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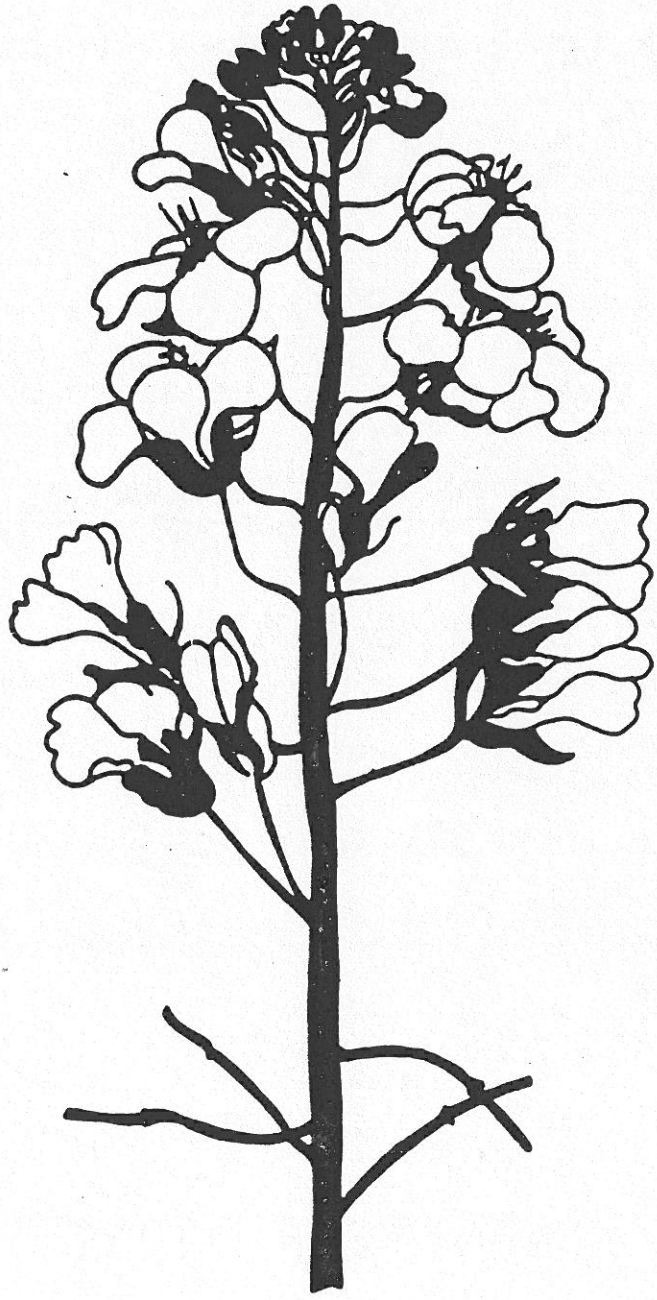

NOVEMBER 1988
EUCARPIA

CONTENTS

Editorial

A gratifying number of articles was again submitted for this issue of the Newsletter. Although space was not available to publish all of these the editors hope that the flow will be maintained for future issues. Articles were selected for publication according to date of receipt and adherance to guidelines.

The distribution by surface mail of the previous issue did not provoke any unfavourable responses and so is being extended this year to include Australia and China.

A.B. Wills retired from SCRI at the end of 1988 and T. Hodgkin will leave for a post at IBPGR, Rome during 1989. They apologise for delays in publication caused by these moves. The future editorship of the Newsletter is to be discussed at the Eucarpia Congress in Gottingen in February - March 1989 and information will be provided with the next call for papers. In the meantime any correspondence should still be sent to the present editors at the address below.

The editors thank Wendy Craig for assistance in the preparation of this issue.

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Generous donations totalling £2967.00 were received from the undernoted companies to whom the editors are deeply grateful.

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REPORT OF INTERNATIONAL CLUBROOT WORKING GROUP MEETING

G R Dixon and H Ikegami

Meetings were held on 21 and 22 August 1988 during the 5th International Congress of Plant Pathology, Kyoto, Japan. Participants represented clubroot interests in Australia, Denmark, Federal Republic of Germany, Japan, Republic of China, UK and USA.

A revised mailing list of active clubroot workers was offerred to those interested, meetings at St. Andrew's, Scotland, (1984) and California, USA, (1986) were reviewed and participation in the 1989 Eucarpia Congress at Gottingen FRG publicised. The offer of committee status within ISPP was accepted. Revival of Clubroot Newsletter was rejected in favour of continued publication in Cruciferae Newsletter, this reaches a far wider audience.

Discussion topics:

Mycology, life cycle and ultrastructure

Electron micrographs of zoosporangia were presented (Tanaka, Japan), linked with discussion of secondary zoospore formation (Matsumiya, Japan). Secondary zoospores are present in root hairs one week after infection even in resistant hosts. The significance of nucleoli failing to form and possible presence of preformed mRNA templates was discussed (Ingram, UK). Possible effects of prolonged storage on resting spore viability was raised as in previous meetings. The relationship between resting spore age and zoospore maturity was examined. ICWG members who possess aged stocks of galls or infected soil were requested to screen these for viability. Pooled results might give an insight into the extent of longevity. The complexity of resting spore walls was referred to by Crute (UK) and Schlosser (FRG). Questions concerning the structure of plasmodia in root hairs and the development of sporangia were examined (Zinkernagel, FRG). Possible stimulation of spore hatching by root exudates from non cruciferous plants (Suzuki, Japan) led to considerable discussion. Others suggested that hatching might also be caused by rhizosphere micro-organisms. Crute (UK) postulated that germination could be triggered over a prolonged period irrespective of external factors in a manner analgous to seed germination. Porter (Australia) argued that resting spores may become highly dehydrated, and rewetting may be extended leading to sporadic germination. Overall there are urgent needs for research concerned with resting spore germination, nuclear and cytoplasmic development in zoospores, mating studies, the role of non crucifer hosts, variation between alternate hosts and cycling of primary zoospores in root hairs.

Inoculum measurement

Use of fluorescent antibody techniques to detect resting spores in soil was illustrated by Aria (Japan). Differentiation of resting spores from particles of soil and attachment to root surfaces demonstrated. No cross reaction with the antibodies and Polymyxa was

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detected hence this is a highly specific test. Lange (Denmark) explained the use of Fluxen B to stain dead spores, commenting on possible quarantine applications.

Pathogenicity and virulence

Conflicting results came from FRG and UK on the specificity of P. brassicae strains towards oil rape and other brassicas. All agreed that cultivars of OSR are highly susceptible. Phenotype expression might be further studied by the use of single spore isolates (Ingram, UK), this should be linked with other studies to elucidate inheritance mechanisms.

Resistance and Genetics

Calabrese breeding studies by Robak (Poland) were communicated by Gabrielson (USA). Chinese cabbage is of major agricultural significance in Japan with urgent necessity of producing clubroot resistant types (Yoshikawa and Yamagishi Japan). Severe crop losses are caused by P. brassica. Cultivars with resistance have been released by Japanese seed companies and the National Research Institute for Vegetables, Ornamentals and Tea (NIVOT). Resistance has been derived from turnip. Other sources of resistance have been identified in cabbage, Brussels sprout, kale, and rutabaga. There is evidence of resistance erosion from 5 locations. A survey of resistance sources in Brassica types was presented by Crute (UK) a new source of resistance has been identified in cattle cabbage from Eire. Discussions at the Crucifer Genetics Workshop Wisconsin, USA, 1987 suggested the formation of 'Plasmodiophora brassicae Recurrent Selection Group' (PBRSG). Those interested should select for resistance in local cultivars. Pollen could be sent to Wisconsin (see HORTSCIENCE, 15, 802-3 for techniques), together with P. brassicae isolates. Morphotypes with resistance would be generated and returned. Interested workers should contact Williams (Madison, USA).

Environment Interactions and Control

Chemical controls in Japan using PCNB, TPN (tetrachloro-isophthalonitrile) and trichlamide were reviewed Ikegami (Japan). Reasons for the unreliability of PCNB due to high spore densities were examined by Horiochi (Japan). A detailed survey of husbandry controls was presented (Zinkernagel FRG). Solarization has provided control and combination with soil fumigation increases effectiveness (Porter, Australia). The role of calcium, boron and pH in this host pathogen interaction were discussed (Dixon, UK, see CROP RESEARCH, 28, 83-95) calcium and pH retard the primary stages of sporogenesis in root hairs, boron also affects cortical stages. Toxopeus (The Netherlands) communicated a suggestion to develop an International Differential Series (ICD). Gratitude was expressed to Dr. D. Astley, Institute for Horticultural Research, UK, for reproduction maintenance and dispatch of ECD series.

Professors Dixon and Ikegami thanked the organisers of 5th ICPP for finance and facilities which permitted these meetings to take place.

BIOCHEMICAL, MOLECULAR BIOLOGICAL AND BIOTECHNOLOGICAL STUDIES ON OILSEEDS

Denis J. Murphy

INTRODUCTION

The Durham Oilseeds Research Group was set up in 1986 with the aim of furthering both fundamental and applied research aimed at the production of improved oilseed crops, both in the UK and overseas. The Group consists of a multidisciplinary team including specialists in the latest techniques of modern molecular biology, immunology, lipid and protein analysis, enzymology and microscopy. Some of the projects with which the Group is involved are listed below.

OLEINS - A NEW CLASS OF HIGHLY ABUNDANT PROTEINS IN OILSEEDS

Storage triacylglycerols in oilseeds are located in organelles, termed oil-bodies, which exhibit many similarities to mammalian chylomicrons. Like the latter, the seed oil-bodies are bounded by a lipoprotein-containing electron-dense membrane, which also contains small amounts of phospholipid (1,2). The protein component of oil-body membranes has been investigated in a wide variety of oleiferous seeds. In the case of the Cruciferae, which includes the cabbages, mustard, rapeseed and radish, the oil-body protein is mostly (60-70%) made up of a single hydrophobic polypeptide of between 18 and 20 kDa, depending upon the species (3). This highly abundant Family of oil-body lipoproteins which we term "OLEINS", constitutes some 20% of the total protein in mature seeds. The major oil-body lipoproteins (oleins) have been purified from a range of Cruciferae and other oilseed species and mono-specific antibodies have been raised against them. A high degree of immunological cross-reactivity was found between oleins within, and in several cases, between different plant Families. Amino acid analysis has revealed compositional similarities. Proteolytic mapping followed by immunoblotting has confirmed the close structural relationship between oleins from different species. A cDNA expression library in the phage λgt_{11} is now being screened for the olein cDNA from rapeseed. Further work will be aimed at characterising in detail the structural and functional properties of this new group of plant proteins and their possible relationships with analogous lipoproteins in animals.

REGULATION OF SEED OIL QUALITY

A very desirable atrribute of a seed oil, from the viewpoint of the end-user, is that it should have a homogeneous fatty acid composition. In many seed triacylglycerols the C_1 and C_3 fatty acids are identical but the C_2 fatty acid is different. This imposes a theoretical upper limit of 66% on the level of a particular desirable fatty acid in the seed oil. The enzyme responsible for insertion of acyl moieties onto the C_2 position is Acyl-CoA:lyso-phosphatidic acid acyltransferase (LPA-AT). This enzyme may play an important role in the regulation of the acyl composition of seed oils. LPA-AT

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has been studied in developing cotyledons of oilseed rape. The major LPA-AT activity in this tissue is associated with a light membrane fraction which on sucrose density gradients co-purified with enzyme markers of the endoplasmic reticulum (ER). The enzyme was specific for 18:1-CoA, rather than 16:0-CoA, which is the opposite specificity of the LPA-AT isolated from leaf chloroplasts. It is concluded that the major LPA-AT activity in rapeseed cotyledons is associated with the ER. The LPA-AT was efficiently solubilised in a CHAPS system and further purified by PEG precipitation, ion-exchange chromatography and preparative electrophoresis. Further studies will be directed towards the isolation of a cDNA clone for the LPA-AT from developing embryos of oilseed rape.

BIOSYNTHESIS OF STORAGE OILS AND PROTEIN DURING EMBRYOGENESIS IN OILSEED RAPE

The timing of the major biosynthetic activities of maturing rapeseed embryos has been studied. Storage oil was measured by gravimetric analysis and by quantitative gas-liquid chromatography. The major soluble seed storage proteins, cruciferin and napin, were quantified by ELISA (enzyme-linked immunosorbent assay) and by densitometric scanning of polyacrylamide gels or immunoblots. The major oil-body lipoprotein, olein, was measured by ELISA and by a PAGE-densitometry. None of these storage proteins has any known catalytic activity and they cannot, therefore, be assayed enzymatically.

The results showed that developing rapeseed embryos initially undergo a 2-3 week cell division phase, during which little or no biosynthesis of storage products occurs. After the 3rd week (post anthesis) storage oils are made as large oil-bodies, lacking an electron-dense delimiting membrane. Napin and cruciferin formation does not begin until week 5-6. The final biosynthetic stage occurs at about week 8 when olein formation starts and the oil-bodies simultaneously acquire a strongly electron-dense boundary membrane. The three different classes of biosynthetic event in rapeseed embryos, i.e. oil synthesis, napin/cruciferin synthesis and oleinsynthesis, are separated by several weeks. We conclude from these and other data, that it is likely that the difference in the timing of these different biosynthetic activities is caused by the existence of several distinct types of seed-specific gene promotor.

STUDIES ON LIPASES FROM OILSEEDS

An anti-lipase antibody has been used to investigate the lipases of cotyledons from germinating seedlings of oilseed rape, Brassica napus, var Mikado. Immunological and enzymatic assays revealed that lipases were confined to germinating seeds and was absent from other tissues, such as developing seeds, leaves, roots and flowers. The antibody totally inhibited microsomal lipase activity at a ratio of 12 µg IgG protein: 10 µg microsomal protein. It was shown by immunoblotting of microsomal, oil-body and total cotyledon proteins separated by gel electrophoresis that, in each case, the antibody specifically bound to a single polypeptide. This polypeptide had an electrophoretic mobility consistent with a molecular weight of 56 kDa.

The appearance and subsequent decline of the 56 kDa polypeptide during the first ten days of germination closely followed that of the lipase activity, both in microsomal protein and total cotyledon protein fractions. An ELISA for the lipase demonstrated that the amount of microsomal lipase present at each stage of germination was proportional to its enzymatic activity. Antibody binding to lipase in microsomal fractions was substantially stronger, both on a protein basis and a lipase activity basis, than the binding to the lipase in oil-body fractions. The enzymatic and immunological activities of the microsomal lipase each co-eluted from sucrose density gradients with an endoplasmic reticulum marker enzyme. The lipases are present in only very small amounts, if at all, in dry seeds and are synthesised de novo after 2-3 days of germination (4,5).

Further studies have shown that the rapeseed lipases exhibited broadly similar substrate specificities (6) and that the castor bean anti-lipase cross-reacted with a single polypeptide of about 60 kDa in at least 10 other species of oilseed (7). Oilseed lipase activity may be regulated by the levels of oleoyl-CoA and free CoA during lipolysis (8). The lipases from germinating rapeseed have been solubilised and are now being purified in our laboratories.

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WISCONSIN FAST PLANTS

P H Williams

Wisconsin Fast Plants is a program involved in the development of educational materials based on rapid cycling brassicas and other species. With support from a U.S. National Science Foundation, WFP kits have been developed which include a self contained growing system (using cool white fluorescent lamps), growing instructions, and specialized genetic stocks of rapid cycling Brassica rapa adapted to the growing system. A number of exercises illustrating various aspects of plant growth, reproduction, physiology, and ecology have been developed for college, high school, and elementery science classes. The WFP educational kits have been licensed for manufacture and distribution to the Carolina Biological Supply Co. by the Wisconsin Alumni Research Foundation.

