Fifth Crucifer Genetics Workshop

April 7–9, 1989

University of California, Davis

PROCEEDINGS

Report No. 4
August 1989

GENETIC RESOURCES CONSERVATION PROGRAM
Division of Agriculture and Natural Resources
UNIVERSITY OF CALIFORNIA

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Edited by

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INTRODUCTION

Carlos F. Quiros

It was gratifying to see the 230+ participants in attendance at the Fifth Crucifer Genetics Workshop. The fact that the number of attendees has increased with each yearly meeting clearly demonstrates that all are profiting from this brief assembly. It is also reassuring to see the increase in participants from all parts of the world. This indicates that the reputation of our meeting is growing outside our borders. Undoubtedly, crop *Brassicas* are gaining in economic importance, not only as sources of oil, but also as vegetable crops. This importance is reflected in the large number of research institutions, governmental and private, practicing *Brassica* research.

When this program was conceived, we tried to cover the most important and current aspects of crucifer genetics. We were fortunate to be able to attract leaders in these fields. This was made possible by the generosity of our sponsors who provided funds to pay travel expenses of invited speakers.

In this volume, we have assembled summaries of the symposium, workshop, and poster presentations to give the reader a fair view of the topics covered by the meeting. For the workshops, we have tried to reconstruct as much as possible of the presentations and discussions, but some of them remain incomplete or missing. Although we included all the abstracts that we received from the workshop speakers and summaries of the general discussions when they were available, it was not possible to present the proceedings of the workshops in a single, uniform format.

The first symposium covered applied aspects of genetics and evolution related to *Brassica* breeding. This encompassed sources of germplasm, assembly of specialized stocks for breeding and evolutionary studies, RFLP mapping and its applications, the status of androgenesis as a breeding tool, and rapeseed breeding in North America. The second symposium presented an overview of *Brassica* biotechnology. The most active research areas in the field, with the highest potential for practical application, were covered, *e.g.*, gene expression, self-incompatibility, cybridization and cms, transformation, and molecular basis of fatty acid composition. The afternoon workshops and poster sessions provided a forum for discussion and expansion of symposium topics. Undoubtedly, the participation of César Gómez-Campo and Toshio Shiga, legendary names in *Brassica* research, was the highlight of the meeting.

The crucifer demonstration plot was a big success. Let's make a tradition of having one at future meetings. A wide array of interesting materials, particularly Portuguese cabbages, made this plot a must to visit.

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Organizing Committee
Carlos F. Quiros, *Chair*
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Although almost everything went as planned, the unexpected hot weather surprised our campus maintenance people who, unfortunately, were unable to change the conference buildings' environmental control settings from heating to air conditioning. For better or worse, all had the opportunity to sample a typical summer day in Davis.

We are eagerly looking forward to the Sixth Crucifer Genetics Workshop at Cornell. Let's work together to make this meeting even more successful and to continue giving it an international dimension.

We thank Dean Charles E. Hess for the support of his office and his participation in opening the meeting. Our special thanks and appreciation go to Nancy Scybert for retyping the summaries and abstracts, to Yvonne Savio for preparing the sponsor posters, to Carolyn Norlyn and her associates in the Campus Events and Information Office for making the meeting run so smoothly, and, finally, to the students and staff who ran the projectors during the presentations.

SPONSORS

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The Department of Vegetable Crops
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The University of California Division of Agriculture and Natural Resources:
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BRASSICA BREEDING, GENETICS, AND EVOLUTION

SYMPOSIUM I

Presiding:

Carlos F. Quiros
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Germplasm of Wild n=9 Mediterranean Species of Brassica

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Following some pioneering collections in the period 1975 to 1977, an IBPGR/FAO-sponsored meeting was held in Rome in 1980 to establish criteria on *Brassica* germplasm collections and to make plans for a series of systematic missions directed to those wild species considered to be the closest relatives to *B. oleracea*. These missions took place between 1982 and 1988.

The 1982 and 1983 missions were held in Greece, the first year in the mainland (Attica, Evia, and Peloponissos) and the second in Crete. They supplied a total of 45 samples of as many populations of *B. cretica* (ssp. *cretica*, ssp. *laconica*, and ssp. *nivea*), together with a number of ecological and corological data including the finding of several new localities. A parallel mission in Turkey provided some additional samples of the same species. *B. cretica* is an almost obligate chasmophytic species, living on limestone cliffs where enough water supply can be secured in summer.

For the 1984 mission, South Italy and Sicily were visited. Beyond any doubt, Sicily contains the highest variability within this group. At least four species (*B. macrocarpa*, *B. incana*, *B. rupestris*, and *B. villosa*) are found in the island, together with at least five additional taxa deserving subspecific status. Interpopulation variability was easily observed for some of the taxa. Some of the collections were particularly abundant, because the plants in this case grew much more often at the base of the cliffs than on the cliffs themselves.

The 1985 mission took place along the Mediterranean coasts of N.W. Italy, S. France, and N.E. Spain. Some 25 samples of *B. montana* were collected. In many instances, this species was found to be somewhat weedy. An attempt to collect wild *B. oleracea* in the Atlantic coast of France yielded poor results because, unlike their Mediterranean counterparts, most pods were still green in July.

The missions of 1986 were devoted to some interesting but so far uncollected areas of Cyprus (*B. hilarionis*), Corsica, Sardinia, and Tunisia (*B. insularis*). Seeds of *B. hilarionis* were collected for probably the first time. The infraspecific variability of *B. insularis* in Corsica was noticeable and thoroughly collected.

No mission was planned for 1987, but an extensive collection of wild *B. oleracea* along the coasts of N. Spain, France, England, and Wales was carried out in 1988, this time during the right season. It yielded numerous samples plus a number of new localities and observations.

The total number of samples collected amounts to 230, which corresponds to as many populations belonging to nine species. It is estimated that the proposed aims have been thoroughly achieved, although some additional missions in the Yugoslavian coast or the Ægean Islands might perhaps be desirable.

According to IBPGR policies, each sample was split into three parts, one of which was conserved in the Universidad Politécnica seed bank which is IBPGR designated for wild crucifers, the second in the University of Tohoku (Sendai, Japan), and the third in a seed bank in the country where each sample was collected. Base collection samples were stored in at least three different places, any risk being thus minimized.

A multiplication program is now underway in order to make the material available for research. In the next catalog of the UPM seed bank, now being prepared and expected to include some 600 species of Cruciferae, an appendix will be added with those *Brassica* populations which have already been multiplied.

Characterization work on these collections is being done in Svalov, and a database referred to this material has been jointly developed in Madrid and Svalov. Some preliminary phytochemical characterization is now being carried out in Barcelona and Madrid.

Cytogenetic Stocks in Brassica: Addition Lines and Genome Evolution

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Brassica crops and wheat share a parallel evolutionary pattern in which amphiploids are derived by hybridization of diploid species. Wheat cytogenetics is perhaps the most advanced in all the crops, where a series of complex stocks have been developed. These include monosomics, nullisomics, and alien chromosome addition and substitution lines. The usefulness of this material in gene mapping and wheat breeding is unquestionable. Besides their practical applications, the development of these stocks in *Brassica* will provide the means to study problems related to the evolution and genetics of these species.

Alien addition lines: In our research program, oriented to study the evolution of the *Brassica* genomes, we are developing a series of these lines. Our emphasis has been the generation of *B. campestris-oleracea* additions (AA+C) from natural *B. napus* and from 'Hakuran' (synthetic *B. napus*) dissecting the *B. oleracea* genome (C). Other addition lines under development in our laboratory are those

dissecting the *B. nigra* genome (B). These are *B. oleracea-nigra* lines (CC+B) from *B. carinata*, and *Diplotaxis erucoides-nigra* (DeDe+B) lines from a synthetic amphidiploid between both species. Also, we are attempting to develop CC+A, BB+C, CC+B, and CC+R addition lines. A few other laboratories are working also in the development of addition lines of *B. napus-nigra* (AACC+B), one in France under the leadership of Anne Marie Chèvre of INRA (Jahier *et al.*, Genome **32**:408, 1989), and the other in Germany under the leadership of Dr. Röbbelen at Göttingen. The objective of these groups is the development of disease-resistant rapeseed. Kaneko *et al.* (Japan J. Breed. **37**:438, 1987) has also dissected the C genome by generating radish-kale additions (RR+C). In addition to the AACC+B lines, Chèvre and her group are developing *B. napus-Diplotaxis erucoides* and *B. napus-hirta* addition lines.

The technique to develop these lines consists of crossing the amphidiploid parent by one of its diploid progenitors. This generates a sesquidiploid, which is a triploid having two complete sets of chromosomes for one of the genomes and a single set for the other genome. During meiosis the chromosomes of the single set remain unpaired and will tend to be eliminated. After several backcrosses it is possible to select individuals with two complete sets of chromosomes of the recurrent species and a single chromosome of the other species. Often, it is not necessary to double chromosome number in the hybrid, since it produces unreduced gametes passing the genome of both parents to the progeny. This was the case for the *B. napus-nigra* lines of Chèvre.

Monosomics and nullisomics: The developement of these lines is in its infancy in *Brassica*. Chang *et al.* (Genome **29**:174, 1987) reported two monosomics and a nullisomic in *B. napus*. The development of these stocks is an area that needs more attention, since along with the addition lines, monosomics will permit the future development of substitution lines. These lines may be extremely useful to study the effect of substituting chromosomes of different genomes on the determination of important traits.

Genome specific markers: We are using isozyme loci and restriction fragment length polymorphisms (RFLPs) as genome specific markers. For the RFLPs, in collaboration with B. Landry, we have identified useful clones from a cDNA library in *B. napus* developed by John Harada. Also, we use genomic clones from *B. oleracea* and *B. napus* libraries constructed in our laboratory (Hosaka *et al.*, unpublished). In addition, we rely on heterologous clones such as rDNA and others. The data generated by the addition lines on the synteny of these markers together with conventional F_2 linkage analysis complement each other. These combined approaches augment the level of polymorphism which could be detected by a single method. Another advantage is that it allows us to compare chromosomes of the same genome extracted either from the diploid species or from the amphidiploids.

Although we are at the stage of gathering information, we can summarize our findings as follows: On the basis of isozymes, rDNA, and genomic markers, we find that the three cultivated genomes, A, B, and C, are mutually and partially homologous. Very few markers

are conserved among these genomes indicating extensive divergence since their origin. 1) Isozyme loci: Although the same number of loci are present in all three genomes, species-specific allozymes are observed at each locus. 2) rRNA genes (work in collaboration with M. Delseny at Perpignan): Although the coding regions in the 18S and 25S regions are highly conserved in *Brassica*, *B. campestris* can be separated from the rest of the cultivated and wild species by the absence of *EcoRI* site 3 in all the repeated units (approx. 6500). Other important differences are the subrepeat size caused by size differences of the intergenic spacers. The *B. campestris* spacer is smaller than the *B. oleracea* and *B. nigra* spacers, which are the same size. RFLPs of the rDNA region disclose similar numbers of common fragments among the diploid cultivated species. This number is approximately half of that observed within any of the species. 3) Genomic clones (Hosaka *et al.*, unpublished): RFLPs of 10 clones disclose mostly genome specificity for distinctive fragments. These markers can be followed up in most instances in the derived amphidiploid species. In a few cases, specific fragments are shared either by the A and B genomes or by the A and C genomes. 4) cDNA: Although we are just starting to test these, they show the same trend as the genomic clones. 5) Other markers: Cruciferin and napin (from M. Crouch) are too polymorphic to reveal possible genomic relationships.

Transmission of alien chromosomes and effects on plant fitness: Most of these data have been collected from alien addition lines of *B. campestris-oleracea* derived from either natural *B. napus* and from Hakuran (synthetic *B. napus*). We observed that adding additional chromosomes to the diploid *Brassica* genomes had little effect on plant survival and fertility, although in some instances they retard development. The fertility of plants with hyperploid chromosome numbers was very high, ranging from 95 to 80%, while transmission had a wide range depending on the alien chromosome. The transmission of alien chromosomes after selfing was slightly lower than in backcrosses, which may be related to inbreeding depression. The hyperploids seem to have a mechanism of nondisjunction by which it is possible to obtain disomic lines from crosses between diploids and individuals carrying one extra chromosome. The resulting disomics transmitted the alien chromosome at a higher rate.

The high fitness of the hyperploids and alien addition lines indicates that interspecific aneuploidy may have played an important role in *Brassica* genome evolution. We know that amphidiploids occur in nature, and we know that these originate from unreduced gametes which are commonly observed in wild *Brassica* hybrids. Hence, it is feasible that sesquidiploid hybrids (interspecific triploids arising as a prior step to amphiploidy) may cross in nature to their diploid parents, thus generating interspecific aneuploids resulting in the addition (or deletion) of chromosomes to establish novel hybrid genomes. These aneuploids may be stabilized in a fashion similar to that observed in the experimental addition lines. By natural selection the successful genotypes may became established as species. Considering the differences disclosed by isozymes, RFLPs, and rDNA among the cultivated genomes, it is possible that they have hybrid origins in which more than one basic genome contributed chromosomes for the formation of new genomes. Thus, it is unlikely that the A, B, and C

genomes originated from a single ancestral genome or archetype in either ascending or descending fashion by merely gaining or losing chromosomes one at a time. Relic homologies detected by allosyndetic pairing (Prakash & Hinata, Opera Bot. **55**:1, 1980), RFLPs and chloroplast (cp) DNA markers (Palmer *et al.*, TAG **65**:181, 1983) may be explained by assuming that the A, B, and C genomes have a common or related ancestral parent as a chromosome donor. For example, the similarity of cpDNA in *B. oleracea* and *B. campestris* suggests that these share a cytoplasm donor species that may have been involved in their origin as the female parent.

Our long term objective is to gain insight into the mechanisms reponsible of the formation of the major *Brassica* genomes and their level of homology.

Rapeseed and Canola Breeding in North America: The US Potential

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The sight of a flowering field of rapeseed is a common site in many areas of the world. Rapeseed and Canola are currently grown on more than 20 million acres which produce 8 million metric tons of oil annually. This oil provides 15% of the edible oil and 100% of the high erucic acid industrial oil consumed worldwide. US Food & Drug Administration (FDA) regulations and a strong dependence on soybean as a source of vegetable oil have historically limited the production of oilseed rape in this country.

Since 1985 when the FDA granted GRAS status to low erucic acid rapeseed (Canola) oil, there has been increasing interest in both the production of this crop and utilization of its unique oil. Canola has low levels of saturated fatty acids, moderate levels of polyunsaturated fatty acids, and fairly high levels of mono-unsaturated fatty acids. Increasingly, the fatty acid composition of Canola oil has been recognized by nutritionists for its benefits in human diets. As domestic consumption of Canola oil increases, it is expected that US production of rapeseed will increase.

Three rates of potential increases of production of Canola in the US were graphed (Fig. 1). The first rate, which assumes that production will increase by only 250,000 acres annually, indicated that total production would approach 3 million acres by the turn of the century. The second rate, which assumes an annual increase of 500,000 acres, estimates that US acreage in the year 2000 would be 5.5 million acres. The third, and most optimistic, estimate assumed that US Canola production would increase by 1 million acres annually. At this rate of increase, there could be in excess of 11 million acres in production. Most individuals familiar with oilseed crops feel that Canola production in the US will increase, but they realize that many variables will influence the actual rate of increase. It is highly possible that US production of rapeseed could exceed 5 million acres if certain limiting factors can be solved.

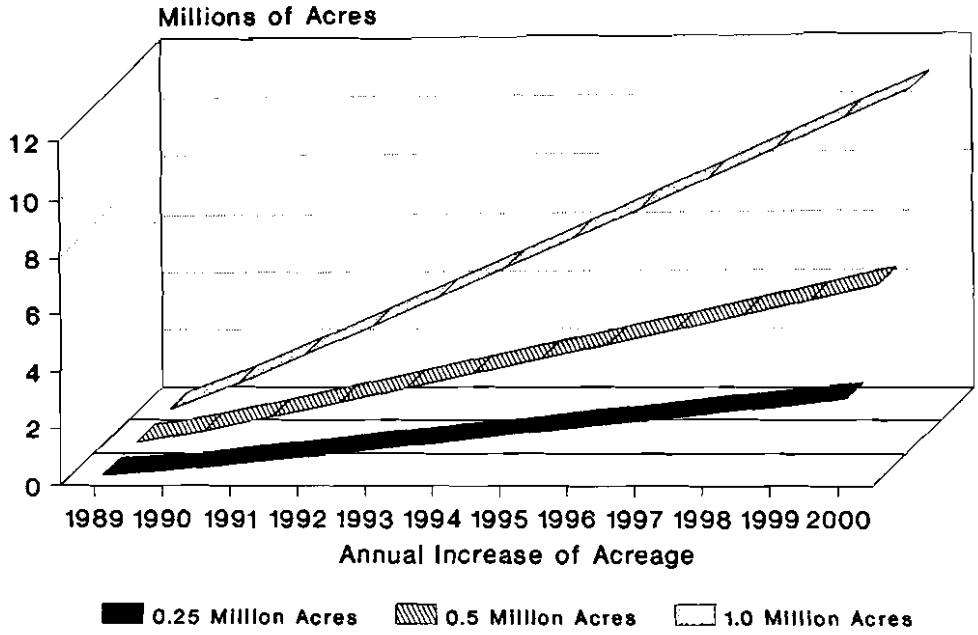

Figure 1. **Three potential rates of increased production of US Canola over the next decade.**

The factors which currently limit expanded production of Canola in the US are: 1) the US farm program; 2) the lack of proven varieties; 3) the lack of grower education; 4) the lack of vertical integration of Canola production into US vegetable oil industry; and 5) inevitable fluctuations in the price of all oilseed crops. Efforts are underway in both private industry and public institutions to solve these problems. This paper emphasizes the efforts currently underway to develop proven varieties of Canola.

Adaptation of US Canola. Much of the continental US will be able to produce Canola if adapted varieties are developed (Fig. 2). The northern tier of states will be able to grow spring cultivars, but high temperatures and pest problems will probably limit production of spring planted cultivars in the rest of the US. Very winter hardy biennial cultivars of either *Brassica napus* or *B. campestris* could be grown in much of the upper Midwest. These cultivars will require 6 to 8 weeks of vernalization. Typical European biennial cultivars, which require 4 to 6 weeks of vernalization, will be adapted across much of the Pacific Northwest and the midsection of this country. Winter annual cultivars, which require only 2 to 4 weeks of vernalization, will be adapted across southern California and the Southeastern states. In the Southern Coastal Plains, fall-planted spring Canola can be produced. In the southern regions of the US, production of Canola as a double crop will help improve the economic competitiveness of this US-grown oilseed crop.

Several Canadian and US organizations have initiated programs to develop Canola-quality cultivars adapted to the US (Table 1). Most of these firms are concentrating on *B. napus* and *B. campestris* as either spring or winter types. All of the firms indicated they felt there would be significant US production of Canola and that hybrids will be commercially available over the next decade. Many of the breeding programs are developing specialty oil types as well as Canola-quality cultivars. In addition, several firms are testing European-derived Canola cultivars for marketing in the US.

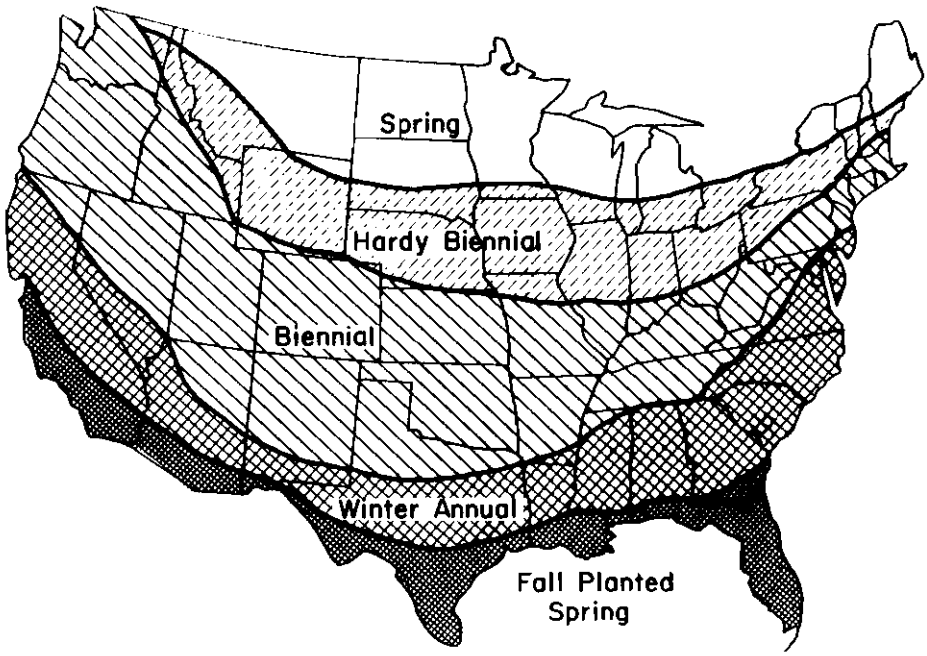

Figure 2. **Potential areas where different types of Canola-quality rapeseed would be adapted in the continental United States.**

Pests of US Canola. There are several pathogens, insects, and weed species which could limit US production of Canola (Table 2). Insects such as aphids, flea beetles, diamondback moths, and the cabbage seedpod weevil will be serious problems in most areas where Canola is grown. A broad range of plant diseases such as black leg, sclerotinia, black rot, and powdery mildew will be serious problems in much of the US. Both broadleaf and grass weeds will need to be controlled to insure successful Canola production except where establishment in the early fall will provide competition with weeds. The lack of registered pesticides will enhance the need for developing tolerance or resistance to as many of these pests as possible.

