solution, centrifuge and decant supernatant. Wash pellet twice with boiling water and pool.

Place DEAE-Sephadex A-25 pyridine acetate form in an Econo column to form a column 18 X 8 mm ID (0.8 mL). Transfer the pooled extract to the column, wash twice with 2 mL water and once with 2 mL pyridine acetate solution. Add 0.5 mL purified sulfatase and let stand overnight. Elute with 4 X 1 mL LC water.

Filter an aliquot of the eluate through a 0.45 micron filter. Inject ca 100 ul onto the LC column and separate with flow and a water (LC solvent A) and water/acetonitrile (LC solvent B)

gradient adjusted to achieve optimal resolution (i.e. flow rate: 1 mL/min, gradient: 5 % B 5 min, increased to 90 % B at 20 min, held at 90 % B till 30 min).

Collect individual desulfo glucosinolates after passage through the UV detector. Take an aloquot to dryness, add 1 mL water, 7 mL sulfuric acid reagent and 1 mL thymol reagent, mix thoroughly and heat in a boiling water bath 35 minutes. Measure the absorbance against a reagent blank at 505 nm (1 cm pathlength). Calculate the micromolar concentration using the micromolar extinction coefficient:

$$\frac{\text{Absorbance}}{\text{Absorption Coefficient}} \times \frac{1}{\text{mL taken to dryness}} = \frac{\text{Micromoles Desulfo Glucosinolate}}{\text{mL LC eluate collected}}$$

Take a second aliquot, add ONPGal, filter through a 0.45 micron membrane and reinject into the HPLC using the established flow and a water and water/acetonitrile gradient (collection volume, aliquot sizes, amount of ONPGal added and reinjection volume are dependent upon the concentration of the desulfo glucosinolate in the sample under study). Calculate the response factor relative to ONPGal as:

$$\frac{\text{Area ONPGal}}{\text{Area Desulfo Glucosinolate}} \times \frac{\text{Micromoles/mL of the Desulfo glucosinolate in the LC eluate}}{\text{Micromoles/mL of the ONPGal solution added to the LC eluate}} \times \frac{\text{mL of LC eluate assayed}}{\text{mL of ONPGal added}} = \text{Relative Response Factor}$$

Determination of Glucosinolate Content

Weigh 0.5 g meal and dry in a forced-air oven at 135 C for 2 hours. Cool in a desiccator over sodium hydroxide pellets. Weigh and calculate moisture as % weight loss.

Weigh 200 mg meal into a tube and heat in a boiling water bath. When hot add 2 mL boiling water, thoroughly wet meal and continue heating for 3 minutes. Add 125 uL barium/lead acetate solution, centrifuge and decant supernatant. Wash pellet twice with boiling water and pool.

Place DEAE-Sephadex A-25 pyridine acetate form in an Econo column to form a column 18 X 8 mm ID (0.8 mL). Transfer the pooled extract to the column, wash twice with 2 mL water and once with 2 mL pyridine acetate solution. Add 0.5 mL purified sulfatase and let stand overnight. Elute with 4 X 1 mL LC water. Add 0.5 mL ONPGal internal standard solution.

Filter an aliquot of the eluate through a 0.45 micron filter. Inject ca 100 ul onto the LC column and separate with the established flow and a water and water/acetonitrile gradient.

Calculate the glucosinolate content in micromoles per gram oil-extracted moisture-free meal:

$$\frac{\text{Area Desulfo Glucosinolate}}{\text{Area ONPGal}} \times \text{Relative Response Factor} \times \frac{\text{Micromoles ONPGal}}{\text{g Meal}} \times \frac{100}{100 - \% \text{ Moisture}} = \frac{\text{Micromoles Desulfo Glucosinolate}}{\text{g Oil-extracted Moisture-free Meal}}$$

Retention times and relative response factors for desulfo glucosinolates separated by high performance liquid chromatography.