WFP has the potential of becoming an important model for science education in which innovations in biological research and teaching are brought together in the classroom to enhance learning through exploration. Representing species of enormous diversity of plant form and utilization, the rapid cycling brassicas with their accelerated life cycle and petite growth habit are ideal organisms for hands-on biology from kindergarten through college. Germinating in less that 12 hours and flowering in 2-3 weeks, these fast plants with their supporting growing systems permit all aspects of plant growth, reproduction, genetics, physiology, and ecology to be explored in the laboratory or classroom in less than a month.

Introduced in the fall of 1986, WFP workshops have been held at various locations in the United States and Canada. Enthusiasm on the part of students, teachers, and communities in which the fast plants have been used in the classroom has demonstrated their potential for enhancing the quality of science education. There is widespread interest in the WFP among plant scientists who see the program as a means of bringing together the most current discoveries in plant biology with information on the development and uses of rapid cycling plants as new instructional materials to enhance biology education.

Central to the WFP program are the rapid cycling experimental seed stocks and supporting Information Documents (IDs) created by specialists from the scientific and educational communities. Technical, experimental, and educational information supporting the uses of living fast-plant materials are available to members of the Crucifer Genetics Cooperative (CrGC) as WFP information documents in printed, electronic, audio, video, and multi-media forms. Numerous IDs suitable for college and precollege biology at all levels are being developed by participants in the WFP program.

Persons interested in the WFP should indicate this on the CrGC Membership Information form (CMI) or write to Wisconsin Fast Plants, Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706 or phone (608)263-2634.

Dept. of Plant Pathology, Univ. of Wisconsin, 1630 Linden Drive, MADISON, WI 53706, U.S.A.

NEEP CROPS: A PROPOSED COMMON NAME FOR BRASSICA RAPA L.

Robert Prescott-Allen

Neep crops is here proposed as a common name for Brassica rapa L., equivalent to cole crops for B. oleracea L.

Neep is an English word for turnip that has fallen from common use except in dialect in Scotland and parts of England. It derives from the Old English náep. The word "turnip" itself is formed from "turn" (= round) + "neep" (Burchfield 1987).

A proposal of choi crops as a common name for B. rapa (Prescott-Allen 1985) has not won acceptance, largely on the grounds that choi (Cantonese for vegetable) is not specific to B. rapa (Toxopeus & Oost 1985). The proposal is now withdrawn.

Neep, however, is specific to B. rapa. It is a traditional English word for the species, but is no longer generally associated with any one form of the species. As such, neep crops is well suited as an umbrella term for B. rapa. Being short and sweet, neep can also be used readily in, for example:

1. Names for sets of cultivar groups: e.g., "seed neeps", "oilseed neeps", "inflorescence neeps", "leaf neeps", "non-heading leaf neeps" (= "tsukena" in Japanese), and "root neeps".

2. Compound formations: e.g., "radineep" as a common name for B. rapa x Raphanus sativus L. (corresponding to "radicole" for B. oleracea x R. sativus) (cf., Toxopeus 1985).

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CULTIVAR GROUP CLASSIFICATION OF BRASSICA RAPA L.: UPDATE 1988

H.Toxopeus, E.H.Oost, H.Yamagishi, R.Prescott-Allen

Cultivar Groups	Shared Character(s)
SEED NEEPS	
Winter Turnip Rape	biennial
Spring Turnip Rape	annual [includes cvs of toria, brown sarson, and European and North American forms]
Yellow Sarson	annual, yellow seeds, siliques usually multivalved
INFLORESCENCE NEEPS	
Broccoletto	early bolting [includes Asian (tsai tai, tsai hsin, saishin, nabana) and European (cima di rapa) forms]
LEAF NEEPS	
Chinese Cabbage	heading or semi-heading; petioles winged
Pak Choi	non-heading, petioles conspicuous, fleshy, not winged
Mizuna	many tillers [includes cvs of mizuna (pinnate leaves) and mibuna (entire leaves)]
Taatsai	flat rosette of many small dark green leaves
Neep Greens	non-heading [includes cvs of yau choi (yóu cài), komatsuna, "non-heading Chinese cabbage", zairainatane, kabuna, raapsteeltjes, and turnip greens]
ROOT NEEPS	
Vegetable Turnip	storage organ (swollen root/hypocotyl) used as vegetable; leaves may also be used
Fodder Turnip	storage organ (swollen root/hypocotyl) and upright rosette of large leaves used as fodder

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We were happy to receive a very substantial input to the discussion on our proposed cv group classification (Toxopeus, Yamagishi & Oost 1987), the result of which is particularly apparent in the comments below. We note with some satisfaction that most of the names and definitions have stood the test well.

For convenient reference, the updated classification is organized into four sets of cv groups with common uses and products: "seed neeps", "inflorescence neeps", "leaf neeps" and "root neeps" (neep crops is proposed elsewhere in this issue as a common name for B. rapa L.). It should be clear that these terms are not an integral part of the classification. The only formal category is the name of the cv group.

Guidelines for naming cultivar groups

The cultivar group is a pragmatic means of classifying crop species (such as B. rapa L.) that are highly polymorphic and include an increasing number of cvs from crosses between infraspecific taxa. The objective is to classify and name the variation of crop forms--including new forms--in a way that is scientifically sound, clear and unambiguous, meaningful (to crop scientists, breeders, seed companies, growers, consumers), and practical.

Article 26 of the International code of nomenclature for cultivated plants-1980 (ICNCP 1980) states that an assemblage of similar cvs may be designated as a group. If used with the specific name and a cv name, the group name should be placed in parentheses: for example, Brassica rapa (Chinese Cabbage group) 'Sakura'. No other guidance is given; but presumably most of the rules for naming cvs also apply to cv groups--in particular: no names in Latin form; and names of not more than three (and preferably one or two) words. The following additional guidelines are suggested:

a. If an unambiguous, well-established name for a cv group already exists, it should be adopted (e.g., Chinese Cabbage).

b. If not, the name should be in a language of the country where the cv group originated and/or was largely developed. Provided it is easy to use internationally, the name should be one that is generally accepted in that country (e.g., Mizuna). When several systems of transcription are available (as in Chinese), the one that is most easily used internationally should be adopted (e.g., Pak Choi rather than Bái Cài [Pinyin system of Mandarin] or Baahk Choi [Yale system of Cantonese]).

c. Alternatively, a name should be formed in another modern language. It should be unambiguous, as short as possible, and easy to pronounce (e.g., Broccoletto; Neep Greens).

Comments on update 1988

Seed neeps. These cv groups remain unchanged.

Inflorescence neeps. Yamagishi (elsewhere in this issue) reports that Asian cvs (formerly Saishin group) and European cvs (formerly Broccoletto group) are alike enough ecologically and morphologically to form one group. Asian and European cvs are organoleptically distinguishable (the latter usually have a stronger flavour); but the difference is not great enough to justify separating the two. Therefore we have combined all inflorescence cvs into one group. We propose **Broccoletto** as the name of the combined group. This name is not tightly (and therefore exclusively) linked with any one form; whereas Tsai Tai, Tsai Hsin, Saishin and Nabana are names for the Asian forms; and Cima di Rapa is apparently the usual name for the European form.

Leaf neeps. Two changes have been made. (1) The shared character(s) entry for **Chinese Cabbage** has been changed to accommodate semi-heading cvs. (2) The name of the leaf turnip group has been changed to **Neep Greens.** This is because we prefer to limit use of the term "turnip" to root neeps, except where it is already well established, as in "turnip rape". Moreover, when used in the same classification as "vegetable turnip", "leaf turnip" implies that cvs in this group are not vegetables.

Root neeps. The shared character(s) entries for these groups have been revised to be somewhat more descriptive.

Comments on the classification are welcome. In particular, we seek advice on the name of the **Taatsai** group. We are not certain that this is the best available transcription. Cao (1986) spells it both Tai Tsai and Ta Tsai.

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'BROCOLETTO' CULTIVATED IN JAPAN

H. Yamagishi

In eastern Asia, there are traditional vegetable groups of Brassica, of which the young inflorescences and flower stalks are used. Recently several F_1 varieties of these groups were released in Japan. Also in Europe a vegetable group of similar usage is known, and called as 'Brocoletto' (Toxopeus), 'Cime di rapa or Broccoli rabs' (Quagliotti). The characteristics of this European type vegetable was compared with Asian ones.

Five strains of 'Brocoletto' were introduced from the Center of Genetic Resources of the Netherlands and cultivated twice. The seeds were sown on August 28th in the field in the first cultivation, and on January 27th in the glass house in the second one. For the comparison Japanese varieties of 'Nabana', 'Saishin' (Both belong to a cultivar group 'Saishin' of Brassica rapa L.:Toxopeus et al.) and 'Kairan' (Brassica oleracea L. var. alboglabra) were cultivated. The investigated characters are shown in Tables 1 and 2.

Although one of the five strains din not initiate buds within 4 months in the field and 2.5 months in the glass house, other four strains flowered. In the field three of the four strains flowered within 3 months after sowing and in the glass house all four strains flowered within 2 months with about 15 leaves. The size of leaves was similar with those of F_1 varieties of 'Nabana and Saishin'. The diameter of the inflorescence and the number of branches were larger than the Japanese varieties, but the general morphology of the inflorescence resembled that of 'Nabana'. The variations of flowering date within the strains were larger than the F_1 varieties (Table 2).

From these, the major strains of 'Brocoletto' showed similar characteristics of flowering and morphology with the Japanese varieties. So, in spite of the different origin, it is thought that there are no reasons to classify the 'Brocoletto' into another group from the Asian one, from the ecological and morphological view points.

Table 1. Characteristics of 'Brocoletto' cultivated in the field.

Strains and Varieties	Days to flowering	Leaf length	Leaf width	Diameter of inflorescence
		cm	cm	cm
87-275	68	46	20	6
87-276	80	47	19	7
87-278	>100	44	20	5
87-279	91	42	18	6
Soyo No.1[a]	76	39	17	4
Chyugoku Saishin[b]	75	45	20	3
Hakushin[c]	72	34	17	6

a: F_1 variety of 'Nabana'. b: OP variety of 'Saishin'. c: F_1 variety of 'Kairan'.

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Table 2. Characteristics of 'Brocoletto' cultivated in the glass house.

Strains and Varieties	Days to flowering		Plant height		No. of leaves		No. of branches	
	M[d]	SD[d]	M	SD	M	SD	M	SD
			cm					
87-275	52	6.4	34	6.6	14	2.7	8	1.5
87-276	54	6.2	37	10.8	15	2.0	9	1.9
87-278	64	3.3	42	5.5	16	1.3	8	1.3
87-279	59	3.9	45	7.4	15	1.3	10	2.6
Soyo No.1[a]	49	2.4	46	10.8	16	1.3	6	1.8
Honkon Saishin[b]	43	2.5	34	6.3	10	0.5	5	0.7
Hakushin[c]	65	4.8	39	4.9	10	1.2	2	0.5

a: F_1 variety of 'Nabana'. b: F_1 variety of 'Saishin'. c: F_1 variety of 'Kairan'. d: Mean value (M) and standard deviation (SD) of the strain or variety.

ACTIVITIES OF THE CRUCIFER GENETICS COOPERATIVE IN 1988

P H Williams

Membership in the Crucifer Genetics Cooperative is more than 1200, representing 48 countries. As of September of 1988 there were 557 subscribing members and 26 sustaining members. During 1987 more than 2000 packets of seed were dispersed. Seventy stocks in 17 species of Brassica, Raphanus, and other crucifers are available. A substantial number of new rapid cycling stocks will be added to the collection in 1988-89. The CrGC has added the Koornneef collection of Arabidopsis thaliana mutants and chromosome markers to its collection and these will be available in 1989. Seed stocks are available for $3.00 U.S. per packet payable to the CrGC. Subscribing members are entitled to 5 packets of seed at no additional cost and receive the Resource Manual, a member list, and annual additions of information documents.

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STUDIES ON THE HYBRIDIZATION AND EVOLUTION OF DIPLOTAXIS DC. (CRUCIFERAE, BRASSICEAE)

Juan B. Martínez-Laborde

The present genomic pattern in Diplotaxis DC. consists of more than 20 diploid species -with six different haploid numbers, namely n = 7, 8, 9, 10, 11 and 13- and only one species -D. muralis, with n = 21- considered to be an amphidiploid resulting from the cross D. tenuifolia (n = 11) X D. viminea (n = 10) (Harberd & McArthur, 1972).
Interspecific hybrids in Diplotaxis seem to occur very seldom in nature. Different species can share the same habitats and even constitute more or less mixed populations in the field, but true intermediates, putatively hybridogenous specimens are very rare. However, artificial crosses have been accomplished by several authors, working with taxa of n = 7 (at the subspecific level) and n = 8 (Gómez-Campo, 1981), n = 10, 11, 21 (Harberd & McArthur, 1972; Sobrino Vesperinas, 1979) and n = 13 (Sobrino Vesperinas, 1985) gametic chromosomes. In most cases the fertility of the hybrids was reported to be very low.
Except for the experiments that led Harberd (1972) to class D. assurgens, D. catholica, D. tenuisiliqua and D. virgata (the latter from the Iberian Peninsula) into four different cytodemes, no reports of crosses among the n = 9 species have been found.

From a number of preliminary crosses among species of Diplotaxis with n = 9 chromosomes, including those mentioned above as well as D. delagei and Spanish and Moroccan populations of D. virgata, several hybrid plants have been obtained. The successful combinations and the materials used are shown in Table 1. In most cases the general morphology of the F1 plants was intermediate with respect to that of the parental species. Although no fertility tests were carried out, the abortive-like anthers and the absence of mature seed in the pods indicated that hybrids were highly sterile.

The considerably low degree of fertility of interspecific hybrids in Diplotaxis reported by previous authors seems to be confirmed by the above results and suggests that strong incompatibility barriers have arisen among the genetic systems of these species. The consideration of chorological aspects shows that allopatric (e g D. ibicensis, from the Balearic Islands, and D. siettiana, from the small island of Alboran) as well as sympatric (e g D. assurgens and D. tenuisiliqua, from Central Morocco) species seem to be reproductively isolated.
Appart from the fact that allopolyploidy might conceivably have taken part in the processes that originated the disploid series in the genus (Harberd, 1976), other ploidy changes and hybridization are almost absent from the genus. Disploidy and reproductive isolation, on the other hand, seem to have played a major rôle in the evolution of Diplotaxis.

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Table 1.- Interspecific crosses performed among n = 9 species of Diplotaxis. Populations utilized are identified with the corresponding accession numbers (letters GC followed by a four figure number) to the Germplasm Collection kept at the Departamento de Biología Vegetal de la Escuela T. S. de Ingenieros Agrónomos, Madrid.

♀ PARENTAL	♂ PARENTAL
D. assurgens GC-1076	D. virgata GC-1099 (Morocco)
D. assurgens GC-1076	D. virgata GC-5540 (Morocco)
D. assurgens GC-1076	D. virgata GC-6691 (Spain)
D. catholica GC-1390	D. delagei GC-6478
D. tenuisiliqua GC-1956	D. virgata GC-5540 (Morocco)
D. tenuisiliqua GC-6532	D. assurgens GC-1076

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FURTHER STUDY ON THE HYBRIDIZATION BETWEEN SYNTHESIZED TRIGENOMIC HEXAPLOID AND CULTIVATED SPECIES IN GENUS BRASSICA

T. Takeda and Y. Takahata

Takeda et al.(1985) reported that the synthesized trigenomic hexaploids (genome AABBCC) could be utilized as a bridge plant to transfer genes from one species to another. However, the crossabilities with monogenomic species were lower than those with digenomic species. In order to improve the crossabilities, we carried out the hybridization of another hexaploid strain (CaC-333) with crop Brassicas and autotetraploids of monogenomic species. Some crosses were also carried out, using the trigenomic hexaploid as a male parent.

The results of hybridization are shown in Table 1. The crossabilities of CaC-333 x crop Brassicas were similar to previous results (Takeda et al. 1985). On the other hand, the crossabilities with monogenomic species increased when the autotetraploids were used as a male parent. This phenomenon was similar to the hybridization of B. napus x B. oleracea. The hybrids could be also obtained in the reciprocal crossing (crop Brassicas x trigenomic hexaploid), though the fertility was always low. This low fertility may be partially due to the production of abnormal pollen in the hexaploid.