Table 1. **Organizations and descriptions of rapeseed programs developing cultivars adapted to the United States.**

Program	Contact person	Growth habit		*Brassica* species*				US test sites
		Spring	Winter	1	2	3	4	
Agrigenetics	L. Sernyk	X	X	X	X	X	–	25
Allelix	I. Grant	X	X	X	X	–	–	20
Calgene	M. Souero	X	X	X	X	–	–	42
Cargill Hybrid	A. Jarvi	X	X	X	–	X	–	7
Conti Seed	G. Buzza	X	–	X	X	X	X	6
Dahenfeldt	L. Lilley	X	X	X	–	–	–	–
DNAP	Z. Fan	X	–	X	–	–	–	21
King Agro	K. Kennema	X	X	X	X	–	–	7
Maccabee	D. Cohen	X	X	X	X	–	–	3
Monsanto	S. Metz	X	–	X	–	–	–	0
Pioneer	D. Gedge	X	?	X	X	–	–	2
Sigco	J. Fetch	X	–	X	X	X	X	9
U. Idaho	D. Auld	–	X	X	–	–	–	21

* 1=*B. napus*; 2=*B. campestris*; 3=*B. juncea*; 4=*B. carinata*

Table 2. **Insects, diseases, and weed species which could limit production of different types of Canola in the United States.**

Pests	Varietal Classification* 1	2	3	4	5	
Insects						
Flea Beetle	X	?	?	?	?	Planting date
Aphids	X	X	X	X	X	Widespread
Seed Pod Weevil	X	?	X	X	X	Potential
Diamond Back Moth	X	X	X	X	X	Location dependent
Maggots, Loopers, & Cutworms	X	?	X	X	X	Potential
Diseases						
Blackleg	X	?	?	X	X	Envir. dependent
White mold	X	X	X	X	X	Moist. dependent
Alternaria	X	?	?	X	X	Wet harvest
White rust	X	X	X	X	X	*B. campestris*
P. mildew	?	?	?	X	X	Dry conditions
Black rot	X	?	?	X	X	Temp. dependent
Nematodes	?	?	?	?	?	Sugarbeet
Viruses	?	?	?	?	?	Aphid trans.
Weeds						
Broadleaf	X	?	?	X	X	
Grasses	X	?	?	X	X	

* 1= Spring; 2=Hardy biennial; 3=Biennial; 4=Winter annual; 5=Fall planted spring

Conclusion. The US could become a significant producer of Canola over this next decade. The application of new techniques in cell biology and molecular biology in conventional plant breeding programs will allow the development of varieties adapted to most production areas in the US. Varieties adapted to the US will require both Canola quality characteristics and multiple-pest resistance.

Genome Analysis in Brassica Using RFLP Markers

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When nuclear DNAs of *Brassica* accessions are digested with restriction endonucleases, restriction fragment length polymorphisms (RFLPs) are generated for genotypes which vary in the position of enzyme recognition sites. Specific RFLPs can be detected using cloned DNAs as probes on Southern blots of total genomic DNA. These RFLPs represent genetic markers, and they can be used to analyze *Brassica* genomes in a variety of ways.

We have used RFLPs to study genetic relationships and evolution within *Brassica* and related genera. The *Brassica* genus is extremely polymorphic for restriction fragment lengths at all taxonomic levels, and this variation has allowed us to conduct detailed phylogenetic analyses. From these analyses and previous studies we

have proposed evolutionary pathways for the cultivated diploid species. We also obtained evidence for polyphyletic origins of amphidiploid species from diploid progenitors and for the influence of maternally contributed cytoplasms on genome change in amphidiploids. Within *B. oleracea*, *B. rapa*, and *B. napus*, we have used molecular markers to study the relationships among cultivated accessions from various subspecies.

Since RFLPs segregate as simple Mendelian factors, they can be used to develop linkage maps. We have constructed detailed RFLP linkage maps consisting of over 250 loci for *B. oleracea* and *B. rapa*. Many duplicated loci were mapped in both species, and the similar arrangement of these loci on different chromosomes suggests that these genomes have evolved in part by duplication of large chromosome segments. Conservation in the linkage arrangement of RFLPs detected by the same set of clones was observed between species, suggesting that *B. oleracea* and *B. rapa* have diverged recently.

RFLPs provide a powerful tool to study and manipulate genes controlling traits of interest. We have used mapped RFLPs to study genes controlling morphological variation in *B. oleracea* and *B. rapa*. In each species, F_2 progenies from a cross of a heading type by a broccoli type were analyzed for segregation of RFLP loci and for variation in leaf, stem, and flowering characteristics, pigmentation, and pubescence. We determined the location of genes controlling these traits, the magnitude of gene effects, and the types of gene action. Many of the traits we analyzed were controlled by genes having major effects, but genes having minor effects also were detected. We were able to dissect the genetic control of complex traits by identifying genes controlling specific components of the traits. For example, we identified several genes controlling different aspects of flowering response, such as requirement for vernalization, days to flower without vernalization, and retention of closed buds before flower opening. The duplication of trait genes within species was analyzed using duplicated RFLP linkage groups. We compared the organization of genes controlling analogous traits between species using RFLP makers with conserved linkage arrangements in the two species. These and similar types of studies may have applications for trait selection in *Brassica* improvement.

Haploidy and Cell Biology of Microspore Embryogenesis

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Anther and isolated microspore culture techniques have been used widely to support plant breeding activities for a variety of *Brassica* crops. There are several important criteria to be considered for reproducible and high embryo production from anther culture. The following have been found to be important variables in anther culture:

a) Genotype of donor plants. Embryogenic capacity of microspores varies greatly amongst cultivars and even

from plant to plant in heterogeneous, outcrossing populations, such as *B. oleracea*.

b) Stage of microspore development. The late uninucleate stage is usually best.

c) High temperature induction. The optimal temperature may vary between 30 and 35°C. The initial reaction of anthers involves a typical heat shock.

d) Duration of high temperature induction. The minimum is 12 h, but 1 or 2 days is usually best.

e) Physiological condition of donor plants. The requirements of the physiological conditions are dependent on species. Some species, *e.g.*, *B. napus*, respond well if the plants are kept at cool temperatures before anther culture. Others, *e.g.*, *B. juncea*, do not respond to low temperature treatment.

f) Composition of the anther culture medium. High levels of sucrose (*i.e.*, 8 to 13%) are essential for the induction of embryogenesis in anther cultures. Plant growth regulators, *i.e.*, auxins and cytokinins, are beneficial for some species such as *B. oleracea*.

Isolated microspore culture techniques have been well developed for *B. napus* but have worked less well for other species. Most factors relevant to anther culture also apply to isolated microspore culture. Excellent results can be achieved by maceration of selected buds or the whole inflorescence. Some cultivars require replacement of the medium to prevent the accumulation of inhibitory substances (1). Temperature requirement and medium composition may differ from anther culture. Optimal temperatures may be lower but may require a longer application. It may be necessary to add cytokinins to the medium.

The conditions required to optimize embryo production from microspore cultures of *B. napus* cv. Topaz have been well defined. Donor plants are grown at 10/5°C (day/night) in a 16 h photoperiod. The isolated microspores are cultured in darkness in a modified Lichter's medium (2, 3) with 13% sucrose at a density of 5×10^4 cells/ml at 32.5°C for three days followed by transfer to 25°C (darkness). It is possible to enrich for embryogenic microspores by careful sizing of bud length and by centrifugation on Percoll gradients. Microspores isolated from 2 to 3.5-mm-long buds and collected from the 24/32% Percoll interface gave the highest frequency of embryo formation, 14% (4). The microspores from longer buds (3.6 to 5 mm), collected from the same interface, produced embryos at a frequency of 8%. However, these two fractions produced morphologically different embryos. The embryos derived from the short buds had a normal root, cotyledon, and hypocotyl and could be easily regenerated into plants, whereas those from the long buds had abnormal cotyledons and only grew roots or malformed shoots. Staining of the microspore nuclei with DAPI (4,6–diamidino–2–phenylindole) showed that the microspores derived from the short buds were uninucleate and those from the long buds were binucleate.

Homogeneous and embryogenic uninucleate microspore populations such as those described above can be exploited to study the differentiation of single gametophytic cells to sporophytic haploid embryos. More specifically, the induction of gametophytic to sporo-

phytic cells can be examined. The induction of embryogenesis occurs during the 32.5°C heat treatment providing the culture conditions outlined above are met. However, if the microspores are incubated at 25°C, the cells continue to develop as gametophytes or pollen. The organization of the cytoskeleton in the developing pollen is typical of gametophytic cells. The microtubules are associated with the nuclear envelope of the uninucleate microspores. At the late uninucleate stage the nucleus and its associated microtubules migrate to the periphery of the cell. Mitosis ensues without a pre-prophase band, which is normally absent in gametophytic cells, and results in an asymmetrical division with a highly condensed generative nucleus and a decondensed vegetative nucleus. On the other hand, a few hours of heat treatment induces a pronounced reorganization of microtubules to the arrangement typical of sporophytic cells. The nuclear-associated microtubules are replaced by cortical microtubules in the interphase microspore. A pre-prophase band of microtubules occurs during prophase. Mitosis proceeds on a centrally located nucleus resulting in a symmetrical division and similarly condensed daughter nuclei. This rapid differentiation of microtubules from gametophytic to sporophytic organization occurs without a prior division. Furthermore, it is not known whether this process requires post-translational modification or the induction of new gene products. Further understanding of the inductive mechanism may provide insight for embryo induction from microspore cultures of recalcitrant species.

This highly embryogenic microspore culture has provided a system for mutagenesis and selection for herbicide resistance. N-ethyl-N-nitrosourea was used to mutagenize the microspore cultures and selection on the herbicide chlorsulfuron (Glean) produced calli and plants which were genetically stable mutants (5).

A technique for transformation of mature embryos with *Agrobacterium tumefaciens* has been developed by C. Phan (Hoechst Canada). After several seconds of chopping in a Waring blender the wounded embryos were co-cultivated with the wild type *A. tumefaciens*. Plants regenerated from embryo-derived callus tested positive for nopaline expression. This method of transformation is being used to obtain transgenic plants with plasmids carrying genes of agronomic importance. Concurrently, transformation of uninucleate microspores and early multinucleate embryoids by means of microinjection is being attempted (B. Huang, Allelix Crop Technologies, Inc.).

Developing, microspore-derived embryos are being used to study lipid metabolism (K. Pomeroy, Agriculture Canada). A dramatic increase in lipid coincides with the development of cotyledons which takes place between 10 to 14 days of culture or during the transition from the globular to the heart stage. This increase in lipid content is represented almost exclusively by the accumulation of triglycerides. Furthermore, the changes in the relative content of the different classes of triglycerides during the development of the gametic embryos parallels the changes in zygotic embryos. The microspore culture system may therefore provide a model system for lipid metabolism during embryogenesis.

The use of anther and microspore cultures continues to be a major activity for many *Brassica* breeding groups. Many oilseed rape breeders are using doubled-haploids extensively and some lines are in

advanced stages of field testing and may soon be released as licensed cultivars (S. Pleines, Hilleshög AB, Sweden). Field studies have shown that the majority of doubled-haploid lines produced from any given F_1 cross are not as useful as the parental lines for breeding but a small percentage appear to be better in many aspects, and there can be advances within a breeding program. Segregation of morphological and biochemical traits generally occurs as would be theoretically expected, however, the selection of some genotype may occur preferentially as a result of the tissue culture procedure. The degree to which plants represent a random sample of the genetic combinations of the parental material requires further study.

Anther and microspore culture techniques have great potential as a tool to support *Brassica* cultivar development and promise to be even more so with ever-increasing numbers of responsive species and advances in selection and molecular techniques for transformation.

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BRASSICA BIOTECHNOLOGY: STATE OF THE SCIENCE SYMPOSIUM II

Presiding:

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Organelle Manipulation by Cybridization: Methods, Results, and Applications

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Induced fusion of isolated protoplasts followed by regeneration of entire plants allows the mixing of cytoplasms and the screening or the selection of recombined forms of cytoplasmic genetic characteristics. These forms are called cybrids for cytoplasmic hybrids. Cybrids may result from either exchange of chloroplasts or mitochondria or recombination between parental organelle genomes.

The introduction of Ogura radish cytoplasm into *Brassica* is an example of an alloplasmic situation (in this case intergeneric) expressing cytoplasmic male sterility (CMS) together with side effects.

The most visible side effect concerns the vegetative organs which are chlorophyll deficient, a disharmony between *Brassica* nucleus and radish cytoplasm which is also found in the reverse combination, without male sterility, when radish nucleus and *Brassica campestris* cytoplasm are combined.

Other side effects concern reproductive organs. There is reduced nectar production and frequently narrower petals which result in the flowers being less attractive to bees and consequently a poor seed set for commercial use. Secondly, abnormal ovaries and external ovules are sometimes found, leading to an inability to develop seed at all. All these side effects rendered this cytoplasm of no practical value in hybrid seed production.

In rapeseed (*Brassica napus*) fusions were performed between a normal cultivar (the spring-type variety Brutor) and another line bearing the Ogura cytoplasm. The two parents differ by both mitochondrial and chloroplastic characters visible at the plant level (respectively male-fertile/male-sterile and green/chlorophyll deficient). Green male-sterile plants were picked from regenerated plants obtained without selection from colonies derived from a mixture of parental protoplasts treated with PEG as a fusogen. These plants possessed the *Brassica napus* chloroplast genome and recombined mitochondrial genomes.

Although these plants were identical concerning the chlorophyll content, they varied in flower morphology. Those having good nectar

production (80% of control fertile plants) and a normal seed set were chosen to introduce their cytoplasm in breeding programs by sexual crosses or by protoplast fusion. Among these new male-sterile cytoplasms, some are able to increase significantly the productivity of the variety.

The same cytoplasms selected in this experiment with *B. napus* were introduced in *B. oleracea* breeding programs by sexual crosses. In the new combinations so obtained (*B. oleracea* nucleus, *B. napus* chloroplast, and recombined mitochondria between *B. napus* and Ogura *Raphanus sativus*), some defects reappeared.

In the meantime, protoplast fusions were performed between normal *B. oleracea* (cv. Cavalier Vert) and *B. oleracea* with Ogura cytoplasm. The same screening method as previously used with *B. napus* allowed us to obtain ten green male-sterile plants from 600 regenerants. Mitochondrial DNA patterns of these cybrids showed a large variability resulting from intergeneric mitochondrial recombinations. Among these cybrids, some showed complete correction of all side effects and are very promising for F_1 hybrid production.

The results emphasize the practical importance of mitochondrial recombination for the improvement of alloplasmic male steriles. In the future it is possible to imagine the use of sources of CMS which are still unusable in agronomical conditions after recombination of their cytoplasmic information with the normal one.

Expression of Members of the S Gene Family

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The self-incompatibility sequences occur as multiple related copies in the *Brassica* genome. We have so far shown that two gene members of this family are expressed. One of these genes is the S-locus-specific glycoprotein structural gene and is referred to as the SLG gene. The other gene has been designated SLR1 and has been studied by postdoctoral fellow Beth Lalonde (Lalonde *et al.*, 1989). The expression of both of these genes has been demonstrated by the isolation of corresponding clones from stigma cDNA libraries, and the use of the respective cDNA probes has revealed similarities and also profound differences in the properties of the two genes. Both the SLG and SLR1 genes show the same general pattern of expression.

The transcripts encoded by these genes cannot be detected in leaf, seedling, or style tissue. They are, however, expressed in the stigma at levels which vary with the developmental stage of the flower buds, with maximal levels of S-transcripts attained at one day prior to flower opening in correlation with the onset of self-incompatibility in the stigma. *In situ* hybridization has shown that both transcripts are exclusively localized in the stigma papillar cells. Differences in the details of expression can be noted, however. Based on their relative representation in cDNA libraries, SLR1 transcripts are approxi-

mately seven times more abundant than SLG transcripts. In addition, and perhaps as a consequence of their higher abundance, SLR1 transcripts are first detected by *in situ* hybridization a full day or several buds earlier than SLG transcripts. Interestingly, stigmas also become competent in sustaining pollen tube growth at approximately the same early bud stage. More substantial and significant differences between the two genes are revealed by sequence and genetic analyses. The conclusion that the SLG gene was involved in determining S-allele specificity was based largely on the demonstration of allele-associated sequence variability and on the co-segregation of the corresponding restriction fragment length polymorphisms with the S-alleles in genetic crosses. In comparisons between different S-allele homozygotes, we had shown that SLG sequences encoded polypeptides that were at most 80% homologous in their most conserved regions and only 40% homologous in a variable region which we suggested to be a determinant of allelic specificity.

Extensive polymorphism and co-segregation with S-alleles has been documented for the S-locus-specific glycoproteins themselves and for the S-related DNA sequences revealed by hybridization with SLG-encoding cDNA probes. In contrast, the analysis of SLR1 sequences isolated from three different S-allele homozygotes has demonstrated that this gene is highly conserved and encodes identical proteins in strains that differ in their S alleles, and cannot, therefore, be a determinant of allelic specificity. This high degree of conservation is reflected in the limited restriction fragment length polymorphism exhibited by SLR1 sequences in a survey of a number of different S genotypes. A clear case of SLR1-associated polymorphism was, however, uncovered in a comparison of two lines of *B. oleracea*, a kale inbred homozygous for the S6 allele and a cabbage inbred homozygous for the S14 allele, and was used to demonstrate that the SLR1 gene lies outside the S locus.

The existence of the SLR1 gene had not been revealed by classical genetic studies. The demonstration that this additional member of the S gene family is also expressed suggests the existence of a family of related proteins in the *Brassica* flower. The primary translation products of SLG and SLR1 are 70% homologous and share common structural features which include the presence of a signal peptide, glycosylation, and the same precise arrangement of eleven cysteine residues at the carboxyl terminus of the molecule. We do not as yet know what the function of these proteins and their involvement in pollen recognition are. The extreme conservation of SLR1 sequences even in different *Brassica* species, and their expression to high levels even in self-compatible strains in which the SLG gene is either not functional or not expressed, suggests for the SLR1 protein product a fundamental role in pollination events in *Brassica*.

Spatial and Temporal Regulation of Genes Activated During Seed Germination

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Seed germination is a critical transition period in the sporophytic life cycle of higher plants during which quiescent embryos undergo a series of differentiation events resulting in the formation of viable young seedlings. Studies of germinating seed physiology have indicated that many of the processes associated with seedling growth are substantially different from those which characterize embryos (reviewed in Bewley and Black, 1983). A major objective of the research in our laboratory is to define the cellular processes that regulate the transition between embryogeny and germination. Our primary approach has been to study events intimately associated with seed germination, and we are studying these events primarily at the level of gene expression. The rationale is that defining the mechanisms involved in regulating genes that encode prevalent products during germination will provide information about the signals that initiate the transition from an embryonic to a postgerminative program of development. In this abstract, we discuss aspects of our work related to germination.

Our studies have provided support for the concept that differential gene activity underlies the morphological and physiological processes associated with germination (Harada *et al.*, 1988a). Specifically, we identified mRNAs, designated postgermination-abundant mRNAs, that are abundant in seedlings but are either undetectable or present at very low levels in immature embryos, dry seeds, and leaves (Harada *et al.*, 1988b). The patterns of mRNA accumulation suggest that the corresponding genes play a role in functions related to seedling growth. Thus, a major shift in the genetic activity of the sporophyte occurs during seed germination. Our current studies focus on two sets of postgermination-abundant mRNAs whose accumulation patterns appear to reflect differences in cotyledon and axis physiology in seedlings. One group of mRNAs accumulates primarily in cotyledons (cotyledon-abundant mRNAs) while another is seedling-axis abundant.