| Glucosinolate | Retention time (min) | Response relative to ONPGal |
|---|---|---|
| 3-Methylsulphinylpropyl | 2.2 | 1.00 |
| (S)-2-Hydroxy-3-butenyl | 2.9 | 0.90 |
| (R)-2-Hydroxy-3-butenyl | 3.2 | 0.95 |
| 2-Propenyl | 3.5 | 0.94 |
| 4-Methylsulphinylbutyl | 3.7 | |
| 4-Methylsulphin-3-butenyl | 4.7 | |
| (R)-2-Hydroxy-4-pentenyl | 6.6 | |
| 5-Methylsulphinylpentenyl | 8.0 | 0.93 |
| 4-Hydroxybenzyl | 8.6 | 0.47 |
| 3-Butenyl | 9.1 | 0.88 |
| 4-Hydroxy-3-indolylmethyl | 9.9 | 0.23 |
| 3-Methylthiopropyl | 11.0 | 1.11 |
| 4-Pentenyl | 12.8 | 0.93 |
| Benzyl | 13.2 | 0.81 |
| 4-Methylthiobutyl | 13.4 | |
| 3-Indolylmethyl | 14.6 | 0.24 |
| 5-Methylthiopentenyl | 16.2 | |
| 2-Phenylethyl | 16.7 | 0.89 |
| 4-Methoxy-3-indolylmethyl | 17.0 | 0.24 |
| 1-Methoxy-3-indolylmethyl | 20.1 | 0.22 |

# High palmitic acid content in summer turnip rape (Brassica campestris var. annua L.)

C. Persson

The removal of the erucic acid from the oil of rape and turnip rape has affected the physical characteristics of the hydrogenated oil since more than 90 % of the fatty acids have a chainlength of 18 carbon atoms. A margarine produced by standard methods from such an oil has a bad texture due to recrystallization. An increase of the palmitic acid ($C_{16:0}$) content creates a bigger variation in chainlength and can therefore solve this problem.

Using the half-seed technique, three seeds with an increased content of palmitic acid were found in 1981. (Persson and Johansson 1983, Jönsson and Persson 1983.) The seeds were found in a breeding-line created by repeated selection for low erucic acid content in the high-erucic variety Sv Torpe. The sums of the palmitic acid and palmitoleic acid ($C_{16:1}$) contents were 11.3 %, 7.0 % and 6.5 % respectively. Lines with a stable palmitic acid content of more than 10 % have been created. Single seeds with a palmitic acid content of 17 % have been found.

The contents of palmitic acid and palmitoleic acid are positively correlated. Normally the palmitoleic acid content is 0.5 % or less but when the palmitic acid content increases to 10 %, the palmitoleic acid content rises to 3 %. Also the linolenic acid ($C_{18:3}$) content is increased but the linoleic acid ($C_{18:2}$) content is somewhat decreased. The oleic acid ($C_{18:1}$) content is decreased.

Heredity is under investigation and preliminary results indicate that one recessive gene is responsible for the increase in the palmitic acid content. The quality of high palmitic acid content has been transfered to double-low material by crossings. A small seed sample of summer turnip material with 11 % palmitic acid in the oil and 26 µmoles glucosinolates in the meal can be obtained from the author at the address below:

Svalöf AB, S-268 00 SVALÖV, Sweden

## References

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# THE EFFECTS OF NITROGEN TOP-DRESSING AND SEED SPACING ON THE SMCO AND $SCN^-$ CONTENTS OF CABBAGE CULTIVARS GROWN FOR FODDER

J.E. Bradshaw

Cabbage (Brassica oleracea var. capitata L.) contains S-methyl cysteine sulphoxide (SMCO, the haemolytic factor) and indole glucosinolates which release the thiocyanate ion ($SCN^-$, a goitrogen) on hydrolysis. Therefore like kale it can cause haemolytic anaemia and goitre in cattle and sheep when fed in large quantities.