As shown in Fig. 1, the combination of the crossabilities of the present study with those from our previous study indicated that, 1) crossability of the hybridization with digenomic species was always higher than that with monogenomic ones, 2) the species containing A genome could be easily hybridized as compared with the species without A genome, 3) higher crossability was obtained when trigenomic hexaploids were used as a female parent, 4) crossability with monogenomic species increased by using the autotetraploids as a male parent. These results recall to us the possibility of the presence of crossability genes in Brassica like Kr in wheat. The hypothesis about inter-specific cross such as Endosperm Balance Number hypothesis (Johnston et al. 1980) and polar-nuclei activation hypothesis (Nishiyama and Yabuno 1978) is likely to be applied to Brassica, too.

We wish to thank Dr. Y. Matsuzawa, Utsunomiya University and Mr. S. Suto, University of Tsukuba for providing the seeds of 4x B. oleracea and 4x B. campestris and the plant of 4x B. oleracea.

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Table 1. Crossabilities between trigenomic hexaploids and cultivated species in Brassica

Cross combination	No. of flowers pollinated	No. of seeds obtained	No. of seeds per pollination
CaC-333(AABBCC) x B.nigra(BB)	177	19	0.11
CaC-333(AABBCC) x B.oleracea(CC)	382	7	0.02
CaC-333(AABBCC) x B.campestris(AA)	824	314	0.38
CaC-333(AABBCC) x B.carinata(BBCC)	311	395	1.26
CaC-333(AABBCC) x B.juncea(AABB)	434	826	1.90
CaC-333(AABBCC) x B.napus(AACC)	312	807	2.59
CaC-333(AABBCC) x B.campestris(AAAA)	65	33	0.51
CaC-334(AABBCC) x B.campestris(AAAA)	57	103	1.81
CaC-334(AABBCC) x B.oleracea(CCCC)	164	48	0.29
B.nigra(BB) x CaC-333(AABBCC)	21	0	0.00
B.oleracea(CC) x CaC-333(AABBCC)	170	1	0.01
B.campestris(AA) x CaC-333(AABBCC)	102	30	0.29*
B.carinata(BBCC) x CaC-333(AABBCC)	65	9	0.14*
B.juncea(AABB) x CaC-333(AABBCC)	28	20	0.71*
B.napus(AACC) x CaC-333(AABBCC)	155	27	0.17*

( ): Genome symbol
*: These are higher than the true crossabilities, because the false hybrid seeds are included in the seeds obtained.

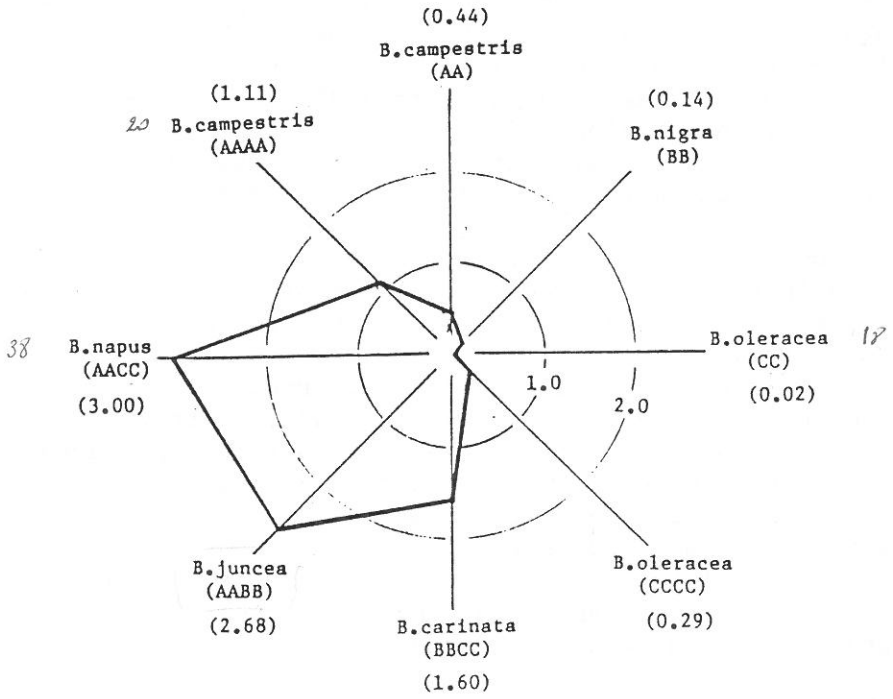

Fig. 1. Mean crossabilities of three trigenomic hexaploids (CaC-333, CaC-334, JO-125) x crop Brassicas based on the present results and previous ones.

PHYLOGENETIC RELATIONS OF B. RAPA AND B. OLERACEA AND THEIR WILD ALLIES REVEALED BY NUCLEAR RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS)

K Song, T Osborn and P H Williams

This is a brief outline of our recent study on the origin of B. rapa and B. oleracea. In previous studies (Song et al. 1988a and 1988b), we hypothesized that B. rapa and B. oleracea originated from a common ancestor which is probably a nine chromosome wild species. The following experiment was conducted to examine this hypothesis and to detect the relationships between cultivated forms and their wild allies.

Thirty-eight accessions were chosen, including ten cultivated and wild B. rapa accessions, nine cultivated B. oleracea accessions, 13 nine chromosome wild populations, and six other species. Thirty-eight nuclear DNA probes and two chloroplast DNA probes were used in detecting RFLPs. A total of 433 fragments were scored across all accessions. Six fragments were found to be common for all accessions, 92 fragments were unique and 335 fragments were phylogenetically informative and thus were used in constructing phylogenetic trees. The data was analyzed using the PAUP microcomputer program as described previously (Song et al. 1988a) and a phylogenetic tree was constructed. Some of the results from the analysis are reported here. 1) With in B. rapa, pak choi, narinosa and Chinese cabbage consisted of a distinct group from turnip and the wild populations, consistent with our hypothesis that B. rapa had two centers of diversity. Chinese cabbage was closely related to pak choi but was separated by a large distance of 45 units, suggesting that Chinese cabbage originated from pak choi, but has diverged considerably. A wild accession collected from India was positioned in the tree between European types and East Asian types, implying an evolutionary pathway of the two centers from Europe to India then to South China. 2) Within cultivated B. oleracea, cauliflower was closely related to broccoli, which clarified some uncertainty in our previous study. Cabbage, Portugese tree kale and Chinese kale had distinct RFLP patterns. All of the thirteen nine chromosome wild populations showed a close relation to cultivated B. oleracea but were far from B. rapa and other species. These wild forms fell into four group: a) B. oleracea and B. alboglabra; c) B. insularis, B. cretica ssp. atlantica; B. cretica ssp. lanconica; and d) B. cretica ssp. nivea, B. macrocarpa, B. montana and B. villosa.

3) Among other wild species, Diplotaxis erucoides and B. fruticulosa were close to each other, whereas Raphanus was related to Eruca but was separated by a large distance of 66 units. Some probes showed Raphanus having similar RFLP patterns to those of B. nigra, suggesting Raphanus may be an intermediate species between B. nigra and Eruca. B. tournifortii was found to be a very interesting

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species in terms of its closeness to both B. rapa and B. oleracea. This species has the shortest distance to the hypothetical common ancestor of B. rapa and B. oleracea (63 units), compared to the five other species examined (78-135 units). Also, B. tournifortii showed almost equal distances to both B. rapa and B. oleracea, which were shorter than the distances between any pair of B. rapa accessions and B. oleracea accessions. This result suggested that B. tournifortii or a close relative could be the common ancestor of B. rapa and B. oleracea. Alternatively, B. tournifortii could be a bridge species. That is B. tournifortii might be derived from one of the nine chromosome wild species and then B. rapa was derived from B. tournifortii or its close relatives.

4) Based on data from 20 single copy probes, it was found that the changes in allele frequency were associated with divergence of species and populations. Very often, different species and/or populations within a species were characterized by different predominant alleles. More detailed results from this study will be reported elsewhere.

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INTERSPECIFIC HYBRIDIZATIONS IN THE GENUS *BRASSICA* FOLLOWED BY IN OVULE EMBRYO CULTURE

E. DIEDERICHSEN & M.D. SACRISTAN

Interspecific crosses were made between the three diploid *Brassica* species *B.oleracea*, *B.campestris* and *B.nigra* in order to resynthesize the amphidiploid species *B.napus*, *B.juncea* and *B.carinata*. This gives us the possibility to combine different sources of resistance against *Plasmodiophora brassicae*, the causal agent of clubroot. The crosses included lines belonging to the European Clubroot Differential (ECD) set, a small number of commercial varieties and two genotypes of *B.nigra* (lines 460 and 2051). Because of its economical importance, the most attempts were done to develop artificial forms of *B.napus*.

Cross efficiency was very high using the in ovule embryo culture, whereas no hybrid plant could be obtained without any tissue culture application.

The results of hybridizations followed by embryo culture are given in table 1:

Maternal Line	No. of Paternal Lines	No. of Pollinated Buds X	No. of Cultured Ovules	No. of Hybrid Plants Y	Efficiency (%) (Y*100/X)
1a) *B.campestris* x *B.oleracea* -*B.napus* resynthesis-					
ECD-01	2	24	103	18	75
ECD-02	3	69	386	47	68
ECD-03	2	10	66	8	80
ECD-04	5	62	1072	60	97
ECD-05	4	49	1071	79	161
'Aarselia'	5	74	749	11	15
'Mosa'	5	26	370	10	38
'Pak Choy'	3	20	438	40	200
Total/Mean a)		334	4255	273	81
1b) *B.oleracea* x *B.campestris*					
ECD-11	4	57	187	14	25
ECD-12	7	80	672	146	183
ECD-13	2	17	166	4	24
ECD-14	3	25	335	2	8
ECD-15	8	119	582	52	44
'Frosty'	4	39	1	0	0
Total/Mean b)		337	1943	218	65
Total/Mean a)+b)		671	6198	491	73
1c) *B.campestris* x *B.nigra* -*B.juncea* resynthesis-					
ECD-04	2	16	117	10	63
Yellow Seeded Sarson	2	6	43	11	183
Total/Mean c)		22	160	21	96

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Maternal Line	No. of Paternal Lines	No. of Pollinated Buds X	No. of Cultured Ovules	No. of Hybrid Plants Y	Efficiency (%) (Y*100/X)
1d) B.oleracea x B.nigra		-B.carinata resynthesis-			
ECD-12	1	4	18	1	25
ECD-13	2	9	53	0	0
1e) B.nigra x B.oleracea					
line 460	1	5	14	3	60
line 2051	1	7	6	2	29
Total/Mean d)+e)		25	91	6	24
1f) B.napus x B.nigra		-Trigenomic Hybrids-			
ECD-07	1	7	45	0	0
ECD-10	1	5	11	4	80
1g) B.nigra x B.napus					
line 460	1	6	5	0	0
line 2051	2	7	9	0	0
Total/Mean f)+g)		25	70	4	16
Total/Mean a) g)		743	6519	522	70

In most cases the crosses were successful in a reciprocal way. The applied in ovule embryo culture system, which has been established by SACRISTAN & GERDEMANN (1986) to overcome interspecific incompability in crosses between the amphidiploid Brassica species, was also successful for the resynthesis of B.napus, B.carinata and B.juncea and even in crosses between rapeseed and B.nigra. Hybrid plants could be identified by their intermediate morphology. In a sample of amphihaploid B.napus hybrids chromosome counts showed the expected chromosome number of 19.

The main influence on cross efficiency was given by the maternal genotype. Differences could be observed between the lines (table 1) and between single plants belonging to the same line, where the efficiency varied from 2% up to 200%. Influences due to the duration of the time between pollination and in vitro culture were hardly to observe. Culture times between 20 to 30 days after pollination seemed to be favourable in any cross combination.

Reference:

SACRISTAN & GERDEMANN (1986): Different Behavior of Brassica juncea and B.carinata as Sources of Phoma lingam Resistance in Experiments of Interspecific Transfer to B.napus.- Plant Breeding 97, 304 -314.

INTERGENERIC HYBRIDIZATION BETWEEN *BRASSICA NAPUS* AND *SINAPIS ARVENSIS* AND THEIR CROSSABILITY

Nobumichi INOMATA

Intergeneric hybridization between *Brassica napus* (2*n*=38) and *Sinapis arvensis* (2*n*=18) was reported by Mizushima (1950, 1952). One F_1 hybrid was obtained and it counted 28 chromosomes in root tip cells. The mean bivalent formation showed 8.5 at the first meiotic division in the PMCs. No pollen fertility was observed and no seed setting was obtained in self-pollination. Some seeds were obtained in open pollination (Mizushima 1952). In the present experiment, many F_1 hybrids between *Brassica napus* and *Sinapis arvensis* were obtained, which were different from the F_1 hybrid produced by Mizushima (1950, 1952). The present paper deals with the production, cytological observation and crossability of the F_1 hybrids.

The materials used in the experiment were *B. napus* subsp. *oleifera* cv. Aomori No. 1 and *Sinapis arvensis* which was provided by the Swedish Seed Association at Svalöf, Sweden and had been kept by sib-mating. *B. napus* was used as a female plant and crossed with the pollen of *S. arvensis*. Ovary culture was done according to previous papers (Inomata 1978, 1985). Pollen fertility was examined by acetic carmine.

The results on the production and cytological observation are shown in Table 1. Nine hybrids were obtained. Three of them were from artificial pollination and six of them were from ovary culture. Ten flowers were pollinated in artificial pollination and fifty ovaries were explanted in the medium. Two and five hybrids counted 28 and 37 chromosomes in root tip cells, respectively. Morphological characteristics of the leaf in the F_1 hybrids was similar to *B. napus* but the leaf had longer petiole and narrower width than that of *B. napus*. The first meiotic division at the PMCs was examined in the F_1 hybrid of plant number four with 37 chromosomes. The frequency of $18_{II}+1_{I}$ was 94% and the rest showed $1_{III}+17_{II}$ in 36 cells observed. The F_1 hybrid of plant number one with 28 chromosomes had low pollen fertility. Other four hybrids had high pollen fertility and they had almost the same as an euploid plant. Other four F_1 hybrids died by flower season.

The results on the crossability of the F_1 hybrids are shown in Table 2. In the F_1 hybrids of plant number one and two, open pollination was done and the seed set percentage per pod was 1.3 and 16.6, respectively. In the F_1 hybrids of plant number four and five, self-, open pollination and F_1 x *B. napus* were done and many seeds were obtained in each pollination. The mean of the seed set percentage per pod was 23.5 in self-pollination, 25.7 in open pollination and 27.3 in the backcross to *B. napus*.

In the present experiment, pollen fertility of the F_1 hybrids with 28 chromosomes had 18.0% and some seeds were obtained in open pollination. It seemed that the difference between the F_1 hybrid obtained by Mizushima and in the present experiment was due to the strain used in the experiment. In the F_1 hybrids with 37 chromosomes

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the chromosome doubling of the genome in S. arvensis might occur just before or after fertilization. As chromosome pairing at the first meiotic division in the PMCs was good, the recombination between the genome of B. napus and S. arvensis might occur easily. It is possible that the genes of S. arvensis can transfer to B. napus and that S. arvensis is an useful plant which enlarge the genetic diversity of B. napus.

Acknowledgment

I would like to thank the Swedish Seed Association at Svalöf, Sweden for providing the seed of Sinapis arvensis, Sv. 64-1023.

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Table 1. Intergeneric hybridization between Brassica napus and Sinapis arvensis by artificial pollination and ovary culture

Plant No.	Origin[1] of F_1 hybrid	Chromo. number (2n)	Pollen[2] fertility (%)	References
1	A. P.	28	18.0	Open pollination
2	A. P.	37	87.8	Open pollination
3	A. P.	37	97.8	-
4	O. C.	37	95.2	Self-, Open pollination, B_1 cross
5	O. C.	-	98.6	Self-, Open pollination, B_1 cross
6	O. C.	37	-	Dead by flower season
7	O. C.	28	-	Dead by flower season
8	O. C.	-	-	Dead by flower season
9	O. C.	37	-	Dead by flower season

1: A. P. shows artificial pollination and O. C. shows ovary culture in vitro. 2: 500 pollen grains were counted.