Analysis of the temporal patterns of mRNA accumulation showed that virtually all cotyledon-abundant genes are expressed similarly. The mRNAs begin to accumulate during late embryogeny, are most prevalent in seedlings, and are at low to undetectable levels in leaves. Because eukaryotic organisms often regulate batteries of genes involved in related developmental processes through a common mechanism, we speculated that the products of cotyledon-abundant genes could play a role in the mobilization of storage reserve macromolecules during postgerminative growth. Evidence to support this hypothesis was obtained by showing that mRNAs encoding isocitrate lyase and malate synthase, two glyoxylate-cycle enzymes involved in lipid mobilization, exhibit patterns of mRNA accumulation that are identical to other cotyledon-abundant mRNAs (Comai *et al.*, 1989). Thus, several aspects of cotyledon development during seedling growth may be regulated similarly. We have also obtained

evidence to suggest that isocitrate lyase and malate synthase genes are coordinately expressed by showing that the mRNAs display identical distribution patterns in seedlings imbibed to two days.

In contrast to the cotyledon-abundant mRNAs, we found that two axis-abundant mRNAs did not exhibit similar temporal accumulation patterns. The difference probably reflects the fact that the axis is a complex seedling part composed of several organs and numerous tissue and cell types. We have focused on one mRNA, designated AX92, that accumulates during early embryogenesis, is abundant in axes of seedlings, and is detected in mature organs of the plant (Harada *et al.*, 1988b). The gene is of particular interest to us because AX92 mRNA appears to accumulate tissue-specifically in the ground meristem and cortex of seedlings (Dietrich *et al.*, 1989). Distribution of the mRNA in meristematic regions of seedlings suggests that the gene is expressed during early stages of cortical cell differentiation. Because differentiation of cortical cells is thought to begin at approximately 7 days post-anthesis, the gene may be a useful marker for tissue differentiation events that occur early in embryogenesis.

Our current efforts are devoted to defining the cellular processes which activate genes encoding isocitrate lyase, malate synthase genes, and the cortex-specific mRNA. All three genes are expressed at high levels in seedlings, but they are also expressed during embryogeny. We believe that determining signals in embryos and seedlings will provide insight into the nature of regulatory events which control the transition between embryogeny and postgermination.

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Characterization of Brassica Seed-Specific Promoters and Their Expression in Transformed Rapeseed

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We are using transformation of *Brassica napus* as a tool to study elements which control gene expression in developing seeds and which can be used to regulate the expression of foreign genes in transgenic lines of rapeseed. Neutral lipid accumulation and storage protein synthesis are two developmentally regulated processes unique to the embryo tissues of *Brassica*. Our studies are currently concentrating on two marker genes for proteins associated with these slightly different phases of embryo development: acyl carrier protein (ACP) and napin.

Acyl carrier protein is an essential cofactor for fatty acid biosynthesis. We have isolated a cDNA clone for ACP that is expressed predominantly in the embryo, although some expression can be detected in leaf tissue. The timing of expression of this ACP gene in seeds closely follows the accumulation of neutral lipids. A genomic clone corresponding to the cDNA has been sequenced.

Napin is one of the two major classes of seed storage proteins in *Brassica*. Other groups have isolated and characterized cDNA and genomic clones from *B. napus* representing members of the napin multigene family. We have isolated a napin cDNA clone from *B. campestris* and sequenced the corresponding genomic clone. Transcription of the *B. campestris* napin gene (named "1-2") or its homologue in *B. napus* ("gNa") is roughly coincident with the peak of lipid synthesis, but is distinctly later than expression of the ACP genes.

DNA sequences flanking the coding region for napin in the "1-2" genomic clone have been used to express an ACP gene in transgenic *B. napus* var. Westar plants. Steady state levels of mRNA transcribed from the chimeric construct were quite high in embryos, but not detected in leaves of the transgenic plants. The level of expression of the gene ranges from 0.1 to 1.0% of the total mRNA. Endogenous ACP mRNA (for all ACP genes) in normal rapeseed embryos is less than 0.1% of the total mRNA, whereas the endogenous "1-2" napin mRNA is 1.0% of the total. Therefore, the level of expression of the chimeric gene appears to be controlled by the napin gene elements. The timing of expression during embryo development for the chimeric gene resembled that of napin, being slightly later than the transcription of the endogenous ACP genes in *B. napus*. All of our observations indicate that the tissue specificity, the timing of expression during embryo development, and the levels of steady state mRNA are controlled by the genomic promoter and transcription termination regions of the napin gene rather than the ACP coding region.

A comparison of the levels and timing of expression of napin ACP promoters is being more closely examined using the *E. coli* β-glucuronidase (GUS) as a reporter gene. Transgenic plant tissues can be easily assayed for GUS activity levels with high sensitivity, and

characteristics of individual napin and ACP promoters can be investigated independently from the other members of their gene families. In general, our results with chimeric GUS constructs suggest that a wide range of levels of gene expression are possible in transgenic lines. Expression levels from a single-gene construct differ widely between transgenic plants, probably due to chromosomal position of the genes after transformation. However, differences observed in endogenous levels of gene expression between the ACP and napin promoters are maintained in transgenic plants on the average. Currently, we are studying a surprising difference in expression between chimeric GUS genes transcribed from the two very similar "gNa" and "1-2" napin promoters. Expression of these two different constructs in transgenic plants may differ due to minor sequence heterogeneities between the promoters and/or translational effects resulting from small differences in the untranslated leaders of the constructs.

Our results make us confident that the regulatory elements from embryo-specific genes in *Brassica* can be adapted to achieve specific expression levels of either 1) genes from other sources encoding fatty acid biosynthetic enzymes that might modify oil content or composition or 2) antisense gene constructs designed to lower expression levels of specific endogenous genes normally expressed in rapeseed embryos (such as the glucosinolate biosynthetic enzymes or the desaturase responsible for linolenic acid synthesis).

Arabidopsis as a Model for Understanding the Molecular Basis of Fatty Acid Composition

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We have isolated a series of mutants of *Arabidopsis* with specific alterations in leaf fatty acid composition. These mutants have provided considerable information on the enzymology of desaturation and on the control of cellular lipid metabolism (Kunst *et al.*, 1988; Browse *et al.*, 1989). More importantly, the mutants have provided a means of directly addressing the question of how the fatty acid composition of thylakoid membranes affects chloroplast structure (Browse *et al.*, 1985; Hugly *et al.*, 1989). I shall briefly summarize our findings in these areas, but my main description will be of a new set of mutants we have isolated which have alterations in the fatty acid composition of their seed oils.

Many crop species produce seed oils in which the fatty acid composition is not ideally suited to the intended use. The application of conventional breeding methods, coupled with chemical mutagenesis, has resulted in the production of new varieties of several species with desirable alterations in the fatty acid composition of seed oil. A notable example is the development of low erucic acid varieties of rapeseed. Similar efforts have resulted in the reduction of the level of polyunsaturated 18-carbon fatty acids in soybean oil and linseed oil. Most of the genetic variation in seed lipid fatty acid composition appears to involve the presence of an allele of a gene which disrupts

normal fatty acid metabolism and leads to an accumulation of intermediate fatty acid products in the seed storage lipids. However, it seems likely that, because of the inherent limitations of this approach, many other desirable changes in seed oil fatty acid composition may require the directed application of genetic engineering methods. Unfortunately, as in many other aspects of plant biology, the lack of specific information about the biochemistry and regulation of lipid metabolism makes it difficult to predict how the introduction of one or a few genes might usefully alter seed lipid synthesis. An additional problem arises from the fact that many of the key enzymes of lipid metabolism are membrane bound, and attempts to solubilize and purify them from plant sources have not been successful.

The fatty acid composition of the *Arabidopsis* mutants are shown in Table 1. Our analysis of the mutants affecting fatty acid desaturation (*fadE*, *fadF*) and fatty acid elongation (*fae1*) revealed that the phenotype of each heterozygote was intermediate between the homozygous mutant and wild-type. This gene dosage effect indicates that over-expression of the wild-type genes (*fadE*, *fadF*, *fae1*) in transgenic plants would be a viable strategy for altering seed oil composition.

***Table 1.* Seed mutants of *Arabidopsis*.**

Mutant line	Gene symbol	Fatty Acid Composition 16:0	18:0	18:1	18:2	18:3	20:1	22:1
Wild type		9	3	15	29	20	21	3
JB9	*fadE*	6	3	57	1	6	22	4
JB21		9	3	31	20	15	18	3
BL1	*fadF*	6	3	19	47	2	17	3
JB20	*fae1*	10	3	24	37	24	2	0
JB11		10	3	13	24	27	20	3

The mutants will also make it possible to clone these genes. Because of its small genome size and low proportion of repetitive DNA, *Arabidopsis* has become a model for plant molecular genetics. A detailed restriction fragment length polymorphism (RFLP) map is available. This, together with cosmid or yeast artificial chromosome libraries, will make it possible to identify and clone the genes for which mapped mutants are available by chromosome walking. Alternatively, the transformation of *Arabidopsis* with components of the corn transposon system Ac/Ds now makes it possible to plan for the cloning of genes for which mutants can be isolated by transposon tagging.

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KEYNOTE SPEECH

Use of Cytoplasmic Male Sterility in the Production of Oilseed Rape F_1 Hybrids

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The CMS (cytoplasmic male sterility) system has the most promising future for F_1 seed production of oilseed rape. During the last two decades, several CMS systems were found from various crucifer species. Among the CMS systems, the *Napus* CMS system seems to have a high probability of success when applied to F_1 seed production systems.

The *Napus* CMS was found independently by Thompson in 1972 and Shiga and Baba in 1971 and 1973 in the progeny of intraspecific crosses. The *Napus* CMS system has the following advantages. 1) There are abundant fertility restoring varieties. This enables the breeder to select the C line which has the higher combining ability from a large population. 2) Its S cytoplasm is native to *Brassica napus*. The chlorosis in low temperature never occurs which is thought to be caused by interaction between exotic cytoplasm and nucleus. 3) The female fertility is high in CMS plants. 4) Selfed seeds can be obtained from the CMS plants. The *Napus* CMS plant can produce viable pollen under high temperature conditions. In Europe and Canada, the oilseed rape undergoes anthesis at higher temperatures and eventually produces pollen. Utilizing these phenomena, the new system for F_1 seed production was proposed. The F_1 seed is produced in Japan and Korea where the *Napus* CMS never produces pollen, and the seed from CMS plants is produced in Europe and Canada.

The disadvantage is that fewer maintainer varieties have been found. Thus breeding the maintainer variety is time consuming. An efficient procedure for breeding maintainers is proposed by the use of asymmetric fusion.

WORKSHOP SUMMARIES

I. Vegetable Crops Breeding

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Mike Dickson, Dept. of Horticultural Sciences, Cornell University reported on his work (Workshop Abstract #5) with CMS material derived from *Brassica nigra* × *B. oleracea* work initiated by Oscar Pearson some 20 years ago. He screened large F_2 populations seeking to improve through selection the poor seed set characteristics of the material. Nectaries have to be attractive in order to have bees work the flowers. Mike has released several broccoli lines. He anticipates the release of a cauliflower line very soon, with perhaps cabbage lines to follow. They might benefit from some testing in West Coast conditions. [A Sluis & Groot representative mentioned some chlorosis was observed in *Nigra* CMS material.]

Dickson also investigated black rot (*Xanthomonas campestris*) resistance in cauliflower and broccoli. The major source of resistance is PI 436606, a cabbage from China. The broccoli lines are advancing more rapidly than the cauliflowers. *Brassica napus* also appears to have a high level of black rot resistance with dominant gene action. He emphasized that juvenile resistance to black rot is separate from adult plant resistance, and that screening is necessary for both.

Virtual immunity with adult plants to lepidopterous insects occurs with the glossy dark green foliage character. There are some materials with normal foliage that give about 60% resistance which could reduce the number of sprays used for control. This work has lead to some serendipitous development of heat tolerance. Mike indicated there are some excellent sources of heat resistance in some broccoli lines, and that material from Wellesbourne lines has shown excellent cold tolerance.

Although the work has not continued, considerable variation for aphid resistance was observed. Considerable variation also was seen for thrip resistance. Few genes appear to be involved in that resistance, and the character might be breed for quickly. Some cultivars already seem to have a good level of resistance.

Interest remains strong in the exotic or specialty crucifers such as the orange-colored cauliflower curd. Mike was hopeful that private firms would increase seed supplies of these commodities as he has had a problem in responding to the demand for samples.

Hans Bongers outlined the breeding objectives for his firm, Nunhems Zaden BV (Workshop Abstract #1). The firm is involved in breeding hybrids of Brussels sprouts and broccoli using the SI system and breeding radish using CMS. Their preference is for a CMS system for all the crucifer commodities. In the future it may be possible to get the Ogura cytoplasm via protoplast fusion into *B. oleracea*.

Anther culture and microspore work is giving good results and partially replacing conventional programs. Doubled haploid production is becoming routine. He would like to see the development of more techniques for *in vitro* screens. Work concerned with transformation for certain characteristics such as insertion of a BT toxin gene is of high interest.

Programs are in place to find resistance to black rot, white blister, and downy mildew. Black rot is the most important crucifer disease problem in Holland. Seed assay is now routine. Black leg is occasionally a problem. A screen for ringspot, *Mycosphaerella brassicicola*, the second most important crucifer disease, is under development at Wageningen [Ernst van den Ende, the project leader for this research, briefly described that effort] as is continuing research for clubroot, *Plasmodiophora brassicae*. While clubroot is not of major importance in Holland, it is in other parts of Europe. However, it is sometimes a problem in Brussels sprouts production in Holland. Because resistance is polygenetic, progress for resistance breeding is not rapid. Mention was made about clubroot-resistant broccoli introduced by Baggett.

Resistance to other diseases, such as light leaf spot, white blister, *Alternaria brassicae*, and downy mildew is developing by means of field selection of tolerance. Downy mildew is more important in greenhouse production. *Alternaria* is difficult to work with and is a problem in seed production, because it can be seed borne. Wellesbourne attempted to develop a toxin screen for resistance, but the program was abandoned because of certain difficulties encountered.

Bongers concluded that there is a high priority given to seeking resistance to all pests and diseases because of increasing restrictions on use of agricultural chemicals. Breeding for insect resistance in Holland is for the moment relegated to public research institutions.

Following this presentation, general discussion brought out the following points:

- Relatively little effort is directed toward breeding for food quality characteristics; other problems command more attention.
- F_1 hybrids appear to be displacing open pollinated lines (OPs) for most crucifers, although the acceptance of F_1 cauliflowers is slow. One person stated that hybrids do not consistently outperform OPs, and the industry perception is that the hybrids are more "slot dependent" than OPs.
- Losses from black rot infections continue to occur. The disease is seed borne, hot water seed treatment is not totally effective, and early development in transplant can result in significant losses. Epidemiology is not completely clear, but weeds seem to serve as a source of primary inoculum in many instances. Black rot survival in soil is important in Holland as a source of inoculum. Seed borne black rot comes in earlier and thus is a greater problem in certain areas than other sources of inoculum. A research program for black rot control was recently begun in Wageningen.
- Mention was made that Osborn will be starting some work in Wisconsin to evaluate the usefulness of RFLPs in screening for resistance to black rot in broccoli and cauliflower.

- Another area of biotechnology contributing to breeding programs is anther culture with the expectation it will become even more important.

Joao Carlos da Silva Dias, Portugal, currently at the Dept. of Plant Pathology at the University of Wisconsin, discussed his work and that of Portuguese colleagues (Workshop Abstract #16) with Portuguese cabbages and kales. He has been expanding the Portuguese landrace collection of these crops. The kales are generally for animal feeding; the cabbages for human use. Cole crop consumption in Portugal is 70 kg per capita, and the Portuguese cabbages account for about 20 to 25% of that amount. These crops are found in Portugal and in other areas with strong Portuguese influence. The germplasm consists of many landraces with high variability within them. Silva Dias is using RFLPs and isozymes to characterize these lines. All accessions appear to have high levels of resistance to downy mildew, which appears to be effective across many races of the pathogen. All the Portuguese types are very susceptible to black rot. Some lines exhibit fusarium resistance. For most Portuguese crucifers, *Alternaria*, ringspot, and powdery mildew are major problems.

Rob Barham, Northrup King Company, discussed the importance of crucifer germplasm collections. A suggestion made during the last Crucifer Crop Advisory Committee meeting was presented to the workshop; that is, a bulk sample be generated of the different collections (*i.e.*, broccoli, cauliflower, etc.) by placing a sample of each PI in a field and allowing random mating. The suggestion was generally opposed because it was felt that it would be difficult to screen for given characters from such a diverse bulk sample.

Another suggestion receiving some, but less than enthusiastic, response was that seed firms should contribute to the regeneration of certain plant introductions in declining supply or quality by including these in breeding cages with noncrossing crops or in separate small cages. Some concern was voiced as to what would constitute a minimum number of plants; about 50 plants was suggested. Barham also summarized the recommendations of the Crucifer Crop Advisory Committee for plant breeding needs. The most important traits to evaluate in the germplasm collection include resistance to black rot, heat stress, lepidopterous insects, and aphids.

Jim McFerson USDA-ARS, Geneva Regional Plant Introduction Station, reported on the status of the Station's crucifer collection. Overall, it is in bad shape. He asked for advice and assistance from the private sector to improve the situation. Geneva, because of its climatic environment, is not a good location for increasing accessions. More than one-half of the material needs to be rejuvenated. He is now collecting data to get a more accurate picture of the condition of the collection. He is targeting the most important Plant Introductions for increase, and expressed a need for help from the seed industry with making some of the increases. The workshop participants expressed the view that the National Plant Germplasm System needs to be better funded so that routine operational and maintenance procedures are provided for adequate and accurate working collections.

II. Molecular Biology and Transformation

Chair:
S.G. Metz

Scribe:
J.M. Fedele

Monsanto Company
St. Louis, Missouri

Transformation:

Brassica napus: As reported by *S.G. Metz* (Workshop Abstract #12), explant source has been hypocotyls of week-old seedlings. Both kanamycin and gentamicin are useful selectable markers; successful selection results have been obtained with both. Hygromycin has not been evaluated, and direct glyphosate selection is not efficient. Transformation efficiency on a per explant basis is 0 to 5%. One, two, and more than two inserts have been used successfully. Regeneration frequency is 80%. Much of the work is subject to regulation. Field trials must be approved by the USDA in the USA and by Ag Canada in Canada. The bioengineered crop will need to be approved. Use of herbicide in the crop will need to be approved.

Brassica campestris: (See Poster Abstract: Facciotti *et al.*, p. 48) The same procedure as that used for *B. napus* will work (hypocotyls of week-old seedlings). Transformation efficiency on a per explant basis is 0 to 9%. Single and multiple insertions have been used. Regeneration frequency is 40 to 50%. This is not expected to affect the rate of transformation.

Brassica oleracea: (See Poster Abstract: Christey and Earle, p. 46) Explant source has been peduncle of Green Comet broccoli. There is a serious problem with explant hpersensitivity to *Agrobacterium tumefaciens*. With kanamycin (50 mg/l) as selection marker, there has been very low efficiency. Addition of Carb (Geopen) to media has helped. Cefotaxime is toxic to *Brassica* explants, but is used in common with vancomycin. There have been no problems with regeneration.

Molecular biology:

C. Makaroff reported on mitochondrial DNA alterations associated with male sterility in Ogura radish (Workshop Abstract #10). Three genes, *atp6*, *atpA*, and *coxI*, have been mapped near breakpoints of rearrangements in the Ogura genome relative to the normal radish genome; the three genes exhibit altered transcript patterns. Also transcribed with *atp6* is an Ogura-specific, 105 amino acid ORF. In most cases short, repeated sequences are found at rearrangement breakpoints. Three possible effects of the Ogura cytoplasm on cytoplasmic male-sterility (CMS) were noted: (1) the altered *atp6* could cause CMS since it is not completely functional; it cannot supply energy demands during pollen formation; (2) ORF 105 is a killer gene; (3) neither is involved in CMS.

E. Earle discussed novel mitochondrial genomes in somatic hybrids. Somatic hybrids of *Brassica napus* were synthesized, containing *B. campestris* chloroplasts and mitochondria from both parents. The mitochondrial genomes were altered; rearrangements were identified in *atp9*, *atp6*, *coxI*, and *atpA*. Novel fragments were found in the altered *atp9*; all versions had the same 11.0 kb fragment, some contained a 20.5 kb fragment. Some fertile plants produced the *ogura atp6* transcript.