The effects of nitrogen top-dressing and seed spacing on the SMCO and $SCN^-$ contents of six cultivars grown for fodder are reported in this note. The more extensive data on dry-matter yield and content have already been published (Bradshaw, 1984). A split-plot design with four replicates was used with nitrogen fertilizer treatments on the main plots (0 and 150 kg/ha four weeks after sowing in addition to 143, 31 and 59 kg/ha of N, P and K respectively applied as a compound fertilizer in the seed-bed), and seed spacings (15, 25 and 38 cm) and cultivars on sub-plots. Each sub-plot comprised five rows 6 m long, with 50 cm between rows. At harvest in mid-October wedges, including outer leaves, were cut from six plants in each sub-plot, bulked, freeze-dried, milled through a 1 mm sieve and then stored in plastic jars with screw-on caps in a deep freeze. SMCO and $SCN^-$ contents were determined as described by Bradshaw & Borzucki (1982).

Nitrogen top-dressing increased SMCO content from 7.10 to 7.84 g/kg dry matter (with S.E. of 0.101) but did not affect $SCN^-$ content.

Seed spacing did not affect SMCO or $SCN^-$ content although there was a statistically significant (P 0.05-0.01) interaction between seed spacings and cultivars for SMCO.

There were statistically significant differences between cultivars for both SMCO and $SCN^-$ (P 0.01-0.001 and <0.001 respectively).

| Cultivar | SMCO | $SCN^-$ |
|---|---|---|
| | g/kg dry matter | |
| Amager Grami | 7.72 | 0.210 |
| Early Drumhead | 7.81 | 0.287 |
| Holland Late Winter | 7.40 | 0.203 |
| January King No. 3 | 7.43 | 0.370 |
| January Prince | 7.88 | 0.413 |
| Utility | 6.60 | 0.165 |
| Mean | 7.47 | 0.275 |
| S.E. for individual cultivars | 0.225 | 0.0122 |

Cultivar Utility had the lowest SMCO and $SCN^-$ contents, and the January King cultivars the highest $SCN^-$ contents as found by Bradshaw & Borzucki (1982) for cabbage heads (i.e. excluding outer leaves). However, the

January King cultivars have a high dry matter content which is a desirable feature of a cabbage for stock feeding, as are the high whole-plant dry-matter yields of cvs Amager Grami and Holland Late Winter and the high head dry-matter yields of cvs Early Drumhead and Utility (Bradshaw, 1984).

If all these desirable characteristics could be combined by plant breeding, (and clubroot resistance introduced), a superior cabbage for stock feeding would result. However, this would require a large programme which would decrease resources for more important crops. Therefore the small fodder cabbage breeding programme which I described in Cruciferae Newsletter No. 8 (page 16) has been terminated now that the initial aim of improving the dry matter yield and content of the foundation population has been achieved.

Acknowledgement

The author thanks Dr M.J. Allison and his colleagues for carrying out the chemical analyses.

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# INFLUENCE OF TIME OF SOWING ON RAPESEED OIL AND MEAL QUALITY

J P Sang and E P Hilliard

In 1981, a new variety of rapeseed (Brassica napus) was released for commercial evaluation in Victoria. This new variety called Marnoo, was bred for higher yield characteristics and low levels of erucic acid and glucosinolates. A questionnaire which was issued to selected growers involved in the commercial production of Marnoo, requested information on crop management systems employed and on yield data. Growers were also requested to supply samples of seed from the crops harvested for chemical quality assessment. Detailed results of this survey have been reported previously by Sang et al (1984). This report is a summary of the chemical evaluation of the seed material supplied by growers during this survey.

In Victoria, rapeseed is normally sown in late Autumn or Winter, depending on seasonal conditions. Sowing times recorded during this survey of Marnoo growers ranged from May to October, depending on soil moisture conditions which varied from one region to another. A total of 29 growers supplied detailed crop management information and seed samples for analysis. A representative sample of the chemical data is shown in Table 1.