Table 2. Crossability of the F_1 hybrids between Brassica napus and Sinapis arvensis

Plant No.[1]	Self-pollination		Open pollination		F_1 x B. napus	
	No. of flowers pollinated	No. of seeds obtained	No. of flowers pollinated	No. of seeds obtained	No. of flowers pollinated	No. of seeds obtained
1	-	-	115	149	-	-
2	-	-	47	780	-	-
4	81	2174	115	2965	20	548
5	61	1163	87	2222	20	543
Total	142	3337	364	6116	40	1091

1: Refer to Table 1.

INHERITANCE OF A PALE YELLOW PETAL MUTANT OF SUMMER RAPE

G Séguin-Swartz

Rape plants (Brassica napus L.) typically bear flowers with lemon-yellow petals, but the occurrence of plants with pale yellow, orange, and pale orange petals has been reported (Sylvén 1927). Sylvén (1927) studied the segregation ratios of three crosses: lemon-yellow X orange, lemon-yellow X pale yellow, and orange X pale yellow petalled plants to determine the inheritance of the different petal colors. F_1 plants from reciprocal crosses had, in all cases, lemon-yellow petals. F_2 generations of the lemon-yellow X orange and lemon-yellow X pale yellow crosses segregated 3 lemon-yellow : 1 orange and 3 lemon-yellow : 1 pale yellow petalled plants, respectively, indicating monogenic differences in petal color in these crosses. The F_2 of the cross orange X pale-yellow segregated 9 lemon-yellow : 3 orange : 3 pale yellow : 1 pale orange petalled plants indicating digenic differences in petal color in this cross. Sylvén (1927) proposed the following genotypes for petal color in B. napus: AABB= lemon-yellow, AAbb= orange, aaBB= pale yellow, and aabb= pale orange. A similar study by Morice (1960) confirmed the inheritance model proposed by Sylvén (1927).

A pale yellow petalled double haploid (DH) line (DH12439) derived from cv. Westar, was observed in 1986 in a field nursery at Saskatoon, Saskatchewan, Canada. To determine the inheritance of the pale yellow petal characteristic, reciprocal crosses were made between Westar-derived DH lines DH12439 with pale yellow petals and DH10930 with lemon-yellow petals.

All F_1 plants from the reciprocal cross had lemon-yellow petals. Segregation for petal color in the BC and F_2 generations approximated 1 lemon-yellow : 1 pale yellow and 3 lemon-yellow : 1 pale yellow segregation ratios, respectively, indicating that the pale yellow petal color was controlled by one pair of recessive alleles at a single locus (Table 1). The observed inheritance pattern in this cross supported the petal color inheritance model proposed for B. napus by Sylvén (1927).

Seed of lines DH12439 (pale yellow petalled) and DH10930 (lemon-yellow petalled) may be obtained from the Defined Brassica Breeding Stock (DBBS) Program of the Agriculture Canada Research Station, 107 Science Crescent, Saskatoon, Sask. S7N 0X2, Canada.

Research Station, 107 Science Crescent, SASKATOON, Canada S7N 0X2

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TABLE 1. BC and F_2 segregation ratios for crosses between a pale yellow petalled line (DH12349) and a lemon-yellow petalled line (DH10930) of B. napus.

Generation	No. plants with petal color		Ratio tested	X^2	P
	lemon-yellow	pale yellow			
BC to pale yellow	42	36	1:1	0.46	.50-.25
F_2 reciprocals combined	222	63	3:1	1.27	.50-.25

Simple inheritance of flower color in *Brassica hirta*

J. Mitchell McGrath

Few, if any, single gene characters have been reported in the yellow mustard *Brassica hirta* (syn. *Sinapsis alba*). In the course of an isozyme survey of *Brassica*, three plants from a single accession obtained from Johnny's Selected Seeds, Albion, Maine, USA were grown in the greenhouse. These three plants differed in the presence and distribution of purple pigment, and bright versus pale yellow flower color. Isozyme analysis revealed variability at PGI (phosphoglucoisomerase), IDH (isocitrate dehydrogenase), LAP (leucine amino peptidase), GOT (glutamate-oxaloactetate transaminase), and PGM (phosphoglucomutase). No variability was detected at 6PGD (6-phosphoglucodehydrogenase) or MDH (malate dehydrogenase). Inheritance of isozymes was not performed.

A single cross between the pale yellow flower color morph and a bright yellow morph was used to develop an F_2 segregating population. Forty-eight bud pollinations of the F_1 resulted in 54 seeds obtained. Not all pollinations were successful, and the modal number of seeds/silique was two. In no instance did the number of seeds/silique exceed four. Fifty-two seeds sown in the greenhouse germinated. Of these, two had albino cotyledons and died. The remaining plants were transferred to the field at 5 weeks after germination, and flower color scored between 7 and 12 weeks post-emergence. Nine plants succumbed to the combined effects transplanting shock, high temperature (excess of 90°F daytime), and dessication. Results of flower color segregation are shown in the following table:

P_1	pale yellow	(self of P_1 pale yellow)
P_2	bright yellow	
F_1	bright yellow	
F_2	32 bright yellow : 9 pale yellow	
	x^2=0.0732	p=0.85-0.75

Inheritance of purple pigment in the stem was monitored but did not conform to Mendelian expectations perhaps due to environmental limitations on expression. One isozyme, LAP, showed developmental specificity of expression as leaves from young plants were positive and post-flowering leaves negative for expression. The F_2 population had reduced vigor with some plants scored as a dwarf phenotype. This phenotype was likely related to inbreeding depression and temperature stress rather than genetic dwarfism.

In conclusion, a single major gene appears to control the expression of bright vs. pale yellow flower color, and the proposed gene designation is *py* for pale yellow, *Py* for bright yellow.. A limited number of seeds are available with the pale yellow trait in the heterozygous condition (*Py/py*).

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GENETICS OF BASAL BRANCHING, ADAXIAL HAIRY LEAVES AND CLUMPING FLOWERING IN MUSTARD

R.D.S.Yadav

Knowledge of the inheritance of a trait is helpful in the production of desired genotypes. The present investigation was therefore undertaken to obtain information on the inheritance of basal branching, adaxial leaf hairs and clumping flowering in mustard (Brassica juncea).

True breeding lines with contrasting features for branching (basal/normal), adaxial leaves (hairy/glabrous) and flowering (clumping/regular) were pair crossed.

In the crosses between basal X normal branching lines, normal branching plants appeared in the F_1. The F_2 segregated 89 normal : 7 basal branching plants, a close fit to a ratio of 15:1 (χ^2=0.18) indicating that basal branching is controlled by duplicate recessive genes.

The crosses made between hairy and glabrous lines gave hairy leaved plants in the F_1 generation. The F_2 segregation was 58 hairy : 22 glabrous showing a good fit to a 3:1 ratio (χ^2=0.27). Therefore hairy leaf is dominant to glabrous and controlled by a single dominant gene.

In the crosses between clumping and regular flowering lines the F_1 plants were regular flowering. The segregation in the F_2 of 118 regular : 10 clumping showed a good fit to a 15:1 ratio (χ^2=0.53), thus, indicating control by duplicate genes.

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BREEDING BRASSICA JUNCEA FOR INCREASED NUMBERS OF SEEDS/POD USING MULTIVALVULARITY

R. A. Foss

During the last 40 years of a breeding programmme for yield and seed quality improvement in Brassica juncea, there have been some dramatic changes amongst all the seed number components of yield except for numbers of seeds/pod, which has remained relatively static. In recent years, therefore, there has been an increasing emphasis on searching for sources of greater seed numbers/pod so as to counteract this putative restriction on yield advance.

Progress was slow until 1980 when some eighteen plants were found amongst a yellow seeded accession from China which subtended multivalved pods. Multivalved pods have previously been reported in B. juncea by Singh, Srivastava and Singh (1973) and Podkolzina (1974). In both cases these authors have described the occurrence of trivalved pods with up to 58% more seeds in them than the normal bivalved type.

Our material showed marked variation in pod morphology associated with a range in numbers of seeds/pod. In general, most pods consisted of four carpellary walls joined along their length enclosing two parallel septa. A row of seeds was attached to both sides of each septum, making four rows of seed in total. Pods from plants with distorted pods contained a withered flower-like structure at the basal end, often projecting as much as half their length and located between the two septa. Pods from plants with smooth undistorted pods contained only the two parallel septa with no such inclusion between them. There were fewer seeds in the inner two rows where the flower-like inclusion was present, so that they generally contained two full and two part rows of seed, giving in total only 25-30 seeds/pod. Pods from plants with the smooth undistorted pods by contrast each contained four full rows of seed, producing in total 35-40 seeds/pod. Normal bivalve pods contain only 18-20 seeds, so the best multivalved lines have double the seed number/pod with no reduction in seed size.

Several years of recurrent selection from the material with the best pods followed by crosses with conventional material has resulted in multivalved progeny yielding nearly as well as the best controls. Further backcrossing will be need to eliminate straw weakness and to improve pod numbers, but so far the multivalved character looks a promising avenue for yield advance in our programme.

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THE RESPONSE OF THE OUTCROSSING RATIO OF WINTER OILSEED RAPE (Brassica napus L.) ON ARTIFICIAL SELECTION

E. RUDLOFF and W. SCHWEIGER

Introduction

Using a method for the estimation of the outcrossing ratio (o.r.) by means of the erucic acid (C22:1) content as a genetic marker (RUDLOFF and SCHWEIGER 1984, 1985) we could show a great inter- and intravarietal variation in winter rape (RUDLOFF and SCHWEIGER 1984). The occurrence of significant differences between varieties imposed the question if it would be possible to increase and to stabilize the o.r. by artificial selection. That would be important for the breeding of high and stable yielding synthetic varieties. This paper is a short report on the results of three cycles of a selection programme started in 1985. A more detailed publication is in preparation.

Material and methods

Each 80 single plants of ten zero erucic breeding stocks A...K were tested in 1985 (generation 0) for their individual o.r. using the method of RUDLOFF and SCHWEIGER (1984,1985). The high erucic tester variety was 'Sollux' and the C22:1 content was analyzed by gaschromatography on seed lots of 1 gram. It was selected into two directions: the plus selection on high C22:1 (i.e. high o.r.) and the minus selection on low C22:1 (i.e. high selfing). Paper bag isolation gave the seeds for the next generation which involved progenies with 15 plants per elite.

Results

The plants of the minus selection had generally less than 10 percent C22:1. In the plus selection are however two cases to distinguish: While in some progenies the highest C22:1 values were clear over 20 percent, other progenies showed maximum values, which were clear below 20%.
As shown in figure 1 the frequency distribution in the minus selection is similar in all generations. The plus selection leads always in generation 1 to significant differences between both directions. The next generations show two clear distinct groups in the plus distribution, resulting from the above mentioned two cases of plus selection. Therefore the biometrical calculations were made for both groups seperately. The right group of the 2nd and 3rd generation shows a clear response to selections. The comparison of the average C22:1 contents of the generations is given in table 1. The level of the C22:1 content is in the minus selection relatively constant.

VEG (P) Malchow/Poel, Institute for Fodder Crop Breeding, MALCHOW/POEL, 2401, D.D.R.

That's not unexpected for the level of the first selection was always very low and could hardly be decreased. The plus selection results in a continuous increasing of the C22:1 content as demonstrated by the right group in generation 2 and 3 and goes up to 25 percent in generation 3. Six lines in that generation have an o.r. of more than 90 percent with variation coefficients (s%) between 9,6 and 17,7 percent.
The results show the effectiveness of artificial selection for rapid improvement of the o.r. in winter rape. It seems to be possible to select lines with high outcrossing which would be very suitable for breeding synthetic varieties.
Further investigations will concern therefore with the suitability of such lines for the synthetic's breeding in winter oilseed rape.

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Table 1: Generation mean and variation coefficient during consecutive generations of selection (C22:1 content)

a) minus selection

	generation			
	0	1	2	3
nr. of individ.	611	258	142	102
$\bar{x}$	9,7	11,2	9,1	8,8
s%	34,9	40,2	36,7	63,6

b) plus selection

	generation					
	0	1	2[1)]		3[1)]	
			left	right	left	right
nr. of individ.	611	520	125	97	93	169
$\bar{x}$	9,7	12,3	11,0	23,5	9,5	25,0
s%	34,9	42,9	24,7	14,7	31,1	20,4

1) the minimum class between both peaks (see fig. 1) is added to both groups

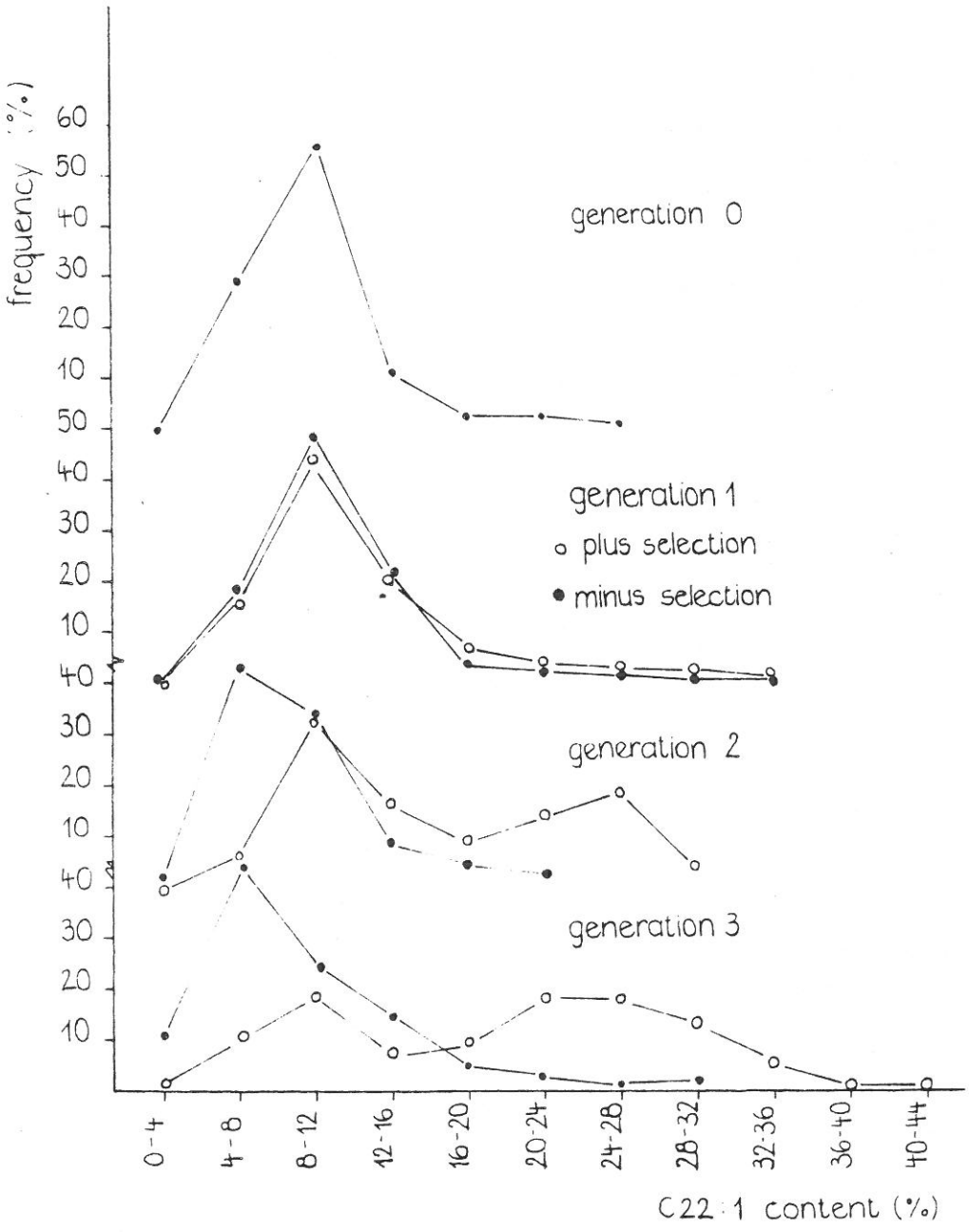

Figure 1: Frequency distribution of the C22:1 content in successive generations of selection

BREEDING *Brassica carinata* FOR LOW ERUCIC ACID CONTENT

O. Fernández-Serrano and L.C. Alonso

The Abyssinian mustard (*Brassica carinata* Braun, 2n=34, BBCC) is a highly interesting species due to its elevated productive potential in countries with a dry climate, as is the case in the Mediterranean area (1). The main problem which has been hindering its development as an oleaginous crop up to now is the high level of erucic acid (22:1) in its oil, as well as the elevated content of glucosilonates in the meal. Recently (2), the existence of a gene was suggested which controls the synthesis of the aforesaid acid in each of the genomes of *Brassica carinata*.