IV. Molecular Markers and Gene Mapping

Chair and Scribe:
T.C. Osborn
Dept. of Agronomy
University of Wisconsin
Madison, Wisconsin

The general theme of this workshop was the development and use of molecular markers, both restriction fragment length polymorphisms (RFLPs) and isozymes, to study genome evolution and genetic control of traits in *Brassica*. The workshop began with a presentation by *Mitch McGrath* on the development and analysis of chromosome addition lines in *Brassica* (Workshop Abstract #11). He reported on the development of several types of addition lines with different genome constitutions and on the analysis of these lines with molecular markers in order to identify the chromosome composition of the lines and to assign markers to particular chromosomes.

The next presentation, by *Keming Song*, dealt with the use of RFLPs for *Brassica* taxonomy (Workshop Abstract #17). He discussed some of the basic considerations in using these markers for taxonomy and then presented data from analyses of cultivated and wild diploid *Brassica* accessions. These data were used to hypothesize the geographic dispersal of cultivated *Brassica* species. A phylogeny also was presented for oilseed cultivars of *B. napus*.

Following Song's presentation, there was some discussion on methodology for scoring and analyzing RFLP data in phylogenetic studies. It was noted that scoring RFLPs as unit characters (Song's method) does not provide as much information as other methods. But it also was recognized that this method is much simpler and allows for screening with many probes which would give better genome coverage and reduce potential bias of individual fragments in the pooled data set. *Suzanne Warwick* noted that there are many unknowns about evolution that affect assumptions related to methodology. She followed this discussion with some brief comments on her research using chloroplast RFLPs for studying *Brassica* taxonomy and evolution.

Scott Figdore presented comparative RFLP linkage maps for *B. oleracea* and *B. campestris* (Workshop Abstract #8). These maps were used to demonstrate the presence of duplicated regions within these genomes and the conservation of linkage arrangements between the genomes. Following this talk, there was discussion about the interpretation of data indicating duplicated RFLP loci. It was noted that one should first consider all duplicated loci in the genome to view the complete picture, then consider subsets that may represent specific evolutionary events. The need for uniform nomenclature of loci and chromosomes was mentioned.

The last presentation was by *Steven Knapp*, and it dealt with methods for analyzing trait loci using molecular markers (Workshop Abstract #9). He presented models based on the use of single markers and sets of linked markers, and demonstrated that the linked marker analysis was more powerful, especially for studies with widely spaced markers.

V. Disease, Pest, and Stress Resistance

Chair:
Richard L. Gabrielson
Western Washington Research
& Extension Center
Washington State University
Puyallup, Washington

Scribe:
G. Stern
Hortinnova Royal Sluis
San Juan Bautista, California

M. Dickson, Cornell University, Geneva, New York: In many places throughout the world, chemicals are no longer effective on lepidopterous insect species, *e.g.*, the diamond-back moth. At Cornell we have two programs in place to develop resistance to the diamond-back moth. Program I employs PI 234599, a glossy leaf cauliflower with complete resistance in the mature plant. It is susceptible during the first 40 days after seeding. Program II seeks to add resistance to "normal leaf" *Brassica oleracea*. There is partial resistance or tolerance in broccoli, cabbage, and cauliflower lines. Most of the F_1s made using these "normal leaf" lines have a rating of around 2 on a scale of infestation ranging from 1 (no infestation) to 5 (severe). Broccoli insect resistant lines may also have some degree of heat tolerance. Material is available by specific, written request to M. Dickson.

M. Whalen, University of California, Berkeley: I am looking for non-host resistance using *Arabidopsis* as host material. I have screened *Arabidopsis* ecotype Columbia (Col-o) for differential response to *Xanthomonas campestris* (Xcc) and *Pseudomonas syringae* (Psm). I found no differential response. I also screened 20 other ecotypes of *Arabidopsis* against Psm and still found no differential response. When I screened Col-o against *P. tomato* (Pst), I got a resistant reaction to Pst and a susceptible reaction to Psm. This ecotype is called Col (Psm(p10656.21)). I believe I have found an avirulent gene in this ecotype. I will be doing resistant × susceptible crosses to find out how the resistance mechanism works. When another fragment of the same size was inserted, either a null reaction or another ecotype with resistance was obtained. Five other ecotypes were thus obtained.

V. Shattuck, University of Guelph, Guelph, Ontario: TuMV is serious problem on rutabagas in southern Ontario. (In the next issue of Plant Disease one can find more details on this topic.) In Ontario the strains of TuMV on rutabaga are the Ontario strain and the Quebec strain (a new strain). Resistance to these strains was found in two fodder rutabagas 'Calder' and 'Sensation'. Resistance is governed by a qualitative trait conditioned by a few genes (less than four but more than one). The main rutabaga variety grown, 'Laurentian', is highly susceptible. Shattuck and cooperators have developed the Quebec 69 line. It is the first rutabaga line to be grown in presence of the C3 strain TuMV and cauliflower TuMV in Ontario. The resistance of the Quebec 69 line is expressed as no symptoms until the plant changes over to reproductive mode. The virus is thus present in a symptomless plant, but it never really creates a major problem. TuMV is a major problem also in canola, but can also affect rapeseed by altering the erucic acid and glucosynalate levels. Cucumber mosaic virus (CuMV) has not been seen in Ontario, but cauliflower TuMV has been. It was pointed out from the audience that CuMV cannot be distinguished from TuMV on rape by symptoms.

C. Thomas, USDA-ARS, Charleston, South Carolina: When we screened the USDA Plant Introduction Station *Brassica oleracea* collection for differentials that can be used for *Peronospora parasitica* (downy mildew), we found high levels of resistance in cabbage and Brussels sprout lines and very little resistance in broccoli, cauliflower, and kohlrabi lines. We want to develop a broccoli–host differential set, in which we can tell which race of mildew is present. Eventually, we will work with plant breeders to develop resistant broccoli, hopefully with some heat tolerance.

M. Ferreira, Dept. of Plant Pathology, University of Wisconsin, Madison: We have proposed a Phenotypic Recurrent Selection Program to develop *Plasmodiophora brassicae* (clubroot) resistance in *Brassica oleracea* (Workshop Abstract #7). We have the following current sources of resistance: cabbage–'Badger Shipper', 'OSU', and 'Resista'; broccoli–OSU lines; and cauliflower–'WaWi' and Chiang's lines. The problems with resistance to *P. brassicae* in *B. oleracea* are 1) polygenic inheritance and 2) plasticity of the pathogen (it can change to overcome resistance in a short time). The Program could involve three methods. (1) Resistant bulk seed would be sent to participants worldwide; participants would select for desirable traits *other than* clubroot resistance; participants would intercross the best plants and return seed from these; the returned seed from all participants would be bulked at the central station (the Crucifer Genetics Cooperative at the University of Wisconsin); the center would screen the bulk material for sources of resistance against a mixture of pathotypes, intercross the best plants, and bulk this seed (RRP); this bulk recombined seed would be sent back to participants; participants would reselect, bulk, and recombine the best plants and return seed to the central station. This cycle would be repeated until participants indicate that further selection is not needed. Our projection is that by March 1998, we could have the third generation of a clubroot-resistant population. We hypothesize that the population would have a wide base of resistance to clubroot. Since the bulk seed will have been selected for important morphological traits, this population should have desirable genotypic qualities. The advantage of this method is (a) that it eliminates the need for seed company participants to have pathologists present, (b) it eliminates the need to control environmental effects that may affect expression of resistance (as would be necessary if all screening were done at the central station), and (c) the resulting population would have been screened against all clubroot pathotypes. (2) This method is similar to the first except that pollen instead of seed would be sent to the central station. (3) This method is similar to the first except that the participants would also screen for clubroot resistance in addition to other characters.

Method 3 is probably undesirable since it could possibly mean developing a more virulent strain, all over the world. It would be better to do all clubroot resistance screening at the central station. The use of rapid-cycle genotypes as sources of resistance was not considered advantageous since it might be difficult to return to good agronomic type with the end population. However, it was pointed out that possible advantages of using rapid-cycle genotypes were: (a) they might provide a better idea of the type of inheritance involved; (b) the hypothesis could be tested in a shorter period of time; and (c) it

would only take two backcrosses to go from the rapid-cycle background to the cabbage agronomic type. It was further urged that rapid-cycle genotypes be considered, because (a) the actual length of time estimated for the Program might be underestimated; (b) if the resistance is being put back into different materials (cabbage, broccoli, cauliflower, etc.), there will still be appreciable backcrossing; and (c) eight years from now enough RFLPs will be sufficiently mapped to facilitate backcrosses.

Ferreira maintained that the primary opposition to use of rapid-cycle genotypes is that one is not exploiting the natural resistance already present in the nonrapid-cycle genotypes. Further comments from the audience included: (1) it might be better to keep the resistance distinct and separate, and distribute it worldwide; (2) a similar approach was tried with corn and worked well for a number of years, however, it ultimately fell apart due to a lack of stability between cooperators and the central station; (3) that if polygenic resistance is sought, a heterogenetic population is not useful. It would be better to just make resistant sources available so that breeders can select resistance to the desired clubroot race. While the proposal may claim to avoid requiring of participants the capability to screen, they will still need to ultimately, if they are putting resistance to a specific race into a specific type; and (4) the proposal seems worth committing to; it represents a beginning.

P. Williams, Dept. of Plant Pathology, University of Wisconsin, Madison: The National Vegetable Research Station at Wellesbourne recently surveyed landraces for clubroot resistance against a wide range of *P. brassicae* races and recommended the following group to be exploited: Bohnerwaldkohl, from Germany, the most resistant source; Binsachesener, from Germany; Horlensod; Ladoskaya, from Poland, nonspecific resistance; Vozazdinska, from Russia; and Badger Shipper.

VI. Hybrids and Population Improvement in Crucifers

No discussion summary was available. Workshop Abstracts #2, #6, #13, and #14 represent presentations made during this workshop.

VIII. Cell Biology

Chair:
P. Arnison
Paladin Hybrids Inc.
Brampton, Ontario, Canada

Scribe:
D. Simmonds
Plant Research Centre
Agriculture Canada
Ottowa, Ontario

P. Pauls discussed possible applications of microspore technology (Workshop Abstract #4). He pointed out that doubled haploid populations are very efficient for inheritance and selection studies because the phenotypes represent a gametic array. For example, erucic acid segregation in doubled haploid F_2 populations were compared. Selection for cold tolerance may be possible in microspore cultures. Embryogenesis in a spring-type *Brassica napus* ('Topaz') decreased when treated at -20°C. On the other hand, embryogenesis in the winter type 'Jet Neuf' was enhanced by a freezing treatment. The F_1 cross (Topaz × Jet Neuf) produced microspores in which embryogenesis was enhanced by a freezing treatment. Flow cytometry is ideally suited to examine the characteristics of microspores during culture. Measurements of cell size and viability can be made simultaneously, and populations that have a greater number of embryogenic cells can be sorted.

L. Holbrook compared gametic and zygotic embryogenesis. He pointed out that evaluation of lipid content by histological staining was unreliable due to the variability in the microspore cultures.

P. Arnison commented on the use of embryo rescue techniques for interspecific hybridization between different *Brassica* species. This approach has been quite successful for the production of hybrids that are not easily achieved by normal sexual means, *e.g.*, *B. napus* × *B. oleracea*, *B. juncea* × *B. campestris*, and *B. juncea* × *B. napus*. Hybrids with $2n=31$ and $2n=43$ chromosomes were recovered. All F_1 hybrids were male sterile. The backcross to *B. napus* followed by embryo rescue resulted in plants with $2n=50$ chromosomes and some pollen fertility. Further backcrosses have greatly restored fertility.

Similar results have been reported by M. Chèvre *et al.* (Workshop Abstract #15). Hybrids have been recovered for somatic hybridizations as well as by embryo rescue. The occurrence of a mechanical female sterility in *B. napus* plants derived originally from anther culture was reported.

IX. Genetics Conservation and Gene Banks in Cruciferae

Chair:
P.H. Williams
Dept. of Plant Pathology
University of Wisconsin
Madison, Wisconsin

Scribe:
P.E. McGuire
Genetic Resources
Conservation Program
University of California

P. Williams: There are over 3,000 species of Cruciferae; who is concerned about conservation of these, who should be concerned? The International Board for Plant Genetic Resources (IBPGR) presented a global mandate for vegetable crop genetic resource conservation, but it is now more than ten years old. An IBPGR report on crucifer crop resources was published in 1980. It identified priority regions for collection and categorized taxa into oilseed and vegetable crops. Primary repositories were to be in centers of primary and secondary diversity with a duplicate in a second site. For example, for leafy vegetable *Brassica* taxa, the primary repository is in Taiwan and the secondary site is the United Kingdom. There are other IBPGR-designated repositories for crucifer material in Beijing, New Delhi, Ottawa, Wellesbourne, Wageningen, Madrid, Addis Ababa, Braunschweig, and Leningrad. There is no IBPGR-designated crucifer repository in the United States. IBPGR has no collections of its own. It can activate advisory groups for specific problems or regions. IBPGR sponsors collections following the priorities that were set out. The procedure followed for such collections is to leave one half of the material in the host country if there is a storage facility. If there is not, IBPGR will work to establish one. There has existed an *ad hoc* IBPGR committee for crucifers since 1980.

S. Kresovich, National Plant Germplasm System, Regional Plant Introduction Station, Geneva, New York: Long-term seed storage in the US is at the National Seed Storage Laboratory (NSSL) in Fort Collins, Colorado. It is also a backup site for IBPGR-national collections. Currently funding for preservation is not the crucial issue. For the three to five thousand accessions possessed by the USDA, the issues are quality and viability. The past history of the collection has been problematic. There are cases where the names associated with the Plant Introduction numbers do not correspond to the material now identified by those numbers. In the past, there was an attempt to make the USDA collection represent a world collection. Perhaps this is not necessary, if a good network exists among sites throughout the world. There is increasing interest in the USDA collection from plant biologists and evolutionary biologists. There is also a research effort at Geneva that is need oriented. Operations are in a rescue mode now and evaluations and enhancement activities are not possible. There is an ongoing attempt to delete redundant material and fill in gaps; however, redundancy was the least of their problems.

In response to questions from the audience, Dr. Kresovich noted that there is a role for groups like the Seed Savers Exchange and the Center for Plant Conservation in the large picture of plant genetic resources conservation. Within the limits of the databases available to him, his unit can direct researchers to other crucifer collections in the world for given materials. He expects greater future interaction with the Crucifer Crop Advisory Committee (Crucifer CAC). P. Williams, chair of the Crucifer CAC, said it will soon be assembling its first report which will document world locations of crucifer genetic resources.

The comment was made that characterization of accessions is important especially for wild species, since they can be difficult to maintain. Dr. Williams responded that all information even if anecdotal was important. He noted that the National Plant Germplasm System (NPGS) was being rejuvenated. The NPGS was reviewed by the Government Accounting Office (GAO) ten years ago, and now the GAO is initiating a ten-year-retrospective review.

Other collections of crucifer germplasm were described. Guy Baillargeon, a taxonomist with the Canadian Government maintains a collection of *Sinapis*, primarily from North Africa, now numbering 1,400 accessions. It includes representatives of the other genera in the Brassicae tribe as well. The Canadian system is in the process of computerizing its inventory, and it should be on line next year. It was noted that an ecotype collection of *Arabidopsis* is maintained at Frankfurt-am-Main, Federal Republic of Germany by the *Arabidopsis* Information Service. Dr. Williams mentioned the Crucifer Genetics Cooperative, which has 1,500 participants, maintains a collection of 150 stocks and serves as a repository for public genetic stocks. He also noted that there are three major repositories in Australia. A representative of the Volcani Center, Israel noted that the gene bank in Israel is rich in primitive varieties and landraces, but that crucifers are not very important. Those that are there are resistant to drought, salinity, and high pH. While European varieties of *Sinapis alba* typically have 40% erucic acid, wild-collected accessions have 48 to 56% erucic acid in seeds. There appears to be a trend; the hotter the place of collection, the higher the erucic acid composition.

An assertion was made that symbionts including pathogens should be collected along with genetic resources. A collection of pathogens of Dutch cabbage is maintained in the Netherlands.

R. Prescott-Allen noted that reserves or protected areas could maintain genetic resources of interest to crop breeders; however, very few such areas have genetic resources protection as their mandate. He would hope to encourage such management in the future. There should be lists made and protocols developed for managers. Currently only in the German Democratic Republic is there any link between *ex situ* and *in situ* maintenance of genetic resources.

Dr. C. Gómez-Campo, Universidad Politécnica, Madrid, Spain reported that the Red Data Book of endangered species for Spain and the Canary Islands is now completed. He considers *ex situ* conservation as an emergency measure. Two species have been saved by *ex situ* conservation; they would be extinct if they were still waiting for *in situ* conservation. In the 200 accessions held in botanical gardens, 20% identification error was found at the species level, 10% at the genus level. Botanical garden holdings account for about 10% of the native flora.

J.C. da Silva Dias, Instituto Superior Agronomia, Lisbon, Portugal reported on the unique situation in Portugal with respect to cabbages and kales. There is no active breeding, and there is high demand for old familiar types which are proving to possess resistance to important diseases. His report is included as Workshop Abstract #3.

WORKSHOP ABSTRACTS

1. CRUCIFER VEGETABLE BREEDING PROGRAM

H. Bongers, Nunhems Zaden BV, Haelen, Holland

Breeding Objectives: Active breeding programs are operated with the following crucifers: Brussels sprouts, broccoli, and radish. For each commodity the intention is the development of hybrid cultivars. The Brussels sprouts and broccoli hybrids are obtained by self-incompatibility and for radish via the use of cytoplasmic male sterility, (CMS). We would prefer a CMS system over self-incompatibility in the Brassicas. In the near future it may be possible to get the Ogura cytoplasm via protoplast fusion in *Brassica oleracea*. Techniques: Anther and microsporeculture – This technique, to obtain rapidly homozygous lines, gives good results and will partially replace the conventional breeding program relatively soon. Micropropagation is possible by means of bud-culture. Where transformation is considered (for instance a BT toxin gene), regeneration systems are achievable. Disease Resistance and Screening: *Xanthomonas campestris* (blackrot) – The detection method for this bacteria is now a routine analysis. *Mycosphaerella brassicicola* (ringspot) – Research is needed for a reliable artificial test for resistance breeding. *Plasmodiophora brassicae* (clubroot) – Research project ongoing at the I.V.T., Wageningen. Resistance to other diseases, such as light leaf spot, white blister, *Alternaria brassicae*, and mildew is developing by means of field tolerance selections. Because of the number of permitted insecticides and fungicides will decrease, we are giving a very high priority to breeding for resistance or tolerance. For this purpose we need techniques for *in vitro* resistance screening.

2. COMPARISON OF MICROSPORE-DERIVED AND SINGLE SEED DESCENT LINES OF OILSEED RAPE (*BRASSICA NAPUS*)

D. Charne and ***W.D. Beversdorf***, University of Guelph, Guelph, Canada

Doubled haploid lines (MD) were produced through microspore culture, and single seed descent lines (SSD) through self-pollination, from two crosses of spring-type *Brassica napus* (Westar/Topaz and Regent/Westar). All lines were evaluated in a replicated test at Elora, Ontario in 1987 and 1988, and data collected for days to flowering and maturity, lodging, and seed yield. Oil and protein contents were also determined on a sample of seed from each plot. Means and variances of MD and SSD progenies were compared within each cross for each parameter. The normality of distribution of each trait was also assessed, and the shapes of distributions compared using a (nonparametric) Mann-Whitney U Test.

There were no significant differences in variance between the MD and SSD progenies in either cross for any of the traits measured. Significant differences in means were detected in two cases: in the Westar/Topaz progenies, the mean protein content of SSD lines was significantly greater ($P<0.05$) than that of MD lines (48.9% vs. 49.2%), while in the Regent/Westar progenies, MD lines showed significantly less lodging ($P<0.01$) than did SSD lines (2.3 vs. 2.6 lodging units, where 1 is upright and 5 is prostrate). These differences were also detected as differences in the shapes of the distributions. These results demonstrate that doubled haploids produced through microspore culture represent a similar sampling of recombinant as do SSD lines, offering similar scope for genetic improvement. This should make haploidy especially attractive in breeding winter-type *B. napus*, where the use of microspore culture can allow a substantial reduction in the length of the breeding cycle.