Table 1

Sowing dates, oil content, erucic acid content and glucosinolate content of Marnoo rapeseed.

| Sowing Date | Oil Content % | Erucic Acid %w/w | Canola Glucosinolate Content (a) |
|---|---|---|---|
| 12/5/81 | 45.3 | 1.3 | 19 |
| 15/5/81 | 45.5 | 1.8 | 23 |
| 2/6/81 | 45.0 | 1.6 | 28 |
| 17/6/81 | 42.4 | 1.3 | 22 |
| 21/6/81 | 42.7 | 1.0 | 21 |
| 8/7/81 | 42.7 | 1.3 | 26 |
| 18/7/81 | 40.7 | 1.1 | 28 |
| 15/8/81 | 36.3 | 1.1 | 29 |
| 10/9/81 | 37.6 | 1.3 | 33 |
| 15/9/81 | 40.2 | 1.2 | 33 |
| 3/10/81 | 42.7 | 1.0 | 42 |
| 4/10/81 | 37.3 | 1.0 | 35 |

(a) micromoles/gram oil free dry basis, sum of 2-hydroxybut-3-enyl, but-3-enyl, 2-hydroxypent-4-enyl and pent-4-enyl glucosinolates.

Results from the complete set of data reported by Sang et al (1984) show that oil content decreased and glucosinolate content increased with later sowing times.

The erucic acid content of the oil ranged from 0.8% w/w to 1.8% w/w and was apparently not effected by time of sowing - while the decrease in oil content with late sowing was expected, as it had been observed previously in Victoria (Wightman, 1976) it was surprising to observe the quite dramatic increase in glucosinolate content of the oil free seed relative to sowing time. Glucosinolate values ranged from 5 up to 42 micromoles per gram in the samples analysed with a correlation between time of sowing and glucosinolate level of 0.72, which is highly significant ($p < .001$). This range in glucosinolate levels was achieved despite the fact that each grower used registered Marnoo seed at sowing which had glucosinolate levels of between 18 and 20 micromoles per gram. While it is accepted by us that factors such as soil nutrient and moisture status and other environmental factors can possibly be influential in determining glucosinolate content of rapeseed, the highly significant statistical relationship which we observed between time of sowing and glucosinolate content of Marnoo crops suggests that in-depth studies are required to elucidate the precise causes of this phenomenon. Trials are currently in progress in this laboratory to investigate the influence of constituent components of environmental and nutrient factors on glucosinolate biosynthesis in supposedly low glucosinolate rapeseed varieties.

References:

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# DATES OF SOWING IN CABBAGE SEEDPRODUCTION BY PLANTS OVER-WINTERING AT THE ROSETTE PHASE

L. Ivanov, Guelly Vitanova

Cabbage seedproduction by plants over-wintering at the rosette phase is an efficient method (3,4). Regional research is recommended for the assessment of optimal dates for sowing seeds directly in the field or for transplant production (4). The method is intended for market seeds in combination with mass negative selection (2,4).

The aim of this study was to assess the favourable sowing dates for the midseason cv. Chelopechenski sin kapak (in the Sofia region) and of the two late cvs Kyosse 17 and Plovdivsko podobreno (in the Plovdiv region) in cases of direct sowing and of planting 35-40-day-old transplants in the field spaced 80 x 40-50 cm. The traditional way of cabbage seedproduction by transplanting the heads in autumn and earthing them up was used as a check.

Observations and measurements were made on the onset of the individual phenophases of the plants, their weight and number of rosette leaves in late autumn, their over-wintering, bolting, seed yield and sowing qualities.

These observations show that plant development until winter sets in was 8-11 days later in case of growing transplants, notwithstanding their date of sowing, than in case of direct sowing. Next spring anthesis of the late cultivars sown July 20th and 30th and of the check was 13 to 18 days earlier than that of the August 20th sowing date. The same difference was evident in the early cultivar grown in the Sofia region sown June 30th and the check as compared to the August 10th sowing date. Plants of the late sowing dates reached anthesis almost simultaneously in both regions.