As far as we know, there have been no published reports on any Abyssinian mustard lines with either medium or low levels of erucic acid (22:1) in their oil.

The target of our work consisted in reducing the erucic acid content in the oil of *B. carinata*, and we employed two lines of research to this purpose.

1) Traditional breeding: An extensive screening among a large number of lines of Abyssinian mustard was carried out. The types lowest in erucic acid were selected by the genealogical method.

2) Interspecific hybridization: A programme of interspecific cross-breeding, including lines of *B. napus* and *B. juncea* without erucic acid in their oil, was started for the purpose of transferring this genetic attribute to *B. carinata* by means of back-crossing. When cytological examination indicated that the genom set of *B. carinata* had been recovered, a self-fertilization and selection programme of types with a low level of erucic acid was applied.

In all cases, the analysis of fatty acids content was carried out by gas-liquid chromatography at Koipe S.A. Laboratory.

Table 1 shows the interval found in the concentration of the main fatty acids in the oil of the Abyssinian mustard population we worked with. It also shows two typical chromatograms of *B. carinata* and the chromatograms of the isolated mutants.

As we can see, there has been a reasonable decrease in the erucic acid content by the two strategies cited above. It is predictable to find further reduction in the following generations of self-fertilized types from the interspecific program. In the event that we have only succeeded in the transfer of low erucic acid genes from one of the genomes "B" or "C", the combination between lines with medium-low content of erucic acid obtained by the two breeding methods might lead to isolation of low erucic types of *B. carinata*.

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TABLE 1: FATTY ACID COMPOSITION (%) OF SEED OILS OF B. CARINATA

LINES	FATTY ACID (%)								
	PALMITIC 16:0	PALMITOLEIC 16:1	ESTEARIC 18:0	OLEIC 18:1	LINOLEIC 18:2	LINOLENIC 18:3	GADOLEIC 20:1	BEHENIC 22:0	ERUCIC 22:1
WILD TYPES:									
BR 9*	4.96	0.97	1.65	14.06	19.00	13.21	10.53	-	35.62
BR 37*	4.10	0.78	1.40	11.06	23.41	9.32	8.86	0.63	40.43
INTERVAL**	2.83 | 12.98	0.084 | 2.08	0.88 | 2.83	9.05 | 27.51	14.59 | 25.82	8.56 | 17.34	3.75 | 15.69	- | 3.15	35.01 | 46.49
MUTANTS:									
TRADITIONAL BREEDING									
BR 27-1-5-1	4.78	0.55	1.64	25.93	22.33	11.10	12.55	0.21	20.64
BR 31-1-4-2	6.25	13.69	2.25	19.26	22.62	13.44	10.34	-	24.44
BR 24-1-1-4	5.08	0.52	1.41	12.21	40.28	11.80	7.16	0.89	18.74
BR 10-4-2-3-5	5.12	1.06	1.90	39.00	20.14	10.00	10.62	-	10.79
INTERSPECIFIC HYBRIDIZATION									
85B196-1(25)	4.91	0.14	1.90	24.77	22.05	14.10	12.00	0.08	18.86
85B116-4(42)	8.96	0.25	3.11	32.46	23.93	8.07	4.44	0.40	17.68
85B27-2(4)	7.09	0.33	2.00	40.91	14.20	10.99	4.71	0.34	18.68
85B116-1-11X-4	5.18	0.08	1.18	46.70	21.30	11.10	3.06	-	11.20
85B155-3-2-8-1	5.21	0.07	1.61	41.00	22.70	11.30	3.66	-	13.80

* Typical types of B. carinata

** Interval found in a large collection of B. carinata

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YELLOW-SEEDED RAPE IN CHINA

Gao Yong-tong

China is one of the countries which has a very long history in the production of rape in the world, a lot of yellow-seeded varieties have been bred in rape by natural and artificial selection. About 27 per cent varieties were yellow-seeded in B. campestris and about 35 per cent varieties in B. juncea, according to 1982 data.

Yellow-seeded campestris is distributed mainly over Sichuan Hunan, Hubei, Henan, Anhue, Jiangsu provinces, and the yellow-seeded juncea is widely dispersed over the Yunnan-Suizhou plateau and the Northwest plateau. In Xinjing, for example, the cultural area of yellow-seeded juncea makes up more than 80 per cent of the total. The yellow seeds in B. campestris and B. juncea usually appear golden-yellow colour coat, no dark spot on the surface of the seedcoat, and the oil contents are 1.73% and 1.60% higher than brown ones respectively, on average.

It was very late that B. napus was introduced into China, so the resources of B. napus were rather poor in China, the varieties which come from abroad in early 1930's were all black-seeded, but it replaced the traditional varieties, ie B. campestris and juncea, in the main productive region gradually, because of high yield.

Dr Liu Houli has been carrying out a program improving the oil content in napus since 1960's. He wanted to introduce the yellow-seeded genes into B. napus from B. campestris to get yellow-seeded napus by crossing napus with campestris, but no discovery before 1975. The first yellow-seeded napus found by Dr Liu originated from B. napus x B. campestris. Later on, other kinds of yellow-seeded materials were discovered from different origins, and a series of research work, including the genetics, breeding, embryology, substantial accumulation in seeds, etc., has been done under the leadership of Dr Liu.

The first yellow-seeded variety '955' in B. napus in China is beginning to be used in the productive region of Central China. '955' has got more than 95% frequency of yellow-seeded plants and more than 46% oil content, while the commercial black-seeded varieties have 38-39%. Its harvesting area was about 10,000 ha in 1988, its yield came up to that of commercial black-seeded varieties, and 5.4 kg more oil can be extracted per 100 kg seeds as compared with black ones (Table 1).

Yellow seedcoat as an important character, has been considered as one of the objects of breeding in B. napus in 1986, some studies on breeding for yellow-seeded napus have been carried out in Sichuan and Jiangsu Academy of Agricultural Science and elsewhere. It can be anticipated that the yellow-seeded napus will be developed more and more in the future.

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Table 1. The comparison between two varieties (1988)

Varieties	955	821
plant height (cm)	144-162	142-162
number of primary branches	5-8	4.3-6.2
siliqua number per plant	261-346	165-305
seeds per siliqua	16-19	19.5
1000-seed weight (g)	3.4	3.6
seed yield per ha (kg)	1823.18	1793.40
oil yield per 100 kg (kg)	37.40	32.00
oil yield per ha (kg)	681.87	573.89

821 - a prevalent commercial variety (black-seeded napus).

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COMPARATIVE PERFORMANCE OF THE GENOTYPES OF INDIAN AND ETHIOPIAN MUSTARD UNDER SEMI-ARID REGION OF INDIA

Hari Singh, D Singh and T P Yadava

Indian mustard (Brassica juncea L. Czern & Coss) is most predominantly cultivated Brassica species in India. By virtue of its higher yields and adaptation, it is widely grown under different agro-climatic conditions and cropping patterns. However, it is susceptable to white rust. Contrary to it, newly revolved genotypes of Ethiopian mustard (Brassica carinata L.Braun.) are completely resistant to white rust. Performance of this new species in comparison to traditional Brassica juncea is reported in the present study. Ten promising genotypes of Ethiopian mustard were evaluated in respect of yield, maturity, oil content and resistance to white rust against RH-30 and Prakash cultivars of Indian mustard during 1983-84 and 1984-85. The experiment was conducted in randomized block design in three replications at Haryana Agricultural University, Hisar. Each entry was represented by a plot of 9 rows each of 6m length. Spacing between rows and plants within rows was kept at 30 and 15 cm, respectively. The experiment was planted in 3rd week of October during both the years.

The results in respect of various attributes studies have been presented in Table 1. The perusal of the results revealed that during 1983-84, four genotypes of Ethiopian mustard, CAR-6, CAR-2, CAR-8 and CAR-5, registered significantly higher seed yield over both the check cultivars of Indian mustard, RH-30 and Prakash. Only CAR-5 and BC-2 could surpass these check varieties in yield by a significant margin during 1984-85. Based on two years average seed yield CAR-5, BC-2,CAR-3, CAR-8, CAR-6, CAR-2 and HC-2 maintained considerably higher yields as compared to RH-30 and Prakash. Oil content was on an average, low in the Ethiopian mustard genotypes. However, BC-2 and HC-2 possessed as good oil content as Prakash and RH-30.

Among, Ethiopian Mustard genotypes CAR-2, CAR-3, CAR-5 and HC-2 were at par in maturity (152 days). Indian mustard cultivars were susceptible to white rust whereas, all the genotypes of Ethiopian mustard showed resistance to this disease. CAR-5, BC-2, CAR-3 and HC-2 were identified as the best genotypes of the Ethiopian mustard in respect of all the desirable attributes.

Acknowledgment

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Table 1: Comparative performaces of Ethiopian and Indian mustard genotypes during 1983-84 and 1984-85.

Genotypes	Seed yield kg/ha		Mean yield Kg/ha	Average oil content (%)	Average maturity (days)	White rust reaction
	1983-84	1984-85				
CAR-5	3517	3045	3281	37.16	153	R
BC-2	2949	3078	3013	40.10	162	R
CAR-3	3489	2448	2968	37.80	152	R
CAR-8	3525	2399	2962	34.90	165	R
CAR-6	3567	2266	2916	36.20	156	R
CAR-2	3551	2263	2907	37.00	152	R
HC-2	3372	2274	2823	39.20	152	R
CAR-1	3199	2239	2719	36.33	154	R
CAR-4	3116	2155	2635	36.49	163	R
CAR-7	2790	2066	2428	36.16	166	R
Prakash	2799	2368	2563	40.16	147	S
RH-30	2790	2459	2654	40.33	139	S
CD at 5%	693.67	415.47	-	-	1.67	
CV %	12.8	10.2	-	-	0.64	

S = Susceptible; R = Resistant;

PERFORMACE OF SWEDE RAPE(*Brassica napus* L.) GENOTYPES UNDER SEMI-ARID REGION OF INDIA

Hari Singh, D Singh and T P Yadava

Indian mustard (*Brassica juncea* L. czern & coss) a prominently cultivated species of genus *Brassica* suffers from the drawbacks of susceptibility to major diseases of *Brassica* viz. *Alternaria* and white rust. Swede rape shows high degree of resistance to white rust and *Alternaria*. In the present study newly evolved promising genotypes of *B. napus* L. were evaluated against important cultivars of Indian Mustard for desirable characters. Ten newly evolved genotypes of swede rape (from the base material introduced from Canada, Sweden, Polland and Japan) and two promising cultivars of Indian mustard, prakash and Varuna were evaluated in randomised block design with three replications at Haryana Agricultural University, Hisar, India during winter season of 1983-84, 1984-85 and 1985-86. Each entry was represented by 9 rows plot of 6m length. The row to row and plant to plant spacing was kept 30 and 15 cm, respectively. The observations of maturity, seed yield, per cent oil content by NMR and reaction to disease were recorded.

The perusal of data in Table 1 indicated that during 1982-83 Midas 2 was statistically better than Varuna in seed yield. HNS-1, Midas-2 and HNS-3 were statistically better in seed yield than Prakash. During 1983-84 however, Varuna registered highest yield of 2481 kg/ha but this yield level was statistically at par with HNS-1, Tower-1, Midas-2 and Regent-1. During 1984-85 season none of the cultivar of *B. napus* could excel Indian mustard check varieties. Based on three years average, HNS-1 and Midas-2 were promising (Table 1). The most interesting feature of *B. napus* cultivars was that except Gulliver-2 all the cultivars possessed higher oil content than *B. juncea* cultivars. Among these Midas-2 and Tower-1 possessed as high as 45.99 and 45.66 per cent oil as against 39.49 and 40.15 per cent in Prakash and Varuna, respectively. All the cultivars of *B. napus* possessed resistance to white rust.

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The financial assistance provided by IDRC, Canada through ICAR, New Delhi for the present study is gratefully acknowledged.

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Table 1: Comparative performnce of newly evolved cultivars of swede rape and Indian Mustard in seed yield, maturity, oil content and reaction to white rust during 1982-83 to 1984-85.

Spp./Genotypes	Seed yield kg/ha			Mean yield kg/ha	Average days to maturity	Oil tent (%)	Reaction to White rust
	1982-83	1983-84	1984-85				
B.napus L.							
HNS-1	1894	2451	2222	2156	151	43.33	R
Midas-2	2094	2256	1779	2043	156	45.99	R
Tower-1	1291	2377	1855	1841	147	45.66	R
HNS-3	1629	1901	1905	1812	155	43.66	R
Regent-1	1435	2180	1504	1706	150	43.33	R
Tower-2	1412	1952	1529	1647	155	42.66	R
Regent-2	1177	2051	1512	1580	148	40.66	R
Guliver-2	1253	1827	1387	1489	157	38.33	R
H1110	896	1849	1382	1369	151	42.45	R
N20-1	-	2047	1353	1133	146	40.33	R
B.juncea cv. Varuna	1754	2473	2431	2219	139	40.15	S
Prakash	1328	2238	2481	2016	147	39.49	S
C.D. at 5 per cent level of significance	275.81	378.59	373.22	-	1.59	-	
C.V. Per cent	11.16	10.55	12.46	-	0.63	-	

R = Resistant; S = Susceptible

SHATTERING RESISTANCE IN INDIAN MUSTARD

Ram Bhajan, Y.S. Chauhan and K. Kumar

Considerable yield losses occur in Brassica oil crops due to rupturing of siliquae before or during harvesting. But the extent of seed losses could be even more severe if harvesting is inevitably delayed, such as in intercropping system. These considerations have placed shattering resistance among major objectives of current breeding programmes. Hence, it is important to identify shattering resistant genotypes for use as suitable donors. Among oleiferous Brassicae, mustard (Brassica juncea L. Czern & Coss) is comparatively more resistant to shattering than other forms (Macleod, 1981; Singh, 1858). The present investigation deals with the field evaluation of mustard genotypes for resistance to shatter.

A total of 26 mustard genotypes, were grown in randomized block design with 3 replications during winter season of 1987-88. The plot size for each genotype was 7.6 sq.m. with a spacing of 30 x 15 cm. Days to maturity was recorded on plot basis for each genotype. Shattering percentage was recorded following "Count shattered siliquae" method (Tomaszewski and Koczowska, 1971) at 10 and 20 days after maturity on 5 randomly tagged plants. Though count of shattered siliquae at 10 days of over ripening (First count) could have been adequate for evaluation but count at 20 days (Second count) was also taken to ensure rigour in screening.

Results of the 'first count' observations showed that NDR 871, Kranti, RSM-107 and NDR-872 possess high degree of resistance to shatter which was also confirmed by 'second count' and mean over both the counts (Table-1). On the other hand, RCC-29, NDR-873 and PR-8705 appeared most susceptible genotypes.

Significant differences among genotypes were observed for per cent shattering at both the counts. Heritability and genetic advance in per cent of mean were of high magnitude to warrant for scope of improvement through selection.