3. PORTUGUESE CABBAGES AND KALES AS GENETIC RESOURCES FOR DISEASE RESISTANCE

J.C. da Silva Dias[1,2], *A. Monteiro*[1] and *P.H. Williams*[2], [1]Instituto Superior Agronomia, Tapada da Ajuda, 1630 Lisboa-Portugal and [2]Dept. of Plant Pathology, University of Wisconsin, Madison

Portuguese cabbages also known as: couve tronchuda, tronchuda, true couve tronchuda, tranxuda, tronchuda kale, Portugal cabbage, dwarf Portugal cabbage, Portugal (sea) kale, sea kale cabbage, large-ribbed or large-veined cabbage, veined cabbage, and Braganza cabbage (English); chou tronchuda, chou (blond) à grosses côtes, chou à large côtes, chou de Beauvais (French); are classified synonymously as *Brassica oleracea* var. *tronchuda* Bailey or *B. oleracea* var. *costata* DC.

Portuguese cabbage is a larger heterogeneous group where the most important landraces or ecotypes are: 'Penca de Chaves' 'Penca de Mirandela', 'Penca da Povoa', 'Couve Gloria de Portugal', 'Couve de Valhascos', 'Couve Portuguesa', and 'Couve Murciana'. As a rule all these landraces are annual plants, with white flowers and characterized as not forming a true head. Rather, the outer leaves fold in and the inner leaves overlap to form a loose pseudohead. Surrounding the pseudohead is a rosette of large, well-developed leaves, with thick petioles. The outer leaves and those of the pseudohead are heavily veined. Landraces differ in ecological adaptation, growth cycle, and morphological characters such as color and shape of the leaves and the size of the veins, the head, and the plant.

One little-grown landrace which is increasing in production yearly is 'Couve grelo', a sprouting cabbage requiring no vernalization. Couve grelo produces no head but the young flowering shoots are consumed as "Portuguese broccoli". Other interesting types are 'Coivao' and 'Couve calcuda' or 'Couve de Condeixa', both with long stalks and a pseudohead requiring more than one year to complete their reproductive cycles. The diversity among types of Portuguese cabbages is sometimes so great that mainly along the central coast region one can find a different landrace in each village! With this great heterogeneity it is not surprising to find that authors differ in their opinions about the classification of the Portuguese cabbage group. Portuguese cabbages appear to be an intermediate group between heading cabbages and the kales with many intergradations.

Most of the known landraces of Portuguese cabbage are used for human consumption. The pseudohead and the outer leaves are very tender. The cabbage is used in soups and several dishes. It is very popular to eat the cabbage with cod at the Christmas season.

'Couves galegas' or Portuguese tree kales (*B. oleracea* var. *acephala* DC) are headless plants, usually with large leaves, having long petioles and a single indeterminate woody stalk that can grow perfectly straight, sometimes reaching more than 2 m in height before bolting. Leaves are picked singly during the period of stem elongation and used for animal and human consumption. Kale leaves are used in the traditional soup "caldo verde". The tree kales are also highly variable with several forms of smooth and curled leaf types. Among the other kales are 'Couve poda', a branching kale that does not flower, and 'orelha de mula', a type with large spatulate leaves, very distinct from other kales. Couve galega is grown throughout Portugal but is mainly found in central and northern parts and in the suburbs of large cities, such as Lisbon and Porto, where it is grown in thousands of small vegetable gardens.

As part of a national program on germplasm preservation, we began two years ago collecting landraces of the Portuguese cabbages and kales. A number of them were in danger of extinction because they were being replaced by other Portuguese cabbages or by imported cabbages. There is no national breeding program for the Portuguese Brassicas which are only traditionally consumed by Portuguese people or in areas of the world with strong Portuguese influence. Because of the limited market potential, commercial foreign seed companies have not been interested in improving the Portuguese types.

Portuguese cabbages and kales have been under cultivation in Portugal perhaps since Roman times or earlier. They were among the first vegetables introduced to the western Iberian Peninsula. For centuries *Brassica* populations have been improved and selected by farmers. Until the present, almost no scientific breeding has been carried out and thus a high degree of morphological and physiological variability can be found among the landraces. For this reason the Portuguese landraces were regarded as an important and promising gene pool for breeding. A survey of some representative landraces for disease resistance, by Monteiro & Williams (1988), confirmed that Portuguese cabbages and kales are an important source of potential resistance against downy mildew, clubroot, and Fusarium yellows. This survey is being continued on other landraces of Portuguese Brassicas, including 'Couve do Algarve', a Portuguese variety of *B. oleracea* var. *capitata* with compact head and yellow flowers which is almost extinct.

4. FLOW CYTOMETRIC CHARACTERIZATION OF *BRASSICA NAPUS* MICROSPORE DEVELOPEMNT IN THE ANTHER AND IN CULTURE

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Microspore culture is an important technique used for the production of doubled haploids in rapeseed breeding programs. The developmental stage of the microspore is an important determinant of success in this procedure. Only microspores in the late uninucleate stage respond to the culture conditions and develop into embryos (Kott *et al.*, 1988, Can. J. Bot. **66**:1658). By flow cytometry the fluorescence and light scatter properties of large numbers of cells can be determined and correlated. This technique has been applied to characterize changes in the properties of microspore cells during their development in anthers and in culture.

Microspores from *Brassica napus* (cv. Topaz) buds at an early stage (1.5.to 2.0 mm), middle stage (2.75.to 3.0 mm), and a late stage (3.5.to 4.0 mm) of development were isolated and examined by flow cytometry. Maturation of the microspores was accompanied by changes in their forward angle light scatter (FALS) and 90° light scatter (90° LS) properties. These measurements are related to the cell size and cell granularity (density), respectively. In particular, samples of cells from young buds had two populations of

cells that could be distinguished on the basis of their 90°-LS properties. Sorting experiments showed that the population with high 90°-LS values consisted predominantly of tetrads. The population with lower 90°-LS values consisted of single cells, presumably freshly released microspores. In samples from larger cells the 90°-LS profile contained only a single peak. However, in these samples the FALS signal was split into two. The two populations represent microspores at different stages of maturation. This study should allow signposts of microspore maturation to be defined that will be used to rapidly assess the effects of environment and genotype on the development of microspores in donor plants for microspore culture.

Although the microspore culture procedure is very productive (several hundred embryos can be produced from a few flower buds), only a small fraction of the potential is realized because only a few percent or less of the microspores that are cultured develop into plants. By far the highest loss occurs during the first few days of microspore culture. The changes in cell viability and cell size in the microspore cultures have been examined by flow cytometry. For these experiments the microspores were stained with the vital stain fluorescein diacetate (FDA) and the light scatter and fluorescence characteristics of freshly isolated microspores (day 0) and microspores that had been in culture for 1, 3, 5, and 7 days were measured. Two populations of cells were distinguished on the basis of correlated light scatter and fluorescence measurements; one which contained cells that were nonfluorescent (dead) and made up 30 to 50% of the total and a population of fluorescent (live) cells. Over the culture period the average size and fluorescence of the live cells increased, but the number of live cells declined. By day 7 only 1% of the microspores that were placed into culture remained alive. The pattern of change is sensitive to environmental influences like cold treatments of microspore cultures. Sorting experiments to isolate large fluorescent cells on day 1 or day 3 resulted in microspore preparations that were enriched in cells committed to embryogenesis compared to a manually plated mixture of stained cells. The flow cytometric procedures allow quantitative assessments of cell structure and function to be made which may have application in studies of plant development and improvement of haploidy procedures.

5. CURRENT VEGETABLE CRUCIFER BREEDING PROJECTS

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Male sterility has been around for 20 years in crucifers, but so far has not become important. Recently we have returned to working with *Brassica nigra* cytosteriles, originally developed by Oscar Pearson. The need was for better seed set and better floral formation. I used large F_2 populations (3000) per crop (broccoli, cauliflower, and cabbage) to try to obtain desirable horticultural traits, good seed set, and normal flower formation. We have released broccoli lines and may soon release a cauliflower line. The Ogura or Raphano-cytosteriles looked promising, but because of the low temperature chlorosis have been set aside. However, it now appears these problems are being overcome by protoplast and mitochondrial manipulations.

6. HYBRIDS AND POPULATION IMPROVEMENT

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Significant high-parent heterosis has been demonstrated in oilseed rape (*Brassica napus* L.) in several recent studies. The development and commercialization of hybrid cultivars could thus allow a substantial increase in both per acre yields and producer returns. A successful hybrid development program has three major components: population improvement, inbred production and testing, and hybrid synthesis and evaluation. The following is a summary of hybrid development strategies being employed by Allelix Crop Technologies in both spring and winter *B. napus* and spring *B. campestris*.

Source populations for inbred extraction were synthesized initially using elite breeding material and conventional cultivars. The performance of these populations for agronomic and quality traits and disease resistance is being improved using both intra- and interpopulation improvement procedures. With both approaches, S_1 and testcross progeny are produced and evaluated, allowing an assessment of both per se performance and combining ability. By using an off-season nursery, and recombining superior S_1s, we are able to complete one cycle of selection per year using this strategy.

Inbreeding is continued in selected families, with expanded testing of progeny produced by crossing S_3 or S_4 lines with one or two inbred testers. Elite S_4 or S_5 lines are intercrossed in a diallel or factorial design, in order to identify both good general combiners and specific, high-performing hybrid combinations. The latter are multiplied, then evaluated in replicated, multi-location tests in order to identify hybrids with sufficient merit for entry into licensing trials.

Introgression of s-alleles or cms cytoplasm/restorer genes into inbreds begins at the S_4 level and continues with three or four backcrosses to the recurrent parent. This allows conversion of superior inbreds to incompatible or male-sterile forms within two years, so that a hybrid is in its finished form at such time as it enters government testing. We use elite inbreds from Cycle 1 populations as testers in successive cycles of selection. For interpopulation improvement, we also use a bulk pollen sample from the opposite population as a broad-based tester.

7. *PLASMODIOPHORA BRASSICAE* RESISTANCE RECURRENT SELECTION PROGRAM (PBRRSP)

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During the session on Disease Resistance and Screening at the Crucifer Genetics Workshop IV, held at the University of Wisconsin, Madison, October 12-14, 1987, part of the discussion was centered on the urgent need to initiate a global research effort to develop broad-based durable resistance against the devastating effects of clubroot, caused by

Plasmodiophora brassicae. A collaborative international program involving private and governmental research institutions engaged in recurrent selection for resistance to *P. brassicae* was proposed. We plan to initiate and coordinate this program to develop resistance to a wide range of *P. brassicae* pathotypes into populations of various *Brassica* species.

The program would be structured as follows. *Brassica* cultivars and landraces regarded as the most effective sources of resistance available would be used to generate the cycle zero (C0) population (within *B. oleracea* this would include, for example, Bohmerwaldkohl, Bindsachsener, Badger Shipper, Ladoskaya, Vazazdinsko, Petibor, Horlensod, etc.). These resistant pollen recipients would have been screened in the Crucifer Genetics Cooperative (CrGC) facilities against a representative mixture of isolates from the localities of participants in the PBRRSP and from the CrGC *Plasmodiphora brassicae* collection. Researchers from different regions and countries would collect pollen from plants of their most desired locally adapted cultivars. The pollen of these cultivars would be sent to the CrGC – University of Wisconsin – Madison, where it would be used to pollinate the resistant plants mentioned above. The seeds harvested would constitute the C0 population, and they would be bulked, sampled, and sent back to the participants who would initiate a new cycle of selection in the field. Samples of seed would also be stored at the CrGC for later comparison with the populations developed in each cycle of selection. Subsamples of the seed would be grown to identify resistant plants to receive pollen coming from the participant sites in the next cycle. This recurrent selection program would continue as long as it was useful to cooperating participants.

We will discuss the details of this and two other alternative phenotypic recurrent selection schemes with those interested in becoming involved with the program. A model of the program from 1989 to 2000 will be presented. This approach to developing a pool of *P. brassicae* resistance from a broad germplasm base would be relatively easy to accomplish. The program would lead to the development of populations of several *Brassica* species with high levels of resistance to a range of pathogenic variation in *P. brassicae*.

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8. COMPARING THE LINKAGE ARRANGEMENT OF RFLP LOCI AMONG POPULATIONS GENERATED FROM VARIOUS *BRASSICA* SUBSPECIES AND SPECIES CROSSES

S. Figdore, M. Slocum, L. Morrison, and *J. Suzuki*, NPI, Salt Lake City, Utah

Using a common set of genomic cloned fragments derived from the cauliflower accession 'Early White', we have constructed RFLP linkage maps in four different F_2 segregating populations generated from crosses involving different *Brassica* subspecies and species. The use of a common set of probes in these mapping efforts has permitted a comparison of the linkage arrangement of RFLP loci in different accessions, providing preliminary insight into genome structure and assessment of the degree of similarity/dissimilarity among the different germplasm sources examined.

A majority (92%) of the probes we identified as useful in detecting RFLPs in *EcoRI*-digested parental DNA accessions hybridized to multiple (>2) restriction fragments when filters were washed in 0.25× SSC and 0.1% SDS at 60°C. Segregation analyses of multiple fragments demonstrated the presence of duplicated loci, located primarily between linkage groups. A strong conservation of the relative linkage arrangement of clusters of duplicated loci was frequently observed among linkage groups, and a conservation of the arrangement of duplicated loci was often observed between populations examined in this study.

A variety of genomic rearrangements were detected among the parental accessions examined. The hybridization patterns of some clones supported the presence of deletion/insertion type rearrangements, duplications, inversions, and translocations. Differences in the linkage arrangement of loci in *B. oleracea* versus *B. campestris* accessions were detected, and could be associated with the generation of 9 versus 10 linkage groups, respectively, in maps of these different species. Differences in the linkage distances between loci in the various populations were also observed. The significance of these findings will be discussed, particularly within the context of the practical usage of these molecular markers in developing linkage associations with quantitative trait loci and other aspects of applied breeding programs.

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9. QUASI-MENDELIAN ANALYSES OF QUANTITATIVE TRAITS USING MOLECULAR MARKER LINKAGE MAPS: A WORKSHOP EMPHASIZING USES OF DOUBLED HAPLOID PROGENY IN RAPESEED

S.J. Knapp, Dept. of Crop Science, Oregon State University, Corvallis

Classical quantitative genetic methods enable estimating and testing hypotheses about genetic variances, heritability, and other population parameters. Quantitative genetics has lacked widely applicable methods for testing hypotheses about the effects of quantitative trait loci (Q loci), so it has been impossible to investigate questions about loci underlying continuous or complex discrete phenotypic distributions. The limitations of classical experimental systems are overcome by molecular marker linkage map experimental systems. Segregating codominant marker loci are used in the latter to estimate the effects of linked Q loci. The idea of using segregating marker loci to make inferences about linked quantitative trait loci is an old one; however, the development of saturated restriction fragment length polymorphism linkage maps and sophisticated methods for estimating Q locus parameters has led to the widespread use of marker methods. These methods enable tests of hypotheses about the effects and location of putative Q loci. The gross objective of marker map experiments is the estimation of Q locus means and linkage map distances between Q and marker loci. I have used the phrase 'quasi-Mendelian analysis' to describe this sort of analysis. Mathematical models for quasi-Mendelian analyses describe the Mendelian genetics of marker and quantitative trait loci as a function of a dependent variable (quantitative trait).

Alternative models and their use in quasi-Mendelian analyses are the subject of this paper. We have described linear and nonlinear statistical models and maximum likelihood methods for estimating Q locus means and map distances in doubled haploid (DH), recombinant inbred, backcross, various testcross, F_2, and F_3 progeny. Our methods

require linked codominant marker loci. Linear model analyses are a convenient and efficient way of estimating Q locus effects, especially when progeny are grown in replicated experimental designs in different environments. Nonlinear model analyses are useful for estimating map distances and Q locus effects, but require substantially greater programming than linear model analyses. The maximum likelihood analyses we have proposed may be executed with standard linear or nonlinear least squares statistical software, *e.g.*, SAS or BMDP. Estimating map distances is more difficult than estimating Q locus means and effects because map distances are less well determined than means, *i.e.*, map distances are estimated with less power than are means. Thus, sample sizes required for efficient mapping of Q loci are greater than those required for detecting Q locus effects. Map distance estimates are environmentally dependent, so it is probably expedient to estimate distances averaged over environments. In certain experimental situations, it may be difficult to biologically or mathematically justify estimating map distances. DH progeny experiments were used to illustrate different problems and analyses because of the importance and widespread use of DH progeny in *Brassica napus* L. breeding and genetics.

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10. MITOCHONDRIAL DNA ALERTATIONS ASSOCIATED WITH MALE-STERILITY IN OGURA RADISH

C.A. Makaroff, I.J. Apel, and *J.D. Palmer*, Dept. of Biology, University of Michigan, Ann Arbor

The Ogura cytoplasm of radish was the first example of cytoplasmic male-sterility in a crucifer. We are interested in identifying the alteration(s) associated with male-sterility in Ogura radish and determining how these alterations result in the male-sterile phenotype. We have studied the structural organization and expression of the mitochondrial genome of Ogura radish and compared it to that of normal radish. Numerous rearrangements and inversions have been identified in the Ogura genome relative to that of normal radish. The transcriptional patterns of several known mitochondrial genes and of rearranged mitochondrial sequences were examined in three nuclear backgrounds. Three genes, *atp6*, *atpA*, and *coxI*, map near rearrangement breakpoints and exhibit altered transcript patterns. These genes have been sequenced and their transcript termini determined. Rearrangements have occurred within transcriptional units for *coxI* and *atp6*, whereas rearrangement breakpoints are located outside the boundaries of the mature *atpA* transcript. Rearrangement 100 bp 5' to the *coxI* initiator methionine has generated a new 5' transcript terminus. Rearrangement breakpoints have been identified both within the coding region of *atp6* and 3' to the gene in Ogura radish. Numerous nucleotide substitutions are predicted to eliminate normal translation of Ogura *atp6*, which is co-transcribed with an Ogura-specific 105 amino acid ORF. Rearrangement breakpoints were mapped 500 bp 5' and 1500 bp 3' to the *atpA* coding region. Differences in the *atpA* transcript pattern are due to altered processing of Ogura-specific sequences. Short repeated sequences are found near most of the rearrangement breakpoints. Although a common sequence does not appear in these repeated regions, their presence suggests that the rearrangements have occurred through homologous recombination.

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11. GENETIC DIVERSITY IN *BRASSICA CAMPESTRIS* (SYN. *B. RAPA*) AND ITS EVOLUTIONARY IMPLICATIONS

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Isozyme sampling of more than 20 accessions of *Brassica campestris*, fertility analysis of sub-specific hybrids, and genetic analysis of isozyme and some morphological traits show this species in the process of speciation. Nei genetic diversity estimates for 10 to 14 isozyme loci (6 of which are monomorphic across the species range) indicate that the historical Euro-centric view of the origin of *B. campestris* is erroneous. Greater diversity is found among the Asian groups. Among the Asian groups, the Indian turnip 'Shelgham' shows the greatest morphological diversity as enlarged hypocotyl and self-compatibility are segregating in the small populations tested. Genetic analysis of turnip formation shows a single major gene controls the presence or absence of enlarged hypocotyl. A scheme of evolution for this species may be origin of a turnip-like progenitor in a large area centered in Afghanistan, independent bi-directional radiation (i) to N China followed by diversification to Chinese cabbage and (ii) towards SE Asia (*i.e.*, the pak choi group). The European radiation has likely occurred later from the turnip followed by the development of oilseed varieties.

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12. ENGINEERED GLYPHOSATE TOLERANCE IN CANOLA

S.G. Metz, G.M. Kishore, H.J. Klee, C.S. Gasser, J.S. Hart, and *X. Delannay*, Monsanto Co., St. Louis, Missouri

The major weed problems associated with producing canola are those of the Cruciferae family; especially wild mustard and stinkweed. If the canola crop were genetically engineered to be tolerant to glyphosate, the active ingredient in Roundup herbicide, a less expensive, more effective, more environmentally sound, and more broadly applicable herbicide alternative would be available to canola producers.

Plants of canola (*Brassica napus*) cv. Westar have been transformed and regenerated according to the procedure described by Fry *et al.* (Plant Cell Reports 6:321-325). Stem segments collected prior to bolting are surface sterilized, cut into 3 mm segments which must be maintained in the proper orientation, and inoculated with a dilute solution of *Agrobacterium tumefaciens* containing the gene conferring tolerance to Roundup herbicide. After a 2-day co-culture period the stems are placed on gentamicin selection media until small shoots are formed. The shoots are excised and placed directly into a soilless mix for rooting.

We have used our transformation system to engineer canola for tolerance to Roundup herbicide. The first plants which have been tested have carried the EPSPs gene isolated from *Arabidopsis*, driven by the CaMV 35s promoter. The gene is inherited in a dominant Mendelian fashion through all the generations tested. Typically the position

effect of the insertion plays a significant role in expression of the trait, with the population giving a bell-shaped curve. R_1 progeny are first tested in the greenhouse and then tested in the field.