Over-wintering of the plants (Table 1) was not related with weight or number of rosette leaves, but depended on age, more precisely on head age. This conclusion is substantiated by the data concerning the over-wintering of the check, the heads of which were formed first, but though they were well earthed up during the winter, had lowest percentage of over-wintering. For the same reason the heads produced by the earliest date of direct sowing of late cultivars and by the earliest date of sowing in both ways of growing the midseason cultivar has lowest over-wintering percentage.

Bolting affected seed yield also. Vernalization of the plants from the last sowing date of the late cultivars in the Plovdiv region (Table 2) was insufficient, therefore their percentage of bolting was low. The same was true for the August 10th sowing date of the transplants in Plovdiv, because transplant development was retarded. As could be expected check plants of the three cultivars bolted normally. In the Sofia region there was no difference in bolting between the two ways of growing for the August 10th date of sowing, since the cultivar is with a shorter vegetation period.

Data about seed yield (Table 2) show that the most appropriate date of sowing for the two late cultivars grown in the Plovdiv region is July 30th both in case of direct sowing and in case of growing by transplants. For the Sofia region the period from July 20th and August 10th is most suitable. In earlier sowing dates and in the check, seed yields are lower, which can be explained by older plant age (4).

Seeds produced from all sowing dates in both regions proved to be of high quality.

Table 1

| Date of sowing | Growing by | $V_1^+$-Plovdiv | | | $V_2^+$-Plovdiv | | | $V_3^+$-Sofia | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant weight kg | Rosette lvs per plant No. | Over wintering* % | Plant weight kg | Rosette lvs per plant No. | Over wintering* % | Plant weight kg | Rosette lvs per plant No. | Over wintering* % |
| 30.VI | 1 | - | - | - | - | - | - | 3.6 | 16 | 65 |
| | 2 | - | - | - | - | - | - | 2.5 | 17 | 65 |
| 20.VII | 1 | 3.2 | 25 | 61 | 3.2 | 24 | 54 | 1.4 | 22 | 79 |
| | 2 | 3.1 | 23 | 85 | 3.1 | 20 | 82 | 1.0 | 22 | 76 |
| 30.VII | 1 | 2.1 | 23 | 85 | 1.9 | 21 | 75 | - | - | - |
| | 2 | 1.5 | 18 | 93 | 1.0 | 17 | 87 | - | - | - |
| 10.VIII | 1 | 1.6 | 19 | 76 | 1.1 | 17 | 78 | 0.5 | 16 | 71 |
| | 2 | 0.9 | 16 | 88 | 0.7 | 14 | 83 | 0.3 | 15 | 69 |
| 20.VIII | 1 | 0.2 | 12 | 90 | 0.2 | 11 | 86 | - | - | - |
| | 2 | 0.1 | 7 | 83 | 0.1 | 6 | 82 | - | - | - |
| 10.VI | check | - | - | - | - | - | - | 5.0 | 20 | 62 |
| 20.VI | check | 4.5 | 26 | 71 | 4.2 | 24 | 74 | - | - | - |
| DF p=1% | | | | 16 | | | 11 | | | 12 |

* 3-year mean, for Sofia region - 2-year

\+ $V_1$ - cv.Kyosse 17; $V_2$ - cv.Pazardzhishko podobreno 16; $V_3$ - cv.Chelopechenski sin kapak

1 - direct sowing
2 - transplants

Table 2

| Date of sowing | Growing by | $V_1^+$ - Plovdiv | | $V_2^+$ - Plovdiv | | $V_3^+$ - Sofia | |
|---|---|---|---|---|---|---|---|
| | | Bolted plants % | Seed yield kg/ha | Bolted plants % | Seed yield kg/ha | Bolted plants % | Seed yield kg/ha |
| 30.VI | 1 | - | - | - | - | 98.3 | 349 |
| | 2 | - | - | - | - | 97.9 | 359 |
| 20.VII | 1 | 99.9 | 567 | 99.7 | 401 | 98.2 | 562 |
| | 2 | 98.9 | 843 | 98.4 | 786 | 97.8 | 521 |
| 30.VII | 1 | 98.9 | 786 | 99.5 | 749 | - | - |
| | 2 | 95.5 | 973 | 97.2 | 844 | - | - |
| 10.VIII | 1 | 99.3 | 539 | 97.3 | 461 | 98.1 | 421 |
| | 2 | 70.3 | 590 | 54.2 | 376 | 97.5 | 541 |
| 20.VIII | 1 | 48.4 | 410 | 39.7 | 345 | - | - |
| | 2 | 39.9 | 267 | 13.9 | 139 | - | - |
| 10.VI | check | - | - | - | - | 98.3 | 329 |
| 20.VI | check | 99.3 | 622 | 98.5 | 521 | - | - |
| DF p=1% | | | 195 | | 173 | | 143 |