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Table 1. Level of shattering percentage for different mustard genotypes and selection parameters

Genotypes/ parameters	Average per cent shattering		Overall mean	Rank*
	First count	Second count		
R K-8701	27.85	30.00	28.93	11
RK -8702	26.17	32.55	29.36	12
AS -101	31.32	36.65	33.99	18
AS -102	24.70	36.82	30.75	15
AS -103	21.97	28.55	25.26	8
PR - 8701	21.65	28.15	24.90	7
PR -8705	37.06	44.79	38.93	24
PR -86-5	27.04	34.48	30.76	16
RAURP-4MDS	22.26	54.54	38.42	23
RAURS-30	28.99	34.82	31.47	17
DIRA-313	18.97	27.47	30.22	6
DIRA-326	26.18	32.77	29.48	13
TM -22	34.51	36.21	35.36	22
NDR-871	9.23	22.35	15.79	1
NDR-872	15.87	24.79	20.33	4
NDR-873	37.79	46.48	42.09	25
RW-1/86-1	24.00	45.40	34.70	20
RW-4/86	26.27	34.49	30.38	14
RW-3/86	25.51	28.91	27.21	10
RSM-106	28.93	40.80	34.87	21
RSM-107	14.41	21.40	17.91	3
RCC-15	24.73	28.03	26.38	9
RCC-29	45.60	47.58	46.59	26
Varuna	16.73	28.29	22.51	5
Kranti	12.16	20.87	16.52	2
Krishna	30.98	38.38	34.67	19
GM	25.41	33.90	-	-
C.D.(at 5%)	4.16	7.20	-	-
Heritability (%)	91.04	76.68	-	-
Genetic advance in per cent of mean	62.75	44.47	-	-

*Rank 1 and 26 indicate the lowest and the highest siliquae shattering, respectively.

Possibilities for indirect selection of head weight in cauliflower using field-trimmed weight or market-trimmed weight.

C R TAPSELL and A WHELTON

Head weight represents one of several yield component characters in cauliflower, and as such is positively selected for in many breeding programmes. Furthermore, with the increasing requirement for overwrapped virtually leafless heads by the multiple supermarket chains in the UK, heavier heads within a narrow size range are required. Conventional measurements of head weight are performed after removal of all leaves, a process which is labour intensive, and destructive. A study was therefore undertaken to see whether the weight of head plus wrapper-leaves as trimmed in the field (field-trimmed weight, FTW), or the weight of head plus inner wrapper-leaves as trimmed for marketing in face-pack crates (market-trimmed weight, MTW), could be used to select indirectly for head weight (HW).

Materials and Methods

Samples were taken of fifteen autumn-cauliflower varieties and five winter-cauliflower varieties grown as part of several randomised block experiments, and for each variety records of HW, FTW and MTW were taken on ten plants on up to three harvest dates. Regression analysis was used to assess relationships between HW & TFW and HW & MTW, and to determine the extent to which these relationships were consistent between varieties and between harvest dates.

Results

A highly significant overall regression relationship of HW on FTW was found (Table 1). When varieties and harvest dates were considered individually, the individual regression slopes of HW on FTW were not significantly different from each other whilst the intercepts were. When the values for the intercepts were estimated after fitting a common regression slope to the data, for any given FTW the HW could vary by up to 380g. Further analysis showed that there were significant differences between intercepts for both varieties and harvest dates, with the former having the greater effect (Table 1).

A highly significant regression relationship of HW on MTW was also found (Table 2). Again, regression slopes for individual varieties and harvest dates did not differ, whereas intercepts did. As with FTW fitting a common regression slope of HW on MTW showed significant differences in interçepts between varieties and between harvest dates, with the former having the greater effect (Table 2). The range of values for intercepts observed for any given MTW on fitting a common regression slope was 330g.

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Discussion

Initially, it appeared that the highly significant relationship between HW and both FTW or MTW would allow for indirect selection of HW. However, on closer inspection this was seen not to be the case. The weight of leaf surrounding a head of any given weight varied considerably between genotypes and to a lesser extent between harvest dates for both FTW and MTW.

Interestingly, many of the autumn-cauliflowers were F_1-hybrids, and these appeared to be no less variable in regression intercepts of harvest dates than the open-pollinated autumn- or winter-cauliflower varieties.

Acknowledgements

The authors wish to thank Mr J C Treble for assistance during the course of this work.

Table 1 Regression of HW on FTW

Item	df	MS	P
Common regression slope	1	10332718	<0.001
Differences in varietal intercepts	19	107700	<0.001
Differences in harvest date intercepts within varieties	28	14989	<0.001
Residual	431	5459	

Table 2 Regression of HW on MTW

Item	df	MS	P
Common regression slope	1	10925670	<0.001
Differences in varietal intercepts	19	104483	<0.001
Differences in harvest date intercepts within varieties	28	12636	<0.001
Residual	431	4378	

STUDIES ON THE RELATIONSHIP BETWEEN SEEDCOAT COLOUR AND PIGMENT CONTENT IN DIFFERENT TYPES OF RAPESEED CULTIVARS*

Hu Xiao-jun

In this paper, the relationship between seedcoat color and pigment content of rapeseed cultivars was studied on the standpoint of plant pigment. The extraction, identification and determination of the pigments in seedcoat were carried out systematically for the first time and a suitable method for the research of seedcoat pigment was presented.

1. The distribution of pigments

The investigation on the distribution of the pigments in embryo and hull to ascertain the pigment substances affecting hull color indicated that in embryo only fat-soluble pigment existed; in hull, however, no fat-soluble pigment but only anthocyanidins were present. therefore, water-soluble anthocyanidin pigments are the main factor to influence the hull color.

2. The identification of pigment component in embryo

The identification of the fat-soluble pigment in embryo by thin-layer chromatographic and spectral methods showed that most of fully ripen seeds contained only lutein; but a very few had also chlorophyll b besides lutein.

3. The extraction of anthocyanidins

The common method used for the extraction of anthocyanidins in plant materials was invalid to seedcoat. Then three factors were tested to decide an appropriate extracting condition. The tempreture was judged for the major factor to affect the extraction of hull pigment according to the right-cross test and the optimal extracting condition was decided through further experiments as: temp.: 80°C, acidic conc.: 5 % and time: 60 min..

4. The separation and identification of anthocyanidins

The separation to the hull pigments by various chromatographic methods made clear that blackseeded Huayou No. 8 (B. napus) was composed of three different anthocyanidins. The three pigments were identified probably as cyanidin, malvidin and delphinidin, in which cyanidin was the main component, based on the following evidence: R_f data in five solvent systems of paper chromatography and spectral data. Mass spectrometric examination also provided a valuble aid for the above identification.

5. The measurement of pigments

The total content of lutein in embryo and anthocyanidins in hull were measured by spectrophotometric method. The analytical results of 45 samples from six different types indicated that lutein content varied from different seed cultivars, with no relation to hull color; but

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anthocyanidin content appeared such a significant diversity according to the difference of hull color that dark hulls contained much higher anthocyanidins than light ones, without exception.

6. The relation between seedcoat color and contents of pigment and oil

The oil and pigment contents in 4 samples from the same cultivars with light and dark hulls from B. napus and B. campestris were determined (Table 1). The amount of oil in the light hulls is much higher and the amount of total anthocyanidins is significantly lower than in the dark hulls; total content of lutein, however, is not different.

Table 1. Comparison of chemical components in light and dark seed

Types	Cultivars	Seedcoat color	Mean content(1) Lutein in embryo (PPm)	Anthocyanidin in hull (m_g/g)	Oil in seed (%)
B. napus	Huayou No.8	Black	21.84	7.117	37.12
		Light brown	19.25	0.367	40.45
		Difference	-2.59	-6.750	+3.33
	Ningyou No.7	Black	22.91	2.504	41.09
		Light brown	23.59	0.282	43.18
		Difference	+0.68	-2.222	+2.09
B. campestris	Leshan golden	Black	14.28	6.243	38.03
		Yellow	16.03	0.242	40.17
		Difference	+1.75	-6.001	+2.14
	501	Black	15.39	5.884	32.22
		Yellow	16.53	0.245	37.27
		Difference	+1.14	-5.639	+5.05

(1) Based on three measurements

* This research program was directed under the leadership of Prof. Dr. Liu Hou-li, Director of the Institute of Crop Genetics and Breeding, Huazhong Agricultural University, Wuhan, China.

Genotypes for High Oleic Acid Content (about 80%) in the Oil of Rapeseed (*Brassica napus* L.)

B. Y. Chen and B. Gertsson

It has been one of the main objectives in rapeseed quality breeding to improve fatty acid composition of the oil. In this regard, it is stressed that high linoleic acid content with two double bonds in its carbon chain is nutritionally desirable. However, the two double bonds of linoleic acid can easily be destroyed at high temperature e.g. when rapeseed oil is used for frying. Another type of fatty acid composition is thus desirable for oil used for frying. For such a purpose, a high oleic acid content with only one double bond in its carbon chain would be preferable. It is also of interest for industrial applications to have a high oleic rapeseed oil (Fick 1983; Friedt 1988).

In Svalöf, we have been screening *B. napus* genotypes for high oleic acid content, using gas chromatography to analyse fatty acid composition. In the F_2 of a cross between a breeding line No7477 and a resynthesized *B. napus* stock No7406, two seeds (EJ120 and EJ142) out of forty seeds analysed were found to contain more than 80% oleic acid (Table 1).

The highest content of oleic acid content was 60.4% among the analysed thirty seeds of the erucic-free breeding line No7477. No7406 was resynthesized from a cross between *B. alboglabra* No4003 and *B. campestris* var. yellow sarson K-151 (for more detail, see Chen et al 1988).The comparison of the fatty acid composition of No7406 with the mid-parent values (1/2 (No4003+K-151)) indicated that the high oleic acid content was trasgressively epistatic over the low content (Table 1). This means that the high oleic acid content must at least be partially due to the intergenomic interaction. It is thus possible in *B. napus* to fix the heterosis caused by the intergenomic interaction. More work is underway to test the extent to which the high oleic acid content can be stabilized.

Acknowledgements: Our sincere thanks to T Säll for his kind assistance in statistical calculations.

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Table 1. Fatty acid composition of *B. napus* accessions EJ120 and EJ142 having high oleic acid content and the relevant material involved in their pedigree

Accession	No of seeds	Fatty acid composition as percent of total fatty acids (M±SE)					
		Palmictic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
B. alboglabra							
No4003	25	3.7±0.1	15.2±0.5	15.2±0.2	10.7±0.3	7.1±0.2	47.1±0.4
B. campestris							
K-151	15	2.4±0.1	11.5±0.4	13.4±0.2	10.0±0.2	4.3±0.2	56.5±0.5
1/2(No4003+K-151)		3.1	13.4	14.3	10.4	5.7	51.8
B. napus			**	***	***	***	ns **
No7406	20	2.7±0.1	19.6±0.7	12.6±0.5	5.3±0.3	6.0±0.3	53.5±0.6
No7477[a]	30	4.8	60.4	22.9	11.2	0.8	0.0
EJ120		3.9	82.8	7.4	3.2	1.5	0.5
EJ142		3.8	83.7	6.5	2.5	2.2	0.8

** and *** differ at 1% and 0.1%, respectively; ns: non-significant.

a: The fatty acid composition of the seed having the highest oleic acid content is presented.

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GENETICS OF OIL CONTENT IN OILSEED RAPE (*BRASSICA NAPUS*)

S K Gupta and K S Labana

Oil is the most valuable component of the seed. The seeds of *Brassica napus* contain 40 to 45 per cent oil. Now, the efforts are being made to increase the quality as well as quantity of oil. The ease and the speed with which the oil content can be measured by wide line nuclear magnetic resonance (NMR) and near-infrared reflectance spectroscopy (NIR) has made increased oil content a prime objective. Therefore, in the present study, an attempt has been made to know the gene action and combining ability for oil content so as to fomulate an appropriate breeding strategy for increasing the oil content.

Materials and Methods: The material comprised of eight genetically diverse genotypes of *Brassica napus* and were crossed in a 8 x 8 diallel set of crosses excluding reciprocals. The parents and hybrids were grown in a randomised complete block design with three replications. The oil content was measured by wide line nuclear magnetic resonance (NMR). The combining ability analysis was done following Griffing (1956a) Method-2 and Model-1.

Results and Discussion: The analysis of variance for combining ability indicated that only gca is highly significant (Table 1). Thus, additive gene effects are important in governing the inheritance of this trait. Thus, to breed for high oil content, which is desirable, selection methods can be followed. The parents Ashai, Lores are the desirable general combiners for high oil content. Therefore, these parents can be used in different selection programmes.

Table 1. Analysis of variance for combining ability

Source of variation	d.f.	Mean squares
gca	7	1.83
sca	28	0.27
Error	70	0.27
$\frac{1}{7}\sum_{i} g_i^2$		0.145
$\frac{1}{28}\sum_{ij} 2\,{}^{2}_{ij}$		-

** Significant at 1 per cent level of significance.

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VARIATION IN GLUCOSINOLATE CONTENT IN DIFFERENT GENERATIONS OF OILSEED RAPE (*Brassica napus*)

K.S. Labana and S.K. Gupta

Glucosinolate content is an important determinant of quality in oilseed rape and most oilseed breeders presently are concentrating their research on the development of varieties with low glucosinolate content. As a knowledge of the variation in glucosinolate content is the first requirement in formulating any breeding strategies, the present study was undertaken. The glucosinolate content was estimated using the chlorimetric method of Brzezinski and Mendelewski (1984). The two crosses were made between similar genotypes having high and low glucosinolate content. In the cross Bronowski x Topa, the parents had minimum glucosinolate content (10-12 μ mol/g). The F_2 had more glucosinolate content (29.29 μ mol/g) as compared to their parents. However, the F_1 had 13.20 μ mol/g glucosinolate content. The backcross generations, viz. B_1 and B_2 had 18.80 and 16.38 μ mol/g respectively.

The parents in the cross GSL-1 x Nikalis had high (89.44 to 94.41 μ mol/g) glucosinolate content. However, the F_1 had 97.50 μ mol/g which exceeded both the parents. In backcross generations, the mean glucosinolate content were comparable to their recurrent parents. The F_2 had the same glucosinolate content as the backcross generations. This study indicates a large variation in glucosinolate content in these materials which can be utilized in inheritance studies so that an appropriate strategy can be formulated for developing low glucosinolate lines in oilseed rape.

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GLUCOSINOLATES IN RAPID-CYCLING BRASSICAS

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

Glucosinolates (GS's) in all plant parts above ground were determined in bulk samples of 12 plants of rapid-cycling populations of Brassica campestris (aa, CrGC 1), B. nigra (bb, CrGC 2), B. oleracea (cc, CrGC 3) B. juncea (aabb, CrGC 4), B. napus (aacc, CrGC5) and B. carinata (bbcc, CrGC 6) (Williams and Hill, 1986). Analytical methodology was described in Carlson, et al, 1981 and Daxenbichler, et al, 1979. Samples were collected by cutting stems at the soil line when each population was beginning to flower. Plants were grown in a growth chamber set at 24C with continuous fluorescent light of 250 $mEs^{-1}m^2$ and were watered daily with 50% Hoagland's nutrient solution.

The table below summarizes the GS contents. Species can be distinguished by GS levels. Those species having the "b" genome (bb, aabb, bbcc) have high levels of allyl GS. Species having the "a" genome (aa, aacc, aacc) have high levels of 3-butenyl GS while species with the "c" genome (cc, aacc, bbcc) have higher levels of 2-hydroxy-3-butenyl and 3-indolylmethyl GS's. B. carinata (bbcc), however, lacks 2-hydroxy-3-butenyl GS and has a very similar GS profile to B. nigra (bb). These results agree with the analyses of Oriental vegetable cultivars (Hill, et al, 1987).

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GLUCOSINOLATES IN CrGC[a] BASE POPULATIONS OF SIX SPECIES OF BRASSICA MEASURED AT THE STAGE OF FIRST OPEN FLOWER (umole/100g fresh wt.)