In 1988 field trials in Canada, engineered canola plants expressed tolerance to Roundup herbicide, however, not at commercial levels. Avenues of interest which may increase tolerance to commercial levels are tissue specific promoters and variant EPSP synthases. In the preliminary trials the yield, oil quantity, oil quality, and maturity were not significantly affected by the tissue culture process.

We have demonstrated the concept of glyphosate tolerance in canola by engineering plants which overexpress the EPSPs enzyme or which express an enzyme which has reduced affinity to glyphosate. In the future, improvements can be made which make this a commercially viable route for weed control in canola.

13. Selection for Combining Ability in *Brassica napus*

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Combining ability analysis for seed yield and days to maturity was carried out on 72 hand-crossed hybrids of spring-type *Brassica napus*. These hybrids were produced using five male inbreds and 15 female inbreds, three of which had been previously selected for general combining ability (GCA). All hybrids were evaluated at two Manitoba locations (Rosebank and Minto) in 1988, along with the check cultivar, Westar. A combined analysis of variance detected significant location and genotype effects, but no genotype × location interaction. Partitioning of hybrids variance into mean squares due to males, females, and males × females showed all three components to be highly significant ($P<0.01$). Combining ability analysis detected significant GCA and SCA variances; however, the GCA variances for both yield and maturity were approximately 20 fold the SCA variances, indicating a predominance of additive genetic variance for both traits. Comparison of the distribution of hybrids in this study with those in the previous study from which some of the inbreds had been obtained (Patel *et al.*, 1987) revealed a substantial increase in the proportion of early-maturing, high-yielding hybrids. These results indicate the importance of selection for combining ability in developing high-performing hybrids of oilseed rape.

14. Combining Ability Analysis of Newly-Developed CMS (A) and Restorer Lines in Spring *Brassica napus*

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Combining ability and hybrid performance for seed yield and maturity were investigated in spring-type *Brassica napus* using pol cms inbreds (A lines) and lines carrying the 'Italy' restorer factor (R lines). A total of 192 hybrids, representing 36 A×R combinations, were evaluated in replicated trials at Rosebank, Manitoba, and Minto, Manitoba, in 1988. Combining ability analysis based on a fixed effects model detected highly significant ($P<0.01$) general (GCA) and specific (SCA) combining ability for both traits. While GCA and SCA variances contributed equally to yield performance, GCA was relatively more important than SCA for days to maturity. The importance of SCA for yield indicates the contribution of nonadditive genetic effects of hybrid performance. More than half of the hybrid combinations outyielded the check cultivar, Westar, and some of these were also earlier maturing than the check. These results indicate that it is possible to obtain high-performing hybrids using the pol cms system. Further selection for combining ability should result in production of hybrids with even greater performance.

15. Cytoplasmic Male Sterility in Cruciferae

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We illustrate the organization of plant breeding research in France through the example of a coordinated program on cytoplasmic male sterility (CMS) in Cruciferae involving several partners. Two main species are concerned in this work: rapeseed (*Brassica napus*) and cabbage (*B. oleracea*). The applied objective is to obtain a CMS system in these crops for production of F_1 hybrid varieties. The fundamental objective is the study of Cruciferae cytoplasmic genomes and mainly mitochondrial genes either having an impact on plant productivity or which are directly involved in CMS.

A. Financial support is obtained from the ministries of agriculture, and research and technology for basic research. Associations like CETIOM (Centre Technique Interprofessional des Oléagineux Metropolitains) and PROMOSOL (Association pour la Promotion de la Sélection des Plantes Oléagineuses) promote this research by direct investments in laboratories and by salaries. Private firms are involved by grants into INRA laboratories and are also performing a part of the project in their own laboratories.

B. The three following objectives are coordinated by INRA:

a) *Identification and improvement of cytoplasmic male sterility sources.* Several CMS sources are studied in parallel: Ogura, Polima, *Diplotaxis*, *B. juncea*, *B. napus*. Alloplasmic male sterilities are obtained by interspecific crosses, requiring embryo culture. The improvement of cytoplasms is performed through protoplast fusion, chloroplast exchange, and mitochondrial recombination permitting agronomical characteristics like chlorophyll content, nectar production, flower and fruit development to be normalized.

b) *Agronomical and molecular characterization of CMS systems.* Cytoplasms obtained by methods described above are studied in different genetic backgrounds of rapeseed and cabbage for seed production (pollination by bees, fruit development, seed set). Each new cytoplasm obtained by protoplast fusion is characterized by chloroplast and mitochondrial restriction profiles with several restriction enzymes.

c) *Studies on the genetic material in devices for hybrid seeds production.* After a first choice among the different cytoplasms, those having superior quality are tested on large

scale experimental devices for hybrid seed production of male sterile or restored F_1 hybrids.

C. Several research institutes are involved in the program. They are represented by Universities (Orsay, Perpignan, Rennes), CNRS (Centre National de la Recherche Scientifique), and foreign institutions (Academy of Agricultural Sciences, Shangai, China; Plant Breeding Institute, Poznan, Poland).

D. Young scientists are engaged in basic research at INRA or CNRS on various aspects of this work through diploma or thesis.

E. The marketing and release of the genetic material obtained is done through contractual collaboration for marketing of varieties among AGRI-OBTENTION, the subsidiary seed company of INRA, and private firms: SERASEM, Clause, Desprez, Gauthier, and Vilmorin.

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16. GLUCOSINOLATE CONTENT AND NUTRITIONAL VALUE OF PORTUGUESE CABBAGE AND KALE

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Cole crops represent about 25% of the Portuguese vegetable production ranking third after potato and tomato. *Brassica oleracea capitata* and *B. o. sabauda* cabbages are over 50% and Portuguese cabbage (*B. o.* var. *costata*) are *circa* 20% of the total *Brassica* crop production. *Brassica* plants play a major role in the Portuguese diet, being a most important vegetable, second to potatoes only. The quite high consumption of *Brassica* vegetables is estimated at 70 kg per person per year, ranking first in Europe and perhaps in the world. These figures motivated research on the nutritive value and glucosinolate content of Portuguese cabbages and kales in order to establish differences between the Portuguese material and the main European cabbage types.

Morphological diversity is very high among the various ecotypes of Portuguese cabbage. This may correspond to quality differences related to chemical composition and glucosinolate content. Quality studies were conducted on representative ecotypes from a large collection, which includes germplasm from the various regions of Portugal. Tests also intended to evaluate the influence of local Vila Real climatic factors on edible quality and different composition of the various plant organs.

In trials conducted in 1988 (to be repeated in 1989), five varieties were used: two types of Portuguese cabbage (*B. oleracea* var. *costata*), one leaf type called 'Galega' (*B. o.* var. *acephala*), one leafy type called 'Nabica' (*B. rapa* var. *napobrassica*), and one hybrid head type (*B. o.* var. *capitata*). The first two varieties are cultivated for the loose head and bud flowers, the *B.o. acephala* and *B. r. napobrassica* varieties are cultivated for the leaves and bud flowers and the *B. o. capitata* for the head. *B. o. capitata* was the control to compare glucosinolate content under Portuguese climate with other countries in Europe. These plants were cultivated during two seasons (Spring-Summer and Autumn-Winter) in order to allow quality characterization and protein, minerals, and total and individual glucosinolates analytical determinations every two weeks along each season.

Among the results already available from the first season (Spring-Summer), it must be stressed the high value of calcium in the leaves of Galega and Portuguese cabbage, over 400 and 350 mg per 100 g of the edible portion, respectively, and the protein content of the leaves, which reaches 2.8 g per 100 g of the edible portion in Galega and Nabica. In the second season (Autumn-Winter), the values have the same tendencies, but the figures are a little higher. As such, we got for calcium in the kale type Galega and Portuguese cabbage over 500 and 380 mg per 100 g of edible portion, respectively. The protein content is over 4.6 g per 100 g of edible portion in the leaves of Galega and about 4.0 g in Nabica. Other mineral (Mg, K, P, Fe, S, Mn, and Zn) contents were also investigated. Petioles are poorer in protein when compared with leaves and heads. For calcium, the highest values are found in leaves, followed by petioles and stems, heads coming last. The second season gave higher values than the first.

Total glucosinolates present a very significant variation ($P<0.01$) between varieties among seasons and between seasons. The levels of these compounds reach maximum value in the heads when compared with the leaves and the Nabica type presents in this case the highest values, around 320 μmoles per 100 g fresh weight. The highest values of all are obtained in the flower buds of Nabica, with 850 μmoles per 100 g fresh weight, the Portuguese cabbage presenting half of this value. Portuguese cabbages have very low glucosinolate content . These varieties also have about 60 μmoles per 100 g fresh weight in the leaves and about 200 μmoles per 100 g fresh weight. However, these figures are season dependent. Total glucosinolates show variation during the day, with the highest value in the afternoon (6 pm) according to a sampling made in two warm consecutive days every four hours from 6 am to 10 pm, local Vila Real Autumn time.

17. Application of RFLP in Evolutionary Studies and Germplasm Evaluation of Brassica

K.M. Song, T.C. Osborn, and *P.H. Williams,* University of Wisconsin, Madison

RFLPs have been used to study genome evolution and phylogeny in *Brassica* and allied genera. Fifty-four accessions including eight diploid species, ten accessions of *B. rapa*, nine cultivated types and thirteen wild forms of *B. oleracea*, and sixteen cultivars of *B. napus* were examined with more than thirty random genomic DNA probes which mapped to eight different linkage groups of the *B. rapa* genome. Phylogenetic trees were constructed by the PAUP microcomputer program based on the RFLP data.

Results from these studies indicated that 1) There exist two basic evolutionary pathways for diploid species: one pathway gave rise to *B. fruticulosa, B. nigra,* and *Sinapis arvensis*, etc. with *B. adpressa* or a close relative as the initial ancestor; and another pathway resulted in *B. oleracea* and *B. rapa* with *Diplotaxis erucoides* or a close relative as the initial ancestor. *B. tournifortii, Raphanus sativus,* and *Eruca sativus* represented intermediate types between the two lineages and might have been derived from introgression/hybridization between species belonging to different lineages. Molecular evidence for an ascending order of chromosome numbers in the evolution of *Brassica* and allied genera were obtained on the basis of RFLP data and phylogenetic analysis. 2) Cultivated *B. oleracea* morphotypes showed monophyletic origin with wild *B. alboglabra* and/or *B. oleracea* as possible ancestors. Various kales in cultivated *B. oleracea* constitute a highly diverse group and represent the primitive morphotypes from which cabbage, broccoli, cauliflower, etc. have evolved. Cauliflower was found to be closely related to broccoli, whereas cabbage was closely related to leafy kales. 3) There was large diversity among the thirteen wild forms of *B. oleracea* examined, representing various taxonomic states from subspecies to species. These wild forms were classified into four groups based on RFLP analysis, represented by *B. oleracea, B. cretica, B. rupestris,* and *B. macrocarpa*, respectively. 4) Within *B. rapa*, Pak choi, narinosa, and Chinese cabbage constituted a group distinct from turnip and wild populations, consistent with our previous hypothesis that *B. rapa* had two centers of domestication. A wild accession collected from India was found to be positioned in the tree between European types and East Asian types, implying an evolutionary pathway from Europe to India then to South China. 5) Much less polymorphism was detected among the sixteen commercial cultivars of oil seeds rape, indicating all of these cultivars have narrow genetic bases. The phylogenetic relationships between these cultivars seem to be correlated to their pedigrees.

Appendix I Workshop Program

Friday, April 7

5:00-9:00pm **Registration**

7:00-9:00pm **Welcoming Social Mixer**

Saturday, April 8

8:00am **Orientation**
D.B. Cohen
Fifth Crucifer Genetics Workshop Chair
Maccabee Seed Company, Davis, CA

Welcome
C.E. Hess
Dean, College of Agricultural and Environmental Sciences
University of California, Davis

8:20am -12:10pm **SYMPOSIUM I**

***BRASSICA* BREEDING, GENETICS, AND EVOLUTION**
Chair: **C.F. Quiros**
University of California, Davis

Germplasm of wild x=9 Mediterranean species of *Brassica*
C. Gómez-Campo
Universidad Politécnica, Madrid, Spain

Cytogenetic stocks in *Brassica*: Addition lines and genome evolution
C.F. Quiros
University of California, Davis

Rapeseed and canola breeding in North America: The US potential
D.L. Auld
University of Idaho, Moscow

Genome analysis in *Brassica* using RFLP markers
T.C. Osborn
University of Wisconsin, Madison

Haploidy and cell biology of microspore embryogenesis
D. Simmonds
Agriculture Canada, Ottawa, Ontario
P. Arnison
Paladin Hybrids, Inc., Brampton, Ontario, Canada

1:45-3:15pm **WORKSHOPS**

I. Vegetable crops breeding
Chair: **V.E. Rubatzky**
University of California, Davis

II. Molecular biology and transformation
Chair: **S.G. Metz**
Monsanto Corporation, St. Louis, Missouri

3:45-5:15pm **WORKSHOPS**

III. Oilseed breeding
Chair: **G. Rakow**
Agriculture Canada, Saskatoon, Saskatchewan

IV. Molecular markers and gene mapping
Chair: **T.C. Osborn**
University of Wisconsin, Madison

5:15-6:15pm **POSTER SESSION**

5:15-6:15pm **VEGETABLE NURSERY VISIT**
Student Farm

7:00pm **BANQUET**

8:30pm **Keynote Speech:**
Use of cytoplasmic male sterility in the production of F_1 hybrids
T. Shiga
Plant Biotechnology Center, Sakata Seed, Japan

Sunday, April 9

8:00am -noon **SYMPOSIUM II**

***BRASSICA* BIOTECHNOLOGY: STATE OF THE SCIENCE**
Chair: **J.J. Harada**
University of California, Davis

Organelle manipulation by cybridization: Methods, results, and applications
G. Pelletier
INRA, Versailles, France

Expression of members of the S gene family
J.B. Nasrallah
Cornell University, Ithaca, New York

Spatial and temporal regulation of genes activated during seed germination
J.J. Harada
University of California, Davis

Characterization of *Brassica* seed-specific promoters and their expression in transformed rapeseed
J.C. Kridl
Calgene, Inc., Davis, California

***Arabidopsis* as a model for understanding the molecular basis of fatty acid composition**
J. Browse
Washington State University, Pullman

1:45-3:15pm **WORKSHOPS**

V. Disease, pest, and stress resistance
Chair: **R.L. Gabrielson**
Washington State University, Puyallup

VI. Hybrids and population improvement in crucifers
Co-Chairs: **J. Patel, I. Grant, D. Charne, M. Elhalwagy,**
Allelix Crop Technologies, Inc., Ontario, Canada

3:45-5:15pm **WORKSHOPS**

VII. Biochemical diversity
Chair: **D.B. Cohen**
Maccabee Seed Co., Davis, California

VIII. Cell biology
Co-Chairs: **P. Arnison**
Paladin Seeds, Inc., Brampton, Ontario, Canada
D. Simmonds
Agriculture Canada, Ottawa, Ontario

IX. Genetics conservation and gene banks in Cruciferae
Chair: **P.H. Williams**
University of Wisconsin, Madison

5:15-5:45pm **CLOSING**
PLANS FOR NEXT CONFERENCE
D.B. Cohen
Maccabee Seed Company, Davis, California

Appendix II Abstracts of Posters

POSTER AUTHOR INDEX

The numbers refer to the following pages.

COSTS AND GENETIC BASIS OF LEAF TRICHOME PRODUCTION IN *BRASSICA CAMPESTRIS*

J. Ågren and D.W. Schemske, Dept. of Ecology and Evolution, University of Chicago, 5630 S. Ingleside, Chicago, IL 60637

Plants may produce both structures, like trichomes and thorns, and chemical compounds that deter herbivores. According to allocation theory, investment in defenses is costly, and the amount of resources devoted to protection against herbivores is expected to depend on the risk and the consequences of herbivore damage. Rapid-cycling *Brassica campestris* exhibits marked variation in the number of trichomes on leaves and petioles. An initial study demonstrated negative phenotypic correlations between the number of trichomes produced and height after 20 d, and between trichome number and earliness of flowering. We are at present performing a selection experiment to test the idea that trichome production is costly to the plant. We are selecting for high and low number of trichomes on the edge of the first true leaf. The prediction is that an increase in trichome production will be associated with a lowered growth rate and fecundity in the absence of herbivores. Through back-crosses we intend to get an estimate of the number of genes controlling trichome production. The realized heritability of the selected trait was 0.37 in the high line and 0.46 in the low line, after the first generation of selection. After two generations of directional selection the mean number of trichomes on the edge of the first true leaf was 69 in the high line, 2 in the low line, and 28 in the control. The number of trichomes on the petiole showed a positive correlated response to the change in leaf trichome number. The increase in trichome production in the high line was associated with a small, but significant, delay of flowering. The results so far demonstrate a substantial genetic component to the variation in trichome production in *B. campestris* and indicate that there may be a trade-off between production of trichomes and allocation to other functions. Planned studies include a comparison of the relative performance of the high and low lines in the presence and in the absence of herbivores feeding on young *B. campestris*.

COMPARISON OF FATTY ACID COMPOSITION OF *SINAPIS ALBA* SEEDS COLLECTED AT VARIOUS LOCATIONS IN ISRAEL

Y. Alber, Z. Yaniv, M. Zur, and D. Schafferman, Dept. of Introduction, ARO, The Volcani Center, Bet Dagan, Israel

Mature seeds of *Sinapis alba* were collected during late spring 1988 at five different sites in the north and center of Israel. Fatty acid analysis of the seed oils showed the following: (a) erucic acid (C22:1) content varied between 43% and 55% of total fatty acids, (b) the lowest concentration of erucic acid was present in seeds collected in the southernmost site, (c) low erucic acid content was accompanied by low oleic acid (18:1) and high polyunsaturated acids (C18:2 + C18.3) content. It is still not known whether these trends indicate genetic variation or an adaptation to climatic conditions.

LINKAGE ANALYSIS OF ISOZYME GENES IN *BRASSICA OLERACEA*

P. Arus, Institut de Recerca i Tecnologia Agroalimentaries, Generalitat de Catalunya, Spain

Progeny from four intraspecific crosses of *B. oleracea* and an interspecific backcross (*B. montana* × *B. oleracea*) × *B. oleracea* were assayed electrophoretically to determine linkage relationships of isozyme genes. Fifteen polymorphic loci including 14 isozymes and leaf color (*g*) were analyzed. Evidence on the genetic basis of six genes not studied previously (one of phosphoglucomutase (*Pgm-3*), 6-phosphogluconate dehydrogenase (*6Pgd-1*), and malate dehydrogenase (*Mdh-1*), and three of aconitase (*Aco-1*, *Aco-3*, and *Aco-4*) was provided. Tests of joint segregation between 87 of the 105 possible pairs of loci identified two linkage groups: one was formed by a cluster of four tightly linked loci (*Pgm-3*–*Pgi-2*–*g*–*6Pgd-1*), and the other by four genes loosely linked (*Aco-4*–*Mdh-1*– *Aco-1*–*Lap-1*). Chi-square tests of goodness-of-fit revealed significantly reduced recombination fractions between five more pairs of genes, but they were not likely to detect true linkage because distance was close to 50 cM and comparisons between the same locus pairs in other crosses did not deviate from random assortment.

ENVIRONMENTAL EFFECTS ON GLUCOSINOLATE COMPOSITION IN WINTER RAPESEED

D.L. Auld, J.B. Davis, K.A. Mahler, and D.S. Engvall, University of Idaho and Washington State Dept. of Agriculture

Winter rapeseed (*Brassica napus* L.) has great promise as a new oilseed crop in the United States if cultivars which have seed meals with less than 30 μmoles g^{-1} of defatted meal are grown. To determine the influence of the production environment on this trait, seed of five differential cultivars grown at 17 locations were analyzed for total glucosinolates. The average glucosinolate levels of the cultivars ranged from 19 to 190 μmoles g^{-1}. Cultivars with low levels of glucosinolates were more stable across environments than high glucosinolate cultivars. Bridger seedlings exposed to a 9-week vernalization period had lower levels of glucosinolates than seedlings vernalized for either 3 or 6 weeks. Addition of sulfur fertilizers had no impact on glucosinolate concentration. Evaluation of glucosinolate levels in experimental cultivars will require evaluation in many environments. Development of cultivars of winter rapeseed that produce low levels of glucosinolates in all production environments is essential for this crop to be successful in this country.