\+ $V_1$ - Kyosse 17; $V_2$ - Pazardzhishko podobreno 16; $V_3$ - Chelopechenski sin kapak

1 - direct sowing
2 - transplants

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# ADVANTAGEOUS EFFECT OF INSECT POLLINATORS ON THE NUMBER OF POLLEN DEPOSITED ON STIGMA AND SEED YIELD IN BRASSICA JUNCEA UNDER ISOLATION CAGES

Ryo Ohsawa and Hyoji Namai

In Brassica juncea (Mustard) which has been considered self-fertilizing plant, Free and Spencer-Booth(1963) reported no relationship between the presence of insect pollinators and seed yields.

We have been trying to estimate the effect of insect pollinators on seed yield in digenomic Brassica crops which have been considered self-fertilizing plant, and observed that the use of insect pollinators in caged plants could improve seed yields in some B.juncea cultivars(Ohsawa and Namai, 1984).

In this paper we described the intimate relationship between the number of pollen deposited on stigma and seed yield in caged B.juncea.

Each caged plot was L3.4m x W1.4m x H1.6m and covered with clean saran screen(#300) containing 12 plants, with two replications. Shimahanaabu(Eristaris cerealis), artificially reared, was used as insect pollinators and released into each cage at a ratio of 0,1,2 and 4 per plant when all plants began flowering. The stigmas were sampled and immediately fixed by acetic alcohol at 11:00 am. of the flowering days for three days. The number of pollen deposited on each stigma was counted microscopically by aceto-carmine squash method.

Fig.1 shows the frequency distribution of the number of pollen deposited on stigma under isolation cages with 0 to 4 insect pollinators per plant. The mean number of pollen deposited on stigma is about 100 in cage without the insect pollinators. In cage with the insect pollinators, the mean number of pollen increased with increasing number of insect pollinators per plant. The stigmas coated with less than 100 pollen were more than 60% of all flowers observed in cage without insect pollinators, and only 5% in cage with four insect pollinators per plant.

Fig.2 shows correlation between the number of pollen deposited on stigma and the number of seeds per flower(seed yield) under isolation cages with and without the insect pollinators. The mean number of seeds per flower is about 13 (60% in seed set percentage) even in the cage without the insect pollinators and about 19(90%) in the cage with four insect pollinators per plant.

Since the number of ovules per flower is about 21 in B.juncea cv.Kikarashina, more than 60% of stigmas were coated with less than five times as many as the number of ovules per flower in cage without insect pollinators, whereas almost all stigmas were coated with more than ten times as many as the number of ovules per flower in cage with sufficient pollinators.

Therefore, using insect pollinators in isolation cage will increase the number of pollen deposited on stigma, resulting in improved seed yields. However, whether the advantageous effect of insect pollinators to seed setting of B.juncea is due to the increase in self-pollination or cross-pollination is a matter for argument.