Brassica species -	campestris	nigra	oleracea	juncea	napus	carinata
Genome -	aa	bb	cc	aabb	aacc	bbcc
Glucosinolate CrGC Stock	1	2	3	4	5	6
Allyl	0[b]	108	38	81	0	226
1- methylpropyl	2	0	0	4	0	0
3-methylsulfinylpropyl	0	0	10	0	0	tr
4-methylthiobutyl	0	0	1	0	0	0
4-methylsulfinylbutyl	4	0	20	0	0	0
3-butenyl	121	0	35	46	16	tr
2-hydroxy-3-buyenyl	0	0	42	0	66	0
5-methylthiopentyl	0	0	0	0	1	0
5-methylsulfinylpentyl	1	0	0	0	16	0
4-pentenyl	4	0	0	0	21	0
2-phenylethyl	7	6	1	4	5	4
3-indolylmethyl	1	2	12	1	14	11
Total [c]	164	182	214	194	199	353
% Glucose account for [d]	86	64	74	70	71	68

[a] CrGC = Crucifer Genetics Co-operative (see Williams and Hill, 1986)

[b] Data are the means of two bulk samples

[c] Calculated from measurement of glucose released upon hydrolysis and using an average molecular weight of 461 for glucosinolates.

[d] Amount of individual GS's relative to glucose obtained as a measure of total glucosinolates.

tr = trace amount measured

GENETIC STUDIES ON THE TOTAL CONTENT OF GLUCOSINOLATES IN SEEDS OF RAPE (Brassica napus L.)

Mou Tongmin and Liu Houli

The inheritance of the total content of glucosinolates (TCG) in seeds and interrelationship between the TCG and 11 agronomic traits and oil content were investigated for six rape (Brassica napus L.) cultivars. The three with higher TCG were bred at home, namely Huayou No.16, Huayou No.13 and 71-39, the rest with lower TCG were introduced from abroad, namely Marnoo, Wesroona and Andor. The main results of these studies are as follows :

1. The average TCG of seeds from F1 plants was greater than the mid-parent value, but lower than the parents with larger value, indicating that the higher value is partially dominant to the lower value. The TCG of the reciprocal F1's, F2's and first backcross generations were insignificantly different to each other, indicating that the cytoplasm from the mother plant does not affect TCG in seeds.

2. From the distributions in the F2 and first backcross generations TCG appeared to be a quantitative character. A small fraction, 1/64, of F2 progeny plants of the reciprocal crosses Huayou No.16 X Marrnoo and Huayou No.13 X Wesroona fell into the range of the lower TCG parents. The fraction in F2's derived from the cross 71-39 X Andor and its reciprocal was 1/16. In the first backcross generations derived from the (Huayou No.16 X Marnoo) X Marnoo and (Marnoo X Huayou No.16) X Marnoo, the lower TCG plants made up 1/8, but in the first backcross generations from the (71-39 X Andor) X Andor and (Andor X 71-39) X Andor, the lower TCG plants made up 1/4. These results indicated that there are three locus differences between Huayou No.16 and Marnoo, and between Huayou No.13 and Wesroona, but two locus differences between 71-39 and Andor. It may be first discovered that two partial dominant pairs of genes control the high TCG

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in some cultivars in Brassica nupus.

3. Gene effects of TCG were estimated from means and mean variances for 4 sets, each including P1, P2, F1, F2, B1 and B2 populations. The results showed that the digenic interaction models are adequate for TCG in 4 sets. The dominant effect (h) and dominant X dominant effect (l) were important for Huayou No.16 and Marnoo, but there was no dominant effect for 71-39 and Andor.

4. The results of the incomplete diallel analysis indicated no significant difference for TCG between the direct and reciprocal crosses. The general combining ability was significantly different among the higher TCG cultivars, but not significantly different among the lower TCG cultivars. The specific combining ability was insignificant. The broad and narrow heritability was 0.96 and 0.93, respectively.

5. Simple, partial and multiple correlation coefficients between the TCG and eleven agronomic traits and oil content were estimated using various generations derived from the cross 71-39 X Andor. The partial and multiple correlation coefficients between the TCG and other characters were insignificant in P1, P2 and RF1 generations. The partial correlation coefficients between the TCG and 1000-seed weight were significant in F2 and RB1 generations. The multiple correlation coefficient between the TCG and all other characters was significant at 5% level in F2 gereration.

Aplication of NIR method for screening of oilseed rape on glucosinolate content.

K.Michalski J.Krzymanski

Inexpensive and fast method of glucosinolate determination in seeds is neccesary for the breeding of new varietes of oilseed rape with very low glucosinolate level. We propose to use the NIR method for screening of large number of seed samples.

NIR spectrophotometer with gratting monochromator (Infrapid-61, LaborMim Hungary) was used to measure reflection spectra. Although samples for measurement are usually grinded, we try to use the intact seeds. It is important for breeding, that seeds are not damaged after measurement and can be used for growing new generation.

For spectrophotometer calibration 50 samples of seeds were taken, with glucosinolate content ranging from 5 to 130 µM/g fat free matter. Because the accuracy of chemical analyses is very important for good calibration of instrument, analyses of each sample were made by the use of several different methods (GC/UV (1,2), HPLC (3), total sulphur content, thymol method (4)). For better accuracy the results obtained by GC/UV analyses for total alkenyl glucosinolate content were mathematically corrected by regression with results of other methods. Reflection spectra were taken off the air dry seed samples (moisture percent <6) and sent by serial port into IBM PC/AT computer. Each sample was measured 7 times.

Special programme "Infreg" was writen for data analysis and calulation of multiple regression. Second derivative of optic density was chosen by programme after search through all mathematics available in Infrapid-61. Then the "Infreg" programme was used to find the best 6 wavelenghts with the highest determination coefficient. Calculations were done by statistical analysis of all wavelenghts in 1300-2400 nm range by 1 nm steps. It was find that the range from 1600 to 1700 nm was the best and sufficient for glucosinolate determination (table 1).

Te obtained determination coefficient for multiple linear regression is high and statistically significant but standard error is still unsatisfactory in particular in lower range of glucosinolate contents. The second calibration was done by using subset of seed samples with glucosinolate content below 40 µM. Narrowed range glucosinolate allowed to improve the accuracy (tab.2).

Conclusions

-Parameters calculated above can be used for the calibration of Infrapid-61 analyser for glucosinolate estimations.

-Analyses of variance demonstrate, that the standard deviation of glucosinolate determination by Infrapid-61 is mainly due to error of measurement. More accurate results may be obtained by taking an average from several measurements.

-Better fitness of regression to chemical analyses may be obtained by dividing the full glucosinolate range in two parts or by using of nonlinear regression.

-Glucosinolate determination in whole seeds of oilseed rape by Infrapid-61 is sufficient precise for the first step selection of breeding materials.

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Table 1. Calibration data based on 350 spectra (7 blocks * 50 samples, second derivative, dλ=14 nm, glucosinolate range 5-130 μM/g f.f.m).

wavelenght (nm)	partial correlation coefficient	Student test for multiple regression
1627	-0.985	2.213 *
1643	0.429	8.956 ***
1603	0.896	5.308 ***
1624	-0.984	5.281 ***
1658	0.280	4.435 ***
1630	-0.978	3.573 ***
standard deviation = 5.9 determination coeff.= 0.985		F=2207.05
analysis of variance standard deviation of measurement = 5.4 standard deviation of regression = 3.1		

Table 2. Calibration data based on 210 spectra (7 blocks * 30 samples, second derivative, dλ=14 nm, glucosinolate content < 40 μM/g f.f.m.)

wavelenght (nm)	partial correlation coefficient	Student test for multiple regresion
1627	-0.849	2.116 *
1643	-0.500	4.238 ***
1624	-0.832	2.924 **
1658	-0.453	1.769 *
1630	-0.825	2.463 **
standard deviation = 4.2 determination coeff.= 0.758		F=69.144
analysis of variance standard deviation of measurement = 3.2 standard deviation of regression = 2.9		

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THE PALLADIUM TEST AND THE THYMOL TEST FOR THE ANALSYIS OF GLUCOSINOLATES IN THE GREEN MATTER OF RAPE (BRASSICA NAPUS L.)

H. Bennert and T. Pauling

There are several quick und reliable methods for the quantitative determination of total glucosinolate (GSL) content in seeds. These methods have not been adapted to the analysis of green matter, so far. But fodder brassica breeders are interested in rapid techniques, as well. In this paper the palladium-GSL complex method (MØLLER et al. 1985) and the determation of glucose derived from GSLs with thymol (McGREGOR and DOWNEY 1986) were tested for their applicability to the analysis of green matter.

Material
Dried green matter of the cultivars Liratop, Akela, Parapluie, Liragrün and 36 fodder rape breeding lines (DSV, Lippstadt), harvested in 1985, was analysed.

Methods
Plants were dried at 80°C and milled to a powder.
1) **Extraction of GSLs**
Glucosinolates were extracted with methanol (70%) followed by a precipitation of proteins with barium/lead acetate.
2) **Isolation of intact GSLs** (see MØLLER et al. 1985)
3) **Isolation of desulfo GSLs**
Glucosinolates were isolated on DEAE Sephadex A25 mini columns (formiate form). The desulfatation took place with purified H1 sulfatase over night. Desulfo GSLs were eluated with HPLC water.
4) **Pd-Test** (see MØLLER et al. 1985)
The eluates of intact and desulfo GSLs were processed in the same way.
5) **Thymol test** (see McGREGOR and DOWNEY 1986)
6) **HPLC of desulfo glucosinolates**
A Knauer HPLC gradient system was used.
Column: Nucleosil C18, 120 x 4,6 mm,
Programm: A = water, B = Acetonitril (20%): Omin - 95%A/5%B,
20min - 5%A/95%B, 25min - 95%A/5%B, 30min - 95%A/5%B,
Detection at 229 nm.

Results and Discussion
HPLC and Pd test determinations of intact and desulfo GSLs were correlated with r = 0,90 and r = 0,98, respectively. This difference is due to different ways of isolation. For intact GSLs isolated from seeds a similar relationship was observed by MARQUARD and SCHLESINGER (1985).
Only a low correlation was found between the thymol test and HPLC values (r = 0,42), caused by a number of analysises which showed significant higher figures with the thymol test than with HPLC. Deviating values could be explained by phosphorylated glucose compounds which may also adsorbate at Ecteola cellulose. Free glucose contained in the green matter is washed quantitatively from the ion exchange material (unpublished results).

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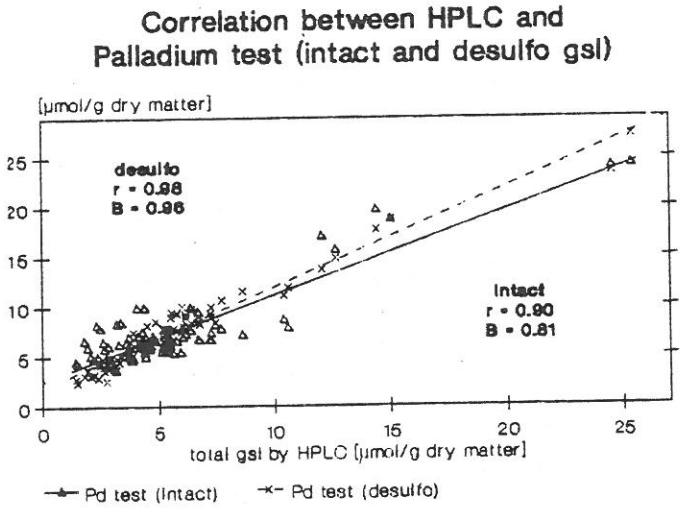
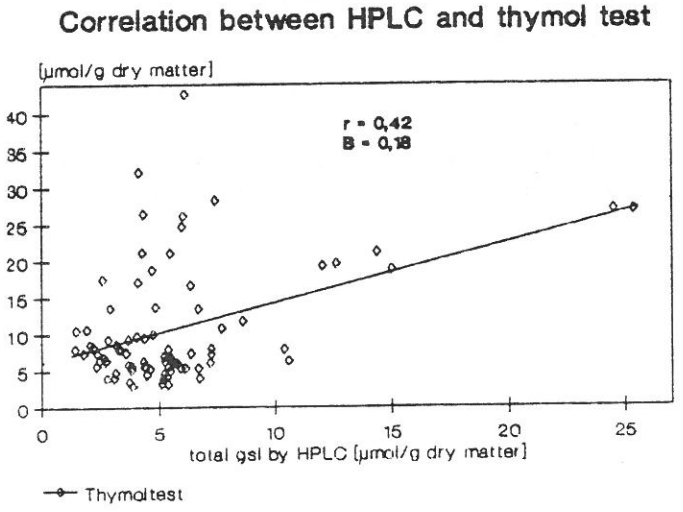
Conclusions

The Pd test has good properties for the analysis of glucosinolates in the green matter of fodder brassicas with a very good correlation to the HPLC values.



The thymol test cannot be recommended for analysing green matter because of interfering compounds in the raw extract.



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A GENETIC ANALYSIS OF QUALITATIVE CHARACTERS IN RAPESEED (BRASSICA NAPUS L)

Hu Zhongli

Five inbred lines of rapeseed were intercrossed to obtain a diallel set including reciprocals. The contents of oil and total glucosinolates were investigated through diallel analyses according to the method presented by Hayman (1954) and described by Mather and Jinks (1982). But in the case of protein content, half-diallel cross analysis was carried out according to the procedure given by Singh (1981). The main results are as follows:

1. The additive-dominance model was perfectly adequate for the genetic analyses of all the characters.

2. The average dominance degrees of oil and total glucosinolate content were partial dominance that tended to increase oil and total glucosinolate content. The estimates of narrow-sense heritability of oil content and total glucosinolate content were 59.6% and 87.4% respectively.

3. Overdominance of protein content was found by degree of average dominance ($\sqrt{H/D} = 1.58$) and Wr/Vr graph. It was observed that overdominance decreased protein content. The estimate of narrow heritability was 42.4%.

4. Oil content showed a highly significant negative correlation with protein content and a nearly significant negative correlation with total glucosinolate content. But total glucosinolate content had a significant positive correlation with protein content.

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EMBRYOGENY OF RAPID-CYCLING BRASSICA RAPA

K Tang and P H Williams

To provide a basis for further studies of embryogeny in rapid cycling Brassica rapa (RCBr), the developmental relationships among silique length, ovule size and embryo growth stages have been examined using the stock CrGC 1-1 from the Crucifer Genetics Cooperative. Plants were grown at 22 C under continual illumination of 250 $\mu Es^{-1}m^{-2}$ and a single pollination was made three days after flowering commenced. Siliques were removed from the plants at 3, 6, 8, 10, 13, 16, 19, 21 days after pollination (DAP) and measured under dissecting microscope. Ovules and embryos were also removed and measured. Silique length increased rapidly during the first 7 days after pollination then slowed. Ovule size increased more slowly reaching maximum at about 13 DAP. After 3 days the embryos had 2-4 cells with a 7-8 cell suspensor. The embryo was in the undifferentiated globular stage until 7 DAP. Differentiation to the heart stage commenced at 7 DAP progressing to torpedo by 11 DAP and walking stick by 14 DAP. By 19 DAP the embryos were fully developed. There was little variation in the developmental stages of embryos whether the flowers were pollinated one or three days after opening. The uniformity in embryo development following pollination suggests that DAP can be used as a relatively reliable 'time line' in studying events in embryogenesis.