MORPHOMETRIC ANALYSIS OF *SINAPIS* L.: THE VALUE OF GENERIC SUBDIVISIONS

G. Baillargeon, Agriculture Canada, Biosystematics Research Centre, Central Experimental Farm, Ottawa, Ontario, Canada, K1A OC6

The value of the sectional classification of the genus *Sinapis* L. proposed by Schulz (1919, 1936), was tested using multivariate statistical analysis and found to be sound. Based on a set of 30 morphological characters, *Sinapis* was divided into the following four discrete entities: 1) sect.

Sinapis (incl. *S. alba* with 3 ssp. and *S. flexuosa*), 2) sect. *Ceratosinapis* (incl. *S. arvensis* with 3 ssp.), 3) sect. *Eriosinapis* (incl. *S. pubescens* with 2 ssp., *S. aristidis*, *S. boivinii*, and *S. indurata*), 4) sect. *Chondrosinapis* (incl. *S. aucheri*). The best discriminators were, in decreasing order of contribution: 1) number of seeds in beak, 2) vestiture of pedicels, 3) length of beak, 4) thickness of septum, 5) length of valves, 6) width of beak below stigma, and 7) length of seeds. Reducing the character set to these seven gave over 95% correct identifications. The delimitation of these four phenetically defined subgroups is well supported by cytological and DNA studies (see poster by WARWICK), so that they appear to represent monophyletic lineages. As a consequence, any phylogenetical study involving *Sinapis* should include at least one representative of each of the four sections. The four groups are morphologically so distinct from each other, that the question arises as to whether the nearest relative of each group is to be found within or outside *Sinapis*, supporting the need for a study of the tribe Brassiceae at the generic level.

APPLICATION OF MICROSPORE CULTURE TO BREEDING WINTER RAPESEED

D.R. Brady, D.L. Auld, and D.A. Erickson, University of Idaho, Moscow, ID

The University of Idaho is investigating the use of microspore culture to develop 40 to 60% of the breeding lines in its rapeseed breeding program. Completely homozygous lines can be recovered by microspore culture in less than two years as compared to a minimum of three years for single seed descent. Minimum population size necessary to recover desirable genotypes is also decreased. The limitations of tissue culture restrict the use of this procedure to high priority breeding lines. Microspore culture is expensive and labor intensive, as well as genotype specific. A study to compare means and distributions of segregating populations of winter rapeseed derived from single seed descent and microspore culture has been initiated in the winter rapeseed breeding program at the University of Idaho.

THE COMBINATION OF ATRAZINE RESISTANCE AND *BRASSICA NIGRA* CYTOPLASMIC MALE STERILITY IN BROCCOLI BY PROTOPLAST FUSION WITH *B. CAMPESTRIS*

M.C. Christey and E.D. Earle, Plant Breeding Dept., Cornell University, Ithaca, NY 14853-1902

Cytoplasmic male sterile (CMS) broccoli plants containing the *Brassica nigra* cytoplasm (BN lines) were developed by PEARSON (1972) and selected for nectary development, normal pistil structure, and improved seed set by DICKSON (1975). These male sterile plants are characterized by petaloid sterility in which 4 or 6 of the anthers are transformed into large yellow petal-like structures with red tips. Stigmatic tissue is often present along the top of these "petals" and ovules are sometimes found along their lower edges. As the cytoplasmic characters of atrazine resistance and male sterility can only be combined by protoplast fusion, mesophyll protoplasts from a BN line were fused with etiolated hypocotyl protoplasts from atrazine resistant *B. campestris* (Candle). From these experiments, 19 colonies that regenerated plants were obtained. All these plants were broccoli-like in phenotype and therefore not somatic hybrids but possibly cybrids. Four plants, all from one colony, were resistant to atrazine as determined by their ability to survive, grow, and root in the presence of 25 μM atrazine, a level at which control plants bleach and show no growth. Protoplasts from these plants have been assayed by the nitro-blue tetrazolium assay (ROBERTSON and EARLE, 1987) as further confirmation that they are atrazine-resistant. These atrazine-resistant broccoli plants all still exhibit the petaloid sterility characteristic of the *B. nigra* CMS. Molecular analysis has confirmed the presence of *B. campestris* chloroplasts in these plants. Analysis of the mitochondrial genome for possible recombination is in progress.

References: DICKSON, M.H. 1975. Hort. Sci. **10**(5):535; PEARSON, O.H. 1982. Amer. Soc. Hort. Sci. **97**(3):397-402; ROBERTSON, D. and E.D. EARLE. 1987. Plant Cell Reports **6**:70-73.

TRANSFORMATION OF *BRASSICA OLERACEA* VAR. *ITALICA* BY CO-CULTIVATION OF PEDUNCLE EXPLANTS WITH *AGROBACTERIUM TUMEFACIENS*

M.C. Christey and E.D. Earle, Plant Breeding Dept., Cornell University, Ithaca, NY 14853-1902

Peduncle explants of four *Brassica oleracea* varieties gave high regeneration rates, with the rapid production of numerous buds. These explants were used to transform a line derived from Green Comet broccoli via co-cultivation with *Agrobacterium tumefaciens*. ***Regeneration***: The use of peduncle explants for the regeneration of *Brassica* was reported in *B. napus* (STRINGAM, 1977). Peduncle explants have been used by others for the regeneration (KLIMASZEWSKA and KELLER, 1985) and transformation of *B. napus* (FRY *et al.*, 1987) but not for *B. oleracea* and *B. campestris*. Peduncle explants from broccoli, cabbage, Chinese broccoli, and cauliflower readily regenerated shoots on the medium used for *B. napus*, with multiple shoots (1 to 35) per explant. With lines derived from Green Comet broccoli (Harris Moran Seed Co.), regeneration rates of over 75% were reproducibly obtained. With cabbage, Chinese broccoli (Guy Lon, Fredonia Seeds), and an atrazine-resistant cauliflower line, regeneration rates of 82-88% were obtained. In contrast, culture of peduncle explants from 3 rapid-cycling lines of *B. campestris* yielded only rare shoots, even though 50 different hormone combinations were tested. Peduncles were removed from plants in the process of bolting and flower buds and pedicels discarded. Explants (0.5 cm) were placed horizontally onto LS medium containing 1 mg/l benzyladenine. After 3 to 4 weeks the entire regenerating ends were excised and transferred to hormone-free medium for further shoot enlargement. Individual shoots were excised and rooted on this medium prior to transfer to soil. The first effect noted on culture was a swelling of the entire explant, particularly the basal end. After 7 days both ends were covered in a small amount of callus, and the surface area of the basal end was approximately 2 times that of the apical end. Shoot regeneration was first noted after 7 to 10 days. After 3 weeks numerous buds were present, usually concentrated on the region closest to the medium. ***Transformation***: Severe browning of the explants after co-cultivation was a problem,

and numerous experiments have only yielded one transformant. This transformant was obtained with strain LBA4404 carrying genes specifying kanamycin resistance and β-glucuronidase (GUS) activity in plants. A tobacco cell suspension feeder layer was used during the cocultivation period. This plant grows and roots on selective levels of kanamycin (50 μg/ml). Leaf explants have regenerated a further 20 kanamycin-resistant shoots on medium containing 50 μg/ml kanamycin. Experiments have indicated that these kanamycin-resistant plants are suitable for use as a marker in protoplast fusion experiments. Protoplasts from these plants form green colonies in the presence of 50 μg/ml kanamycin. In contrast, protoplasts from control plants form rare yellow colonies which do not survive. The GUS assay (JEFFERSON *et al.*, 1987) was positive on petiole sections from the kanamycin-resistant plants, with staining concentrated in the vascular region. Southern analysis has not yet been completed.

References: FRY, J., A. BARNASON, and R.B. HORSCH. 1987. Plant Cell Reports **6**:321-325; JEFFERSON, R.A., T.A. KAVANAGH, and M.W. BEVAN. 1987. EMBO J. **6**:3901-3907; KLIMASZEWSKA, K. and W.A. KELLER. 1985. Plant Cell Tissue Organ Culture **4**:183-197; STRINGAM, G.R. 1977. Plant Cell Letters **9**:115-119.

AN EFFECTIVE PLANT REGENERATION FROM HOMOGENIZED TISSUE DERIVED FROM MICROSPORE EMBRYOS OF *BRASSICA NAPUS* L.

P.V. Chuong*, B. Asdpen, Hoechst Canada, Inc., Ottawa, Ontario, Canada, and W.A. Keller, Plant Research Center, Agriculture Canada, Ottawa, Ontario, Canada, K1A OC6
**Present address: Sungene Technologies Corp., 2050 Concourse Dr., San Jose, CA 95131*

Twenty-day-old embryos obtained from microspore cultures of *Brassica napus* were cultured in B5 liquid medium on a rotative shaker at 120 RPM for 7 to 10 d to induce further development to seedling-like structures. These structures were homogenized with B5 medium in a kitchen blender at low speed for 4-10 sec. After the centrifugation of the homogenized mixture at 100 × g for 5 min, the liquid phase was decanted and the homogenized tissue was plated on solidified B5 medium supplemented with 0.1 mg/l GA3. Large numbers of regenerants including plantlets, secondary embryos, organs, and calli could be observed after 2 wk of subculture. The quality and the number of regenerants depended on the amount of homogenized tissue plated on the regeneration medium, the duration of homogenization, and the developmental stage of the original seedling-like structures. Homogenized tissue plated at high density tended to develop into abnormal structures whereas that at low density regenerated complete healthy plantlets. A range between 50 to 300 structures could be usually recovered from 1 to 3 g of freshly homogenized tissue. Shoots/plantlets could be easily recovered through organogenesis or secondary embryogenesis after subculturing these abnormal regenerants. With the high frequency of structure regeneration in a short period of time and the ease of plantlet/shoot and secondary embryo recovery from the primary regenerants, the embryo tissue homogenization technique has a great potential in a range of biotechnology applications such as mutation induction, selection for herbicide/disease resistance/tolerance, and *Agrobacterium*-mediated transformation in *Brassica*.

GENOTYPIC AND ENVIRONMENTAL VARIATION OF GLUCOSINOLATE CONTENT IN RAPESEED

J.B. Davis, D.L. Auld, and K.A. Mahler, University of Idaho, Moscow, ID

The University of Idaho has been coordinating the Winter Rapeseed National Variety Trial since 1985. Many rapeseed (*Brassica napus* L.) cultivars have been grown and tested for quality traits and agronomic performance at numerous locations in the continental United States. As part of this trial, six cultivars with differential glucosinolate levels were grown at eight locations during the 1986-87 and 1987-88 crop years and were tested for seed glucosinolate content. Three of the cultivars contained high levels of glucosinolates, two had intermediate levels, and one had a low level of glucosinolates. The ranking of the cultivars remained relatively constant across environments, but within cultivars, the glucosinolate content varied between environments. The cultivar-by-environment interaction was also significant. The magnitude of the F-values in the statistical model suggest that genetic factors had a greater impact than the environment on glucosinolate content in winter rapeseed.

PLANT REGENERATION FROM ISOLATED MICROSPORES OF *BRASSICA CAMPESTRIS*

D. Facciotti and J. Turner, Calgene, Inc., 1920 Fifth St., Davis, CA 95616

The embryonic competence of isolated microspores of *B. campestris* was examined with respect to genotype and the environmental conditions under which microspore donor plants were grown. Plants of the varieties R-500, Candle, and Tobin were grown under long-day conditions at four different temperatures: 13°C/18°C (night/day), 22°C, 24°C, and 28°C. Embryogenesis was observed mainly with Tobin. It also occurred to a lesser extent with Candle, while no embryos were ever recovered from R-500. For both Tobin and Candle, most embryos were formed from plants maintained at relatively low (22°C and 13°C/18°C) temperatures. Plants were regenerated from Tobin embryos only. Some lines were maintained under *in vitro* conditions by shoot cuttings.

OIL BIOSYNTHESIS IN GAMETIC AND ZYGOTIC EMBRYOS OF *BRASSICA NAPUS*

D. Facciotti, S. Fujimoto*, N. McDonald, and K. Lardizabal, Calgene, Inc., 1920 Fifth St., Davis, CA 95616, *UCD Undergraduate Student, Calgene Intern Program

Oil biosynthesis was studied in zygotic and gametic embryos of *B. napus* cv. Cascade with respect to triglyceride, oil composition, and embryo developmental stages. The pattern of oil biosynthesis was remarkably similar in both gametic and zygotic embryos. Tryglicerides were synthesized from the early cotyledonary stage, characterized by the

formation of the cotyledons and a relative abundance of saturated fatty acids. Lipid content and the synthesis of unsaturated fatty acid rapidly increased during the next phase only, which is characterized by rapid cotyledonary growth. The oil composition was independent of the environmental conditions and appeared to be determined by the developmental stage of the embryo. This was observed in both gametic and zygotic embryos. In conclusion, gametic embryos should provide an excellent model system for the study of oil biosynthesis at the molecular level.

SOMATIC REGENERATION AND RECOVERY OF TRANSGENIC PLANTS FROM *BRASSICA CAMPESTRIS*

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The *in vitro* responsiveness of hypocotyl, petiole, cotyledon, root, and leaf tissues to form shoots and plants was studied in the following *B. campestris* varieties: Ante, Candle, Emma, Span, Sylvi (winter variety), and Tobin. Tissue explants, dissected from one-week-old seedlings, were incubated (3 to 15 days depending on the explant origin) on B5 or MS media supplemented with 2,4-D (1 mg/l) and subsequently transferred to B5 medium containing BA (3 mg/l) and zeatin (1 mg/l). This two-step culture procedure, with respect to hormones, was necessary to induce shoot formation from hypocotyl explants with a frequency (percent explants forming at least one shoot) of 20 to 30%. A higher and reproducible frequency (50 to 90%) was observed with these explants only when the second-step culture medium contained $AgNO_3$ (5 to 10 mg/l). In the presence of $AgNO_3$ petiole, leaf, and cotyledon explants also produced shoots, though at a lower and less producible frequency (0 to 30%). With root explants shoot regeneration (max. 1%) was possible only after a long (6 to 15 days) first culture step in the presence of 2,4-D. Regenerated shoots were rooted in B5 medium supplemented with IBA (1 to 2 mg/l). The resulting plants were potted and transferred to the greenhouse. Despite notable morphological differences displayed at the vegetative stage, these plants (>300 plants tested) produced seeds (bud pollination selfing) with an oil profile remarkably similar to that of the mother plants. With respect to shoot formation, the varieties Emma and Span produced the most consistent results. Emma was used in further transformation experiments. Transgenic plants were obtained from hypocotyl explants (Emma) co-cultivated (48 h during the first culture step) with *Agrobacterium tumefaciens* EHA 101/7001. The *Agrobacterium* strain used (disarmed, binary vector) carried both NPTII (kanamycin resistance) and GUS (glucuronidase) marker genes controlled by CaMV 35S and *mas* promoters respectively. Following cocultivation, shoots were regenerated and rooted under selective conditions in the presence of kanamycin (25 mg/l). Explants were sampled and assayed for NPTII and GUS activities and later, the insertion of these genes in the plant genome was also confirmed by Southern blot analysis. Transformation efficiency (percent explants forming at least one transformed plant), in terms of NPTII activity, ranged from 0.1 to 9.0%.

T-DNA TAGGING OF DEVELOPMENTAL GENES OF *ARABIDOPSIS THALIANA*

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More than 800 transformed lines of *A. thaliana*, segregating for a kanamycin resistance marker, have been screened for altered phenotypes that segregate in a manner consistent with Mendelian predictions. These transformed lines were generated by the seed infection method as described by FELDMANN and MARKS (MGG, **208**:1, 1987). This methodology precludes variants induced by the tissue culture process (somaclonal variants). Mutants observed so far include those affected in size (dwarf, miniature, and runt, along with a number of other as yet undescribed small plants), flower (agamous and reduced fertility) and trichome (glabrous) morphology, embryo- and seedling-development (lethals), and pigmentation (albino, yellow-green, early yellowing). A number of these mutants have been shown to be tightly linked to the T-DNA insert (dwarf, FELDMANN *et al.* Science **243**:1351, 1989; glabrous, MARKS *et al.*, University of Nebraska, Lincoln, pers. comm.; agamous, YANOFSKY *et al.*, Caltech, pers. comm.). The disrupted genes have been isolated and used as probes to isolate the wildtype genes. Complementation of mutant plants with the wildtype genes is in progress. Here we describe additional mutants recently isolated including a miniature, runt, and lines with reduced fertility. Miniature exhibits a dramatic reduction in size, small purplish leaves, roots that are short and appear to be deficient in elongation but not formation, and reduced seed set. Runt has rosette leaves that are curled initially, yellowish petioles in comparison to the wildtype, and delayed bolting.

FILTER PAPER DISC METHOD FOR CLUBROOT SCREENING

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A method of screening *Brassica* seedlings for resistance to *Plasmodiophora brassicae* Wor. based on the Wisconsin Fast Plant Growing System has been developed. First a diamond-shaped wick is inserted through a hole in the base of the planting cell or minipot (7 cc volume), then the bottom 1/3 of the cell is filled with a soil mix (perlite:sand:soil 1:1:1). Two Osmocote™ pellets (N:P:K 14:14:14) are added to each cell and the remainder of the pot is filled with finely screened PB mix (black organic peat soil:Jiffy Mix™ 1:1). A filter paper disc (0.5 cm) loaded with 10^7 freshly harvested cysts of *P. brassicae* is placed in the PB mix just above the mid-section of each pot. Seeds are germinated in Petri plates with wet filter paper and transplanted to the wells just as the root is emerging. Control of soil humidity is provided through capillary watering of the wick to a water mat extending to the reservoir of the WFP growing system. The method is designed for space efficient (122 plants/m^2) and reliable screening 35 days after sowing the seeds. The procedure has been found to be reliable and repeatable under a range of environmental conditions that are conducive to good plant growth. Cysts remain

viable for over 6 months when stored dry on filter paper discs at 4°C. The filter paper disc method is an efficient way of storing, exchanging, and using *P. brassicae.*

FLOW CYTOMETRIC CHARACTERIZATION OF *BRASSICA NAPUS* MICROSPORE DEVELOPMENT IN THE ANTHER

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Microspore culture is an important technique used for the production of doubled haploids in rapeseed breeding programs. The developmental stage of the microspore is an important determinant of success in this procedure. Only microspores in the late uninucleate stage respond to the culture conditions and develop into embryos (KOTT *et al.*, 1988, Can. J. Bot. **66**:1658). By flow cytometry the fluorescence and light scatter properties of large numbers of cells can be determined and correlated. This technique has been applied to characterize changes in the properties of microspore cells during their development in anthers and in culture. Microspores from *B. napus* (cv. Topaz) buds at an early stage (1.5 to 2.0 mm), middle stage (2.75 to 3.0 mm), and a late stage (3.5 to 4.0 mm) of development were isolated and examined by flow cytometry. Maturation of the microspores was accompanied by changes in their forward angle light scatter (FALS) and 90° light scatter (90° LS) properties. These measurements are related to the cell size and cell granularity (density), respectively. In particular, samples of cells from young buds had two populations of cells that could be distinguished on the basis of their 90°-LS properties. Sorting experiments showed that the population with high 90°-LS values consisted predominantly of tetrads. The population with lower 90°-LS values consisted of single cells, presumably freshly released microspores. In samples from larger cells the 90°-LS profile contained only a single peak. However, in these samples the FALS signal was split into two. The two populations represent microspores at different stages of maturation. This study should allow signposts of microspore maturation to be defined that will be used to assess rapidly the effects of environment and genotype on the development of microspores in donor plants for microspore culture.

SCREENING FOR HEAT TOLERANCE AND HOLDING ABILITY IN BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*)

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Holding ability has become an economically attractive attribute to select for in the development of hybrid broccoli in recent years. A continued increase in consumer demand for this member of the *B. oleracea* species has expanded its production into regions with warmer and often quite variable growing conditions. Another driving force to increase the holding period is the desire by large-scale growers to optimize their harvest potential in a once-over cutting operation. Forty hybrid broccoli lines (fourteen named releases and twenty-six experimental lines) were evaluated at the Long Island Horticultural Research Laboratory at Riverhead, New York in 1988. By allowing floral buds to reach full expansion before harvest, holding periods were quantified that reflected the maximum genetic potential of each genotype under a high temperature environment. By cross checking the best holding values with harvest uniformity data and several key quality parameters, the top eight lines were selected for the 1989 advanced replicated trials. First season results indicate some very promising heat tolerance with mean holding values in the 4 to 6 day range under mean high temperatures of 32+°C during maturation. Several of the top selections also showed excellent harvest concentration allowing for an average of 2-3 days to cut 80-100% of the plant population.