## References

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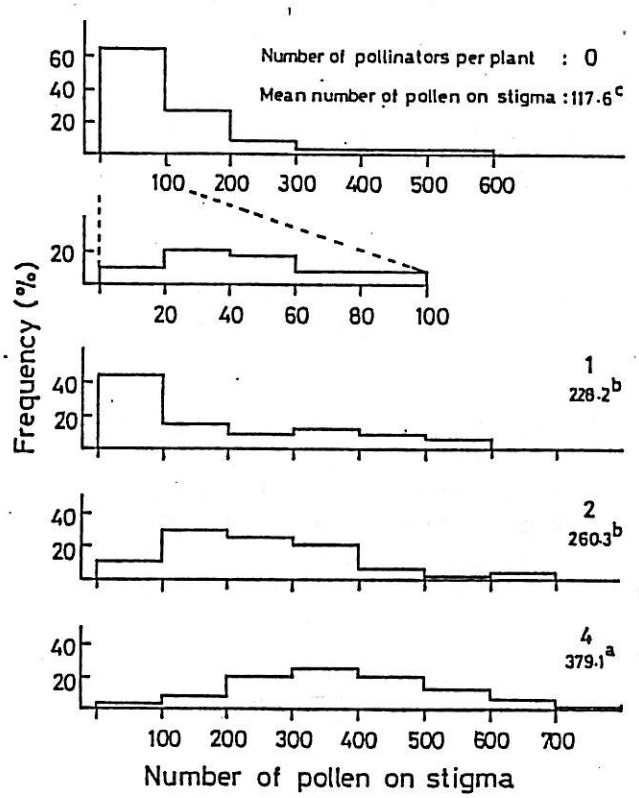


Fig.1 Frequency distribution of number of pollen on stigma in each cage.
Means followed by the same letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

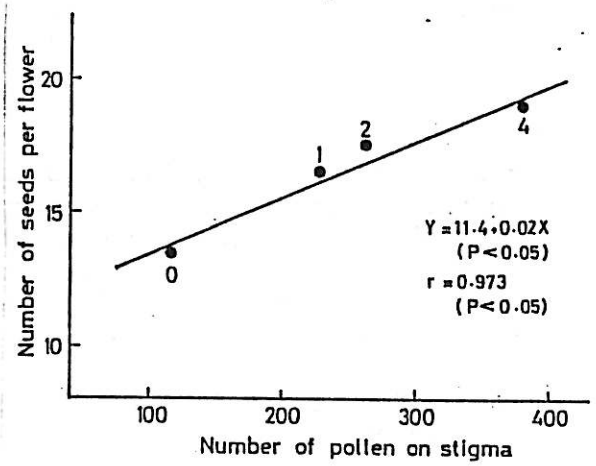


Fig.2 Correlation between number of pollen on stigma and number of seeds per flower.
Each number of 0,1,2 and 4 means number of pollinators per plant in each cage with 12 plants, respectively

# THE DEVELOPMENT OF A CULTIVAR DATABASE FOR SWEDE

F N Green and P J Winfield

We propose to build a database on Swede cultivars, primarily as an aid to cultivar registration.

In the course of registration tests for Distinctness, Uniformity and Stability (DUS) of Swede cultivars in the UK, a large amount of information has been recorded over a period of eight years. This information has been collated for cultivars registered on the UK National List or the EEC Common Catalogue, and it is intended to transfer this to a microcomputer to form a database.

Twenty-one morphological characters have been recorded at the vegetative stage and these are listed in a paper by us in the Proceedings of Better Brassicas '84 conference, published by the Scottish Crop Research Institute. In addition a limited amount of data has been collated on characters recorded at the reproductive stage.

A collection of approximately eighty cultivars, which were not tested for DUS since they pre-dated registration in the UK, was sown in 1985 to examine the range of variation in morphology. Data recorded on these cultivars will also be added to the proposed database.

The database will hold information on synonyms, supplier, breeder, parentage, stock number and year of receipt; reference to photographs seed quantity and genebank storage will also be included. Space has also been allocated for adding general comments.

It is intended that this morphological database could be expanded by adding sections on performance, genetics, disease resistance, pest resistance, susceptibility to herbicides etc using the cultivar name as the common access. We recognise that many characters are influenced by environmental factors (site and season), but information on those which are not, will be extremely valuable. Such a database could be used by breeders, registration workers, seedsmen and growers; descriptions could also be provided for Genebanks.

Anyone interested in participating in this project are welcome to contact the authors.

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