Table 1. Embryogenesis in rapid-cycling Brassica rapa (CrGC 1-1)[a]

DAP	Silique		Ovule		Stage of embryo development
	length(cm)	width(cm)	length(cm)	width(cm)	
3	1.74±0.268	0.09±0.014	0.05±0.012	0.04±0.006	2-4 cells
6	4.59±1.118	0.19±0.024	0.11±0.027	0.08±0.017	globular
8	6.17±0.974	0.26±0.060	0.15±0.007	0.10±0.005	globular
10	5.81±1.005	0.36±0.002	0.18±0.016	0.14±0.026	heart
13	6.35±0.891	0.42±0.058	0.21±0.009	0.19±0.009	early walking stick
16	6.43±1.299	0.42±0.059	0.21±0.013	0.19±0.067	late walking stick
19	6.68±0.907	0.44±0.033	0.20±0.007	0.18±0.067	mature
21	6.09±1.780	0.43±0.065	0.26±0.013	0.19±0.010	mature

[a]Each datum represents 30 silique, 25 ovules and 25 embryos measured.
DAP = days after pollination.

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DEVELOPMENT OF TRISOMICS OF RAPID-CYCLING BRASSICA RAPA

K Tang, C B Hill and P H Williams

The development of trisomic stocks of rapid-cycling Brassica rapa (RCBr) has been continued since our earlier report [Hill, Tang and Williams, Cruciferae Newsletter, 1986].

The karyotype of RCBr has been determined using an improved chromosome preparation technique. RCBr consists of 2 pairs of metacentric, 3 pairs of submetacentric, 2 pairs of subtelocentric, 2 pairs of telocentric and 1 pair of satellite chromosomes.

Fifty-six aneuploids were obtained from crosses of 3N X 2N and 50 primary trisomic plants were obtained from 117 progenies of (2N + 2) X 2N, (2N + 3) X 2N, (2N + 4) X 2N. According to the karyotype of RCBr we have identified 6 different trisomics, representing chromosomes 5, 6, 7, 8, 9 and 10 respectively. Seeds of the trisomics have increased for 1-3 generations with the exception of trisomic 7 which did not produce seed. Trisomy in RCBr is not accompanied by distinctive differences in phenotype. Transmission rate of trisomics is dependent on both the particular chromosome and on the pollen parent. For example, when the trisomic 6 was crossed with Brassica rapa, Saichin group, cv. Flower Pak Choy, the transmission rate was 28%, but when the same plant was crossed with B. rapa, Brocotetto group, cv. Hong Tsai Tai, the transmission rate was 46%. The transmission rate was much higher when the trisomic was the female than when the trisomic was the male.

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Hill, C.B., Tang, K. and Williams, P.H. 1986. Development of primary trisomic of rapid-cycling Brassica campestris. EUCARPIA, Cruciferae Newsletter 10:30.

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Table 1. The transmission rate of trisomics in rapid-cycling Brassica rapa (RCBr)

Female	Pollen	Generation examined	No. of progenies examined	No. of trisomics obtained	Percent trans.
Trisomic 10					
211873-22	CrGC 1-9	first	25	9	36
211873-20	**	first	48	17	35.4
211873-20	Stored pollen	second	34	1	2.9
Trisomic 9					
211873-12	**	first	44	12	27.3
Trisomic 8					
211870-18	**	first	19	3	15.8
	**	second	33	5	15.2
	Flowering pak choy	third	38	8	21.1
2N plant	**	third	40	1	2.5
Trisomic 6					
211862-12	**	first	38	14	38.9
	2N from Flower Pak Choy	second	35	10	28.6
	2N from Hong Tsai Tai	second	39	18	46
Trisomic 5					
211808-4	2N from 21229	first	44	4	9.1
	2N from Hong Tsai Tai	second	43	8	18

** means pollen from 2N plant of the same line as trisomic.

CHROMOCENTRE COUNTS AS AN AID IN DETERMINING THE PLOIDY LEVEL IN CRUCIFERS

BIJAY S. SINGH and N. DAYAL

Crucifers are characterized by the presence of dark staining hetropycnotic bodies called chromocentres in their interphase nuclei. Chromocentres represent the pericentric heterochromatic regions of chromosomes. Crucifers may be suitably modelled for studies of chromocentres. Over the past 15 years in this laboratory, we have made extensive cytogenetical studies on chromocentre frequency (cfr) and its distribution in radish and other crucifers (Dayal, 1975, 1986; Dayal et.al., 1982, 1983). We have also shown that the mean cfr is positively corelated with the diploid number of chromosomes in crucifers (Dayal and Kumar, 1984).

In experimental production of polyploids by means of colchicine technique, polyploid plants are usually screened on the basis of morphophysiological studies such as leaf characteristics, stomatal size and index, floral characteristics, delayed flowering, size of pollen grains, fruit and seed and finally confirmed by chromosome counts. All these methods are time consuming and lengthy procedures and requires, specially for cytological analysis, considerable technical skill. In contrast to these methods, polyploid plants can easily be screened by determining the mean cfr in colchicine treated plants (C_1) of crucifers. Mean cfr in them can be easily and quickly determined by counting the number of chromocentres in the interphase nuclei. It requires no special cytological skill. In this method receptive cells of the stigma are selected for the count of chromocentres. For this styles from mature flower buds are fixed in acetic alcohol (1:3 v/v) mordanted with a few drops of $FeCl_3$ and stained and squashed with 1.5% acetocarmine. Chromocentre counts are made only in well squashed receptive cells. 20 cells per plants are scored and their mean value is computed (Dayal, 1975).

By means of chromocentre counts we have been able to screen 34 colchiploid plants from a total of 735 seedlings of radish (*Raphanus sativus* L.) treated with 0.25% solution of colchicine (Make-LOBA-CHEMIE INDOAUSTRANAL, CO., BOMBAY).

In population plants belonging to the variety "Japanese White" of radish mean cfr was found to be 14.0 ± 0.12. In contrast to this, colchicine treated radish plants (C_1) showed significantly a much higher

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mean cfr 22.5 ± 0.18 (Table 1). Thus, in tetraploid plants mean cfr increased by 1.5 times when compared to their diploid ones. Plants having higher mean cfr were also found to possess all other characteristics of polyploids such as increased number of serretions in the leaf margin, thicker and fleshy leaves, higher stomatal index and larger stomata, delayed flowering and larger size of lower and floral parts, pollen grains and seeds when tested against the diploid population plants. These plants were confirmed to be autotetraploids by studying the chromosome behaviour during meiosis and determining the gametic chromosome number (n=18).

Table-1: Chromocentres per nucleus in diploid and colchitetraploid radish

Materials	No. of plants	Chromocentres/ nucleus M ± S.E.	CV %
Diploid (2n=18)	10	14.0 ± 0.12	8.5
Tetraploid (2n=36)	34	22.5 ± 0.18	23.5

Our earlier studies on radish and other crucifers have shown that mean cfr is characteristic for a species or even for a population and depends upon chromosome length, genome size, genetic factors etc. Here we suggest that mean cfr can very well be exploited as a suitable and confirmatory cytological method for determining the level of ploidy in radish.

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APPLICATION OF INDUCED PARTHENOGENESIS IN RAPESEED BREEDING

Luo Peng Wu Suhui

Parthenogenesis is the development of an individual from an egg without fertilization. The individuals thus produced are homozygous in heredity and potentially valuable in plant breeding. Many investigators (Olsson et al., 1955: Stringam et al., 1973; Luo et al., 1976), accordingly, studied parthenogenesis and its application.

Rapeseed is one of the most important crops in China, especially in Sichuan. Studies on parthenogenesis and its application for increasing seed yield of rapeseed have been conducted for several years. The results follow.

1. Parthenogenesis was induced in rapeseed by treatment with several chemicals, physical agents, and pollen of several species of Brassica. Interspecific pollen and the pollen treated with 5000r γ-rays were effective. Especially effective was the interspecific pollen of B. campestris, which induced parthenogenetic haploids at a frequency of 1.25%. Thus interspecific pollination with B. campestris is a satisfactory means of inducing parthenogenesis in rapeseed.

2. Induced parthenogenetic haploids of rapeseed are similar to their mother plants in shape, but are smaller in stature. Both the leaves and stomata are smaller with fewer chloroplasts in the guard cells. During the flowering season, the early buds are often sterile, and flowers are smaller, with small short stamens. The pollen grains are poorly developed. and of very low viability. There are fewer pods per plant and they are poorly developed. The chromosome number of the cells of the root tips and young leaves of the induced parthenogenetic haploids is 19. Some cells have a number of 38, which may be a result of diploidization. Meiosis of pollen mother cells is irregular. Lagging chromosomes and chromosome bridges are often observed. This irregular behavior often leads to sterility of the parthenogenetic haploids. The production of the parthenogenetic haploids may be the result of psudogamy induced by interspecific pollination.

3. Parthenogenetic homozygous inbred lines of rapeseed were produced as follows. Young buds of induced parthenogenetic haploids were treated with 0.2% colchicine solution and cultivated under good agricultural conditions. The haploids were then diploidized with an increse in fertility. Certain parthenogenetic haploids, when isolated, yielded a few homozygous inbred seeds. From these seeds a few homozygous inbred lines, such as 3529 resulted. Parthenogenetic inbred lines of rapeseed were similar to their mother plants in shape and uniformity. They grew normally and were male fertile. The

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chromosome number was 38,and their offsprings were uniform in characters . Plants grown from the open-crossed seeds of the parthenogenetic haloids were also similar in shape to the mother plants. They grew vigorously and were male fertile . The chromosome number of their body cells was 38, but they lack uniformity.

4. Studies of hybrid rapeseed of parthenogenetic origin were conducted. A parthenogenetic inbred line was crossed with many rapeseed cultivars and inbred lines, with resultant cross combinations. Tests were conducted with the local cultivar Silan 302 as the standard. The combinations of 3529 X Oro and 3529 X Swiss were the most promising, from which the hybrid rapeseed of parthenogenetic origin was selected. The hybrid rapeseed of parthenogenetic origin was a good combination. It is highyielding, good in oil quality and resistant to adverse environments. The production of hybrid seeds with the available technology was then achieved by growing seed and pollen parent plants in alternating rows. During the flowering season, the flowers of the mother plants were emasculated by the chemical ($CH_2As_3H_2O$). At the harvest the hybrid seeds are gathered from the seed parents. Yields of 600-750 kg/ha. of hybrid seeds were obtained.

5. Hybrid rapeseed of parthenogenetic origin was field evaluated. In 1986-1988, The regional test of Sichuan province was conducted with the local cultivar Silan 302 as the standard for comparison. Average yield of the hybrid rapeseed was 2,132 kg/ha., while that for Silan 302 was only 1,781 kg/ha. Hybrid rapeseed outyielded the standard by 19.7 %. The field production data of hybrid rapeseed were collected. The average yield of the hybrid rapeseed was 2,693 kg/ha., while that of the standard Silan 302 was only 2,342 kg/ha. The hybrid rapeseed outyielded the standard by about 15.0%. Hybrid rapeseed of parthenogenetic origin is now cultivated on over 6,000 ha. in several districts in Sichuan.

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PRELIMINARY STUDY ON THE RADISH CYTOPLASMIC EFFECTS AND PARENTAL SELECTION OF "AIJIAOHUANG" CMS (OGURA)

I. Radish Cytoplasmic Effects

Hao Xiuming and Cao Shouchun

The cytoplasmic effects on main F_1 hybrid economic traits of non-heading Chinese cabbage (Brassica campestris L. ssp. chinensis (L.) Makino) Aijiaohuang CMS with radish (Raphanus sativus L.) cytoplasm (Ogura, 1968) are investigated. Usage of the CMS on F_1 hybrid breeding is still discussed.

When introduced to our university, the source of CMS has been backcrossed with 3 generations of Chinese head cabbage (Brassics campestris L. ssp. pekinensis (Lour) Olsson) and 2 generations of another non-heading Chinese cabbage cultivar Wayiao. This source of CMS then was transferred into Aijiaohuang in conventional manner by successive backcrossing with male fertile inbred line of Aijiaohuang AB for 4 generations. Now, the economic traits of Aijiaohuang CMS are stable and its seed-setting after pollination by hand is normal as non-heading Chinese cabbage.

Using Aijiaohuang CMS and its maintainter as maternal parents, 5 spring cultivated type inbred lines and 5 autumn-winter cultivated type inbred lines of non-heading Chinese cabbage as parental parents, 20 F_1 hybrids were made. According to the cultivar property, three field trials with randomized block design of four replicates were conducted. In summer cultivated period, maternal parents, 5 autumn-winter cultivated inbred lines and their 10 F_1 hybrids were cultivated. In winter-spring cultivated period, maternal parents, 5 spring cultivated inbred lines and their 10 F_1 hybrids were planted. In autumn-winter cultivated period, all the materials were used.

We explored the radish cytoplasmic effects by comparing the average number of total F_1 hybrids with radish cytoplasm ($\bar{x}A$) and that of total F_1 hybrids with non-heading Chinese cabbage ($\bar{x}B$) in each trait. The ratio of average number difference ($\bar{x}A - \bar{x}B$) to the average number of all hybrids ($\bar{x}A + \bar{x}B)/2$ expressed the effect degree of radish cytoplasmic effect on each trait. The results were as follows:

The radish cytoplasmic effects on F_1 hybrid traits varied with different cultivated periods and diverse parental nuclear genetic background. The negative effects in

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autumn-winter cultivated period were least. In this period, the average number of ten hybrids with radish cytoplasm was significantly higher than that with non-heading Chinese cabbage cytoplasm in vitamin C content in fresh blade and weight ratio of blade / petiole, and significantly lower in plant weight, plant width and height, number of leaves, single leaf weight, the fresh leaf weight and chlorophyll per unit area and specific leaf weight. There was no significant difference in vitamin C content in fresh petiole, dry matter and soluable solid substance content in fresh blade and petiole and plant diseases. Among above traits having significant radish cytoplasmic effect, the effect degree $(\bar{x}A - \bar{x}B) / ((\bar{x}A + \bar{x}B)/2) \times 100$ in plant weight (53.9), single leaf weight (39.73) and weight ratio of blade / petiole (40.73) was largest, that on plant height (15.89), number of leaves (17.29) and chlorophyll content per unit area (23.47) was middle, and other traits below 8. Because the nutrent content of fresh blade is 1-2 times higher than that of petiole, we may sum up the radish cytoplasm effects as two opposite respects. One is decreasing the plant weight and its related characters. Another is increasing the nutrent in per unit weight vegetable. The value of Ogura's source CMS on non-heading Chinese cabbage F_1 hybrid breeding depends on the balance of this two respects.

In summer and winter-spring two trials, the cytoplasmic effect property on main economic traits was similar to that in autumn-winter cultivated period except that no difference existed on weight ratio of blade / petiole in winter-spring period. The negative effect degree on plant weight, number of leaves and single leaf weight grew by 18-83% in summer cultivated period, and 13-33% in winter-spring cultivated period than autumn-winter cultivated period.

The radish cytoplasmic effect difference among some parental nuclear genetic background attained the significant level at 5% in plant weight and width, weight ratio of blade / petiole, single leaf weight, number of leaves, chrolophyll content and specific leaf weight.

Further attempt on fine quality F_1 hybrid breeding using this sourse of CMS in non-heading Chinese cabbage is necessary and valuable.

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PRELIMINARY STUDY ON THE RADISH CYTOPLASMIC EFFECTS AND PARENTAL SELECTION OF "AIJIAOHUANG" CMS (OGURA)

II. Parental Selection

Hao Xiuming and Cao Shouchun

One Aijiaohuang CMS Line with radish (Raphanus sativus L.) cytoplasm and its maintainer, 10 cultivar inbred lines and their 20 F_1 hybrids of non-heading Chinese cabbage (Brassica campestris L. ssp. chinensis (L.) Makino) were used as experimental materials in this research. An experiment with randomized block design of four replicates was carried out in autumn - winter period in which the radish negative cytoplasmic effects were least. Main economic characters involving vitamin C, soluable solid substance and dry matter content in fresh blade and petiole, plant weight, signle leaf weight, number of leaves, weight ratio of blade / petiole, the fresh leaf weight and chlorophyll content per unit area and specific leaf weight of the experimental materials were evaluated. The purpose of this study is exploring the parental selection nature of Aijiaohuang CMS. The results obtained from analysing the relationship between parents and F_1 hybrids and combining ability through contant parental regression according to Griffing (1950) model were as followed:

(1) All the characters of F_1 hybrids