EVALUATION OF USDA COLLECTION OF OILSEED *BRASSICAS* FOR PHENOTYPIC VARIATION AT MOSCOW, ID AND TIFTON, GA

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Identification of rapeseed accessions with desirable traits can lead to the development of new rapeseed cultivars. An important source of genetic variation for cultivar improvement are the USDA germplasm repositories. Two replications of 208 oilseed *Brassica* accessions from the USDA collection of Ames, Iowa and 9 additional control cultivars were planted at Tifton, Georgia and Moscow, Idaho in the fall of 1987. Essentially, all the lines survived at Tifton. At Moscow, only one *B. campestris*, eight *B. napus*, and the four *B. juncea* lines did not survive the winter. There was considerable variation in plant heights, time of flowering, stem coloration, intensity of flower color, and agronomic performance. It is hoped that these evaluations will identify accessions adapted to these potential production areas as well as locate useful marker genes.

ARABIDOPSIS PLANTS WITH ALTERED SEED STORAGE LIPIDS OBTAINED BY CHEMICAL MUTAGENESIS

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To identify the genes that effect desired changes in the FA composition of rapeseed (*Brassica napus*), we are studying *Arabidopsis*, a diploid crucifer with a small genome that is easily transformed and regenerated. The approach involves "transposon tagging," whereby genes associated with FA biosynthesis are first identified using chemical mutagenesis. Chemically induced mutant plants are then crossed with plants carrying transposons. Progeny are screened for phenotypes resembling the chemically induced mutant parents, indicating that these individuals would be carrying the transposon at the same locus. Transposon-specific probes can then be used to identify the location of the gene. We have run FA analyses on about 2000 pedigreed M_3 seed collections from EMS mutagenesis and have found over 60 phenotypes in which the composition differed from wild type in at least one FA by greater than 4 standard deviations. After re-screening siblings of the phenotypes first identified, three important heritable mutants were chosen for transposon tagging: G30, lacking 18:3; 4A5, deficient in 18:2 and 18:3; and 9A1, lacking all FA's greater than C18. Characteristics of these and other mutants and progress in obtaining plants carrying transposons will be discussed.

EVOLUTIONARY TRENDS IN THE *BRASSICA OLERACEA* CYTODEME: CYTOGENETIC AND MOLECULAR EVIDENCE

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A series of 25 genomic and cDNA RFLP probes and 8 isoenzyme loci were employed to survey the genetic polymorphism of 8 cultivated types and 10 wild species. Extensive polymorphism was disclosed by the molecular markers. They were useful to confirm the close evolutionary proximity of the wild to the cultivated species in this cytodeme. However, no clear trends were found to assign a specific wild species as ancestor of a specific cultivated type. It was possible to follow up specific markers from more than one wild species in accessions of the same cultivated type. In general, the polymorphism of the wild species was represented in the cultivated forms. Therefore, the wild and cultivated species of the *Brassica oleracea* cytodeme form a continuum of closely related species serving as a gene pool in the development of the different cultivated types. Morphological differentiation of the wild species may be the result of geographic isolation by colonization of different territories and islands in the Mediterranean Basin. Pollen fertility in the analyzed F_1 populations falls into two distinct classes (low and high fertility). Observations of meiotic behavior of chromosomes in the F_1 hybrids show a direct relationship between low fertility and karyotypic changes such as chromosomal translocation. Six different F_2 populations from the different fertility groups are now being analyzed for linkage relationships. Distortion of expected segregation ratios for the markers will measure the level of evolutionary divergence among these species.

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SEGREGATION AND REARRANGEMENT OF MITOCHONDRIAL DNA IN SOMATIC HYBRIDS PRODUCED BETWEEN DIFFERENT SPECIES WITHIN BRASSICACEAE

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Three different combinations of somatic hybrids have been produced between more or less closely related species within the family of Brassicaceae. The somatic hybrids *Brassica oleracea* (+) *B. campestris*, *B. napus* (+) *B. nigra*, and *B. napus* (+) *Eruca sativa* have been confirmed as complete or partial hybrids after isoenzyme analysis. Chromosome number, fertility, and chloroplast genotype have also been studied (SUNDBERG *et al.*, 1987; SJODIN and GLIMELIUS, 1989; FAHLESON *et al.*, 1988). In this study mitochondrial DNA (mt-DNA) was isolated from the F_1 generation of the hybrids, and the restriction patterns of the mt-DNA were analyzed. The segregation of the mt-DNA is examined in order to study if differences in the mt-DNA segregation can be found between the different combinations of hybrids. The mt-DNA segregation will also be compared with the segregation of the chloroplast genotype in the hybrids. The extent of mt-DNA rearrangements is investigated in the hybrids, and the different hybrid combinations will be compared. We will study if specific regions of the mt-DNA are involved in the rearrangements.

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SECONDARY EMBRYOGENESIS IN *BRASSICA NAPUS* SSP. *OLEIFERA*

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The secondary embryogenesis potential of *Brassica napus* ssp. *oleifera* cv. Primor was maintained for over seven years. Many factors were found to affect secondary embryogenesis in culture. Secondary embryogenesis was significantly suppressed when strength of iron in the medium was reduced. Absence of micronutrients and vitamins had no effect on secondary embryogenesis. A reduced strength of macronutrients decrease secondary embryogenesis significantly. Secondary embryogenesis was significantly reduced when only part of the secondary embryoid was cultured. Incorporation of activated charcoal, gamma irradiation, etc. suppressed secondary embryogenesis.

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SCREENING *BRASSICA* GERMPLASM FOR FATTY ACID COMPOSITION

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The University of Idaho has initiated a program to systematically evaluate *Brassica* germplasm for fatty acid composition. The identification of rapeseed accessions which produce unique oils would allow increased production of this premium quality oilseed crop. Thus far, over 2100 *Brassica* accessions representing eleven species from twenty countries have been analyzed for seven common fatty acids found in rapeseed. The range of fatty acids found in the USDA Ames, IA germplasm collection are typical of all accessions analyzed. This collection of 364 accessions contained *B. napus*, *B. campestris*, and *B. juncea* genotypes. The ranges of fatty acids are: palmitic acid (16:0): 1.6-9.2; stearic acid (18:0): 0.0-7.2; oleic acid (18:10): 11.0-57.8; linoleic acid (18:2): 8.4-26.4; linolenic acid (18:3): 3.3-14.8; eicosenoic acid (20:1): 1.9-17.7; and erucic acid (22:1): 0.7-54.3. All ranges are expressed as percent of the methyl esters. Knowledge of the distribution and range of the fatty acid composition in known *Brassica* accessions will allow for breeding of specific traits into new varieties.

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DISSECTION OF THE *BRASSICA OLERACEA* GENOME WITH ALIEN CHROMOSOME ADDITION LINES

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The development of single *B. oleracea* chromosome addition lines from the resynthesized *B. napus* cultivar Hakuran is in progress. Syntenic gene arrangements have disclosed six of the nine possible addition lines, and additional DNA markers have been identified for at least one other syntenic group. Results of chromosome distribution in the second backcross generation obtained by gross chromosome counting and by transmission of chromosome-specific markers are in accord, indicating little segregation

distortion. However, distortion is observed in the third backcross generation. Of the six syntenic marker groups, few markers are present on more than one chromosome. An exception are the genes coding for 18S-25S ribosomal RNA, which are present on two (and perhaps three) chromosomes. Spacer variation between these two rDNA chromosomes has been maintained during the evolution of *B. oleracea*. Comparison of one *B. oleracea* synteny group with linkage analysis in *B. campestris* (syn. *B. rapa*) shows strong conservation of gene sequences. Other syntenic groups are currently being analyzed.

PATHOGENICITY GROUPING OF *LEPTOSPHAERIA MACULANS* ISOLATES BASED ON CULTIVARS OF *BRASSICA NAPUS*

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As part of a study into the pathogenic variability in *Leptosphaeria maculans* (Desm.) Ces & de Not. from different geographic regions, we studied the pathogenicity of 39 isolates from N. America, Europe, and Australia against a range of *Brassica napus* var. *oleifera* (oilseed rape) cultivars. Isolates could be categorized into four pathogenicity groups (PG) based on differential pathogenicity on cotyledons of Westar, Quinta, and Glacier. PG1 isolates can be distinguished by lack of virulence to Westar. PG2 isolates are virulent only on Westar but tend to give slightly more susceptible interaction phentoypes on Quinta than on Glacier. PG3 isolates are virulent on Westar and Glacier and intermediate on Quinta. PG4 isolates are virulent on all three cultivars. Using these cultivars as differentials we have examined about 70 single ascospore isolates from oilseed rape debris from Saskatchewan and Manitoba, Canada and from Western Australia and New South Wales, Australia. All Canadian isolates tested were PG2 types whereas isolates from Australia varied from PG2 through PG4. Isolates from Western Australia produced abundant pseudothecia, whereas Manitoba isolates failed to produce pseudothecia when paired. The significance of these results will be discussed with respect to blackleg disease of oilseed rape.

CHARACTERIAZTION OF CDNAS FOR RADISH CRUCIFERIN AND NAPIN STORAGE PROTEINS

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A cDNA library was prepared from radish immature seed mRNA. This library was screened with total homologous cDNA and cDNA prepared from dry or germinated seed mRNA. In this way, a number of clones corresponding to abundant mRNA in the immature seeds was selected. These clones were grouped on the basis of cross-hybridization and hybridization with heterologous probes from rapeseed cruciferin and napin. The identity of cruciferin and napin clones was confirmed by hybrid release translation experiments and sequencing. Napin is coded by two sets of closely related genes whereas cruciferin genes are more divergent. Two major cruciferin cDNA families, which poorly cross-hybridize in stringent conditions, were observed. One is more than 90% homologous to that described in rapeseed. Members of the other family range between 60 and 70% homology with the preceding one. cDNA probes corresponding to different members of these small gene families were used to detect variability in radish and rapeseed.

RAPESEED GENETIC RESOURCES IN CHINA

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China is one of the places of origin of *B. campestris* L. and *B. juncea* L. and possesses large rapeseed genetic resources. Twenty-three hundred rapeseed germplasm materials have been identified and evaluated at the Institute of Oil Crops Research where they are stored at 5°C (medium-term storage). Some varieties or lines are widely used in breeding programs. Some are already in production directly in various regions of China; however, others still need to be evaluated. According to the identification and primary evaluation of these lines in the US, some of the germplasm materials have desirable characteristics, *i.e.*, maturity, high oil content, cold resistance, resistance to some diseases, etc. which will be used in breeding to generate improved cultivars.

ASSESSMENT OF RAPID-CYCLING *BRASSICA* AS A BIOASSAY FOR DETERMINING EFFECTS OF TOXIC CHEMICALS

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An assessment of rapid-cycling *Brassica* (*Brassica rapa* CrGC 1-1 and *Brassica juncea* CrGC 4-1) to be used as test species in a plant bioassay to determine the toxicity of hazardous materials has been initiated. Various morphological and phenological measurements throughout the plant's life cycle have been examined to determine their utility as quantifiable endpoints. These include fresh and dry biomass; various stem and leaf measurements; and floral and reproduction parameters. Data from initial studies involving exposure to selected herbicides will be presented and will include examining the sensitivity of various endpoints to the chemical stress. Data will also be presented from studies in which rapid-cycling *Brassica* was substituted as test species in two bioassays (120 hour, Critical Life Stage, Seed Germination and Root Elongation Bioassays) routinely used at ERL, Corvallis, for toxicity assessment of samples from Superfund sites.

CHARACTERIZATION BY MOLECULAR MARKERS OF *DIPLOTAXIS ERUCOIDES* – *BRASSICA NIGRA* ADDITION LINES

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In an attempt to characterize the *B. napus* genome (B), chromosome addition lines were constructed by backcrossing an interspecific hybrid of *D. erucoides* × *B. nigra* to *D. erucoides*. Five monosomic and two double monosomic addition lines were identified by various isozyme loci (THIS

et al., 1988). The restriction fragment length polymorphism between *D. erucoides* and *B. nigra* was assessed by screening digested total genomic DNA of the two species using *B. napus* genomic clones (HOSAKA *et al.*, in preparation) along with other probes of diverse origin including a rDNA clone (pTA 71, APPELS and DVOŘÁK, 1982). The polymorphic probes were then used to screen the monosomic addition lines and to localize these markers on specific chromosomes. So far, we have observed the following synteny groups:

- chr 1: GOT1, napin gene
- chr 2: 6PGDH1, 6PGDH2, TPI1, pB485
- chr 3: PGM3, pTA71
- chr 4: MDH2, pTA71, pB177, pB485
- chr 5: pB488

Additional progenies are being generated in order to obtain the complete series for the *B. nigra* genome.

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Evaluation of *Brassica oleracea* var. *capitata* Plant Introductions for Resistance to Race 2 of Downy Mildew

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Evaluations for resistance against race 2 of downy mildew, incited by *Peronospora parasitica*, were conducted on 325 US Plant Introductions (PI) classified as *Brassica oleracea* var. *capitata* (cabbage). Five commercial cabbage cultivars (Rio Verde, Headstart, Market Prize, Bravo, and Sanibel) and two commercial broccoli (*B. oleracea* var. *italica*) cultivars (Green Duke and Waltham 29) were included as checks in all tests. Plants were inoculated at the two-expanded-leaf stage with 5.0×10^3 conidia per ml. Inoculated plants were incubated in a dark 16°C dew chamber for 24 h and were then placed in a 22°C growth chamber with a 12 h photoperiod. On the seventh day after inoculation, plants were returned to the dew chamber for 30 h. Individual plants were then rated for downy mildew reaction phenotype at nine days postinoculation on a 0-9 scale of increasing disease severity. An overall disease index (DI) was calculated for each entry. In 77 of the tested PIs from 2-100% of the plants had reaction phenotypes ≤3. The DIs for 24 PIs were significantly lower than the DI for the most resistant cabbage check, Headstart (DI=5.9). Eight of these PIs (199948, 199949, 263056, 357374, 418984, 418986, 418987, and 418988) were highly resistant, because all plants had a reaction phenotype of ≤3. However, several of these eight may be incorrectly classified as *B. oleracea* var. *capitata*.

Inheritance of Isozymes in *Brassica campestris*

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The inheritance of 12 isozyme coding loci in *Brassica campestris* is reported. Four cultivars of *B. campestris*: Candle, Torch, DH 716, and a rapid-cycling line were used. Plant extracts were prepared by crushing 25 to 30 mg fresh leaves in 0.04 ml of extraction buffer (0.1M Tris-HCl with 1% w/v reduced glutathione pH 7.5). Horizontal starch electrophoresis was used for most of the enzymes. Vertical polyacrylamide electrophoresis was employed for GOT. The inheritance of isozymes for eight enzymes was studied: phosphoglucoisomerase (PGI), leucine aminopeptidase (LAP), shikimic dehydrogenase (SDH), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), acid phosphatase (APS), alcohol dehydrogenase (ADH) and glutamate oxalacetate transaminase (GOT). The loci *Pgi-1*, *Pgm-1*, *Adh-1*, *Adh-2*, *6Pgd-1*, *Got-2*, *Got-4*, and *Got-5* were monomorphic. *Pgi-2*, *Lap-1*, *Aps-1L*, and *Pgm-1* were polymorphic with three allozymes each, whereas loci *Pgm-3*, *Sdh-1*, and *Sdh-2* had four. *Got-1* and *Got-3* were polymorphic with at least two allozymes each, whereas *Got-2*, *Got-4*, and *Got-5* were monomorphic. The alloenzymes in the polymorphic genes segregated in a Mendelian fashion. Most of these enzyme loci have been reported in *Brassica oleracea*.

Improvement in Earliness and Firmness of a Cauliflower Inbred Through Protoplast Regeneration

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Self-pollinated progeny of plants regenerated from leaf protoplasts of cauliflower inbred NY7642B were grown along with the parental line and commercial cultivars. Most of the protoplast-derived lines matured earlier than 7642B. Plants differing in earliness and selected on the basis of size, color, shape, and firmness were self-pollinated; their progeny were again earlier than 7642B. In a replicated field trial, the self-pollinated progeny of one of these plants were evaluated for earliness, firmness, color, shape, overall quality, seed set, and yield. These progeny were significantly earlier and firmer than 7642B; they had better overall quality and were at least as good as 7642B in the other characteristics.

Molecular Systematics of the Genera *Sinapis* and *Raphanus* in Relation to Major *Brassica* Lineages

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Comparative restriction site mapping of chloroplast and nuclear ribosomal DNA of ten *Sinapis* taxa, two *Raphanus* species, and representatives of two major *Brassica* genomes was carried out by filter hybridization of ^{32}P-labelled *Brassica* chloroplast DNA fragments and wheat and radish ribosomal DNA to blots of total genomic DNA. The analysis of 19 restriction endonucleases revealed four major

lineages, each distinguished by 10 to 30 polymorphisms. There was little or no variation among members of each lineage. These included: I) *S. alba* L. ssp. *alba* (n=12), [*Brassica alba* = *B. hirta*] and ssp. *mairei* (H. Lindb.) Maire, and *S. flexuosa* Poir. (n=12); II) *S. aristidis* Coss. (n=9), *S. boivinii* Baill. (n=18), *S. indurata* Coss. (n=9), and *S. pubescens* L. (n=9) ssp. *pubescens* and ssp. *virgata* (Battnd.) Baill.; III) *S. arvensis* L. (n=9) and *B. nigra* (n=8); and IV) *S. aucheri* (Boiss.) Schultz, *B. campestris* L. (= *B. rapa* L.) (n=10), *R. raphanistrum* L. (n=9), and *R. sativus* L. (n=9). Sectional classification of the genus *Sinapis* by Schultz (1936) was supported by the molecular data. Lineage I and III corresponded to the annual Sections SINAPIS and CERATOSINAPIS, respectively, and lineage II to the perennial Section ERIOSINAPIS. Anomalous in the genus, *Sinapis aucheri* (Section CHONDROSINAPIS), is morphologically and cytologically unique (n=7), but its restriction sites were indistinguishable from the *B. campestris* and *Raphanus* lineage. The genus *Raphanus*, which has a controversial position within the Tribe Brassiceae, clearly belongs with the sub-tribe Brassicinae.

Cloning and Expression of Acetolactate Synthase Genes in *Brassica napus*

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Acetolactate synthase (ALS) is the first enzyme in the pathway of synthesis of the animal-essential branched-chain amino acids. It is the site of action of three classes of herbicides, and point mutations in ALS genes confer resistance to these herbicides. The important agronomic species *B. napus* contains three or more highly ALS-homologous sequences. We have isolated DNA fragments that correspond to two of these genes. One of these genes is a genomic *Xba I* fragment (3.3 kb in length) that contains 780 bp of 5'-promoter region, and a 2 kb open reading frame which does not appear to be interrupted by introns. Site-directed mutagenesis of this sequence at codon 172 followed by re-introduction into plants via *Agrobacterium* results in increased tolerance to sulfonylurea herbicides. A second ALS gene has been isolated from a *B. napus* seedling cDNA library. Both ALS sequences contain unique regions which distinguish them from the others in *B. napus*. These sequences have been used as probes to examine if and where they are expressed in *B. napus*. These probes also indicate that the genomic clone sequence originated in one of the progenitors of *B. napus*, the diploid species *B. campestris*; whereas the cDNA clone sequence appears to have originated in the other progenitor, *B. oleracea*.

Tendencies in Fatty Acid Composition of Various Cruciferous Seeds Native to Israel

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Seeds from four Cruciferae species, native to Israel, have been examined for fatty acid composition. The goal was to identify from the wild gene collection species rich in fatty acids useful for industry. Species rich in erucic acid (C22:1), such as species of *Crambe* and *Sinapis*, are low in polyunsaturated linolenic acid (C18:3) as well as in the saturated palmitic acid (C16:0). The same negative correlation is observed in species low in erucic acid (species of *Sisymbrium* and *Lepidium*), in which 44% and 42% linolenic acid was found, respectively. No relation was found between the content of erucic and oleic acids, as opposed to the situation in cultivated rape seeds.

Manipulation of the *Brassica* Cytoplasm by Protoplast Fusion

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Characters such as male sterility and resistance to the triazine herbicides are genetically associated with different cytoplasms in the *Brassicas*. These traits can play an important role in the production of F_1 hybrids in the oilseed rape form of *B. napus*, and to the potential improvement of this crop. Protoplast fusion allows the production of novel combinations of cytoplasmic organelles to produce cytoplasmic hybrids or cybrids. Many of the various cybrids we have produced are currently at the fourth backcross generation in our breeding program. With few exceptions, the cybrids are generally agronomically normal. Whereas no DNA recombination has been detected in our cybrids, the 11.3kb mitochondrial plasmid has been demonstrated to "migrate" from one fusion parental chondriome to the other, intimating the potential for "transformation" of mitochondrial DNA.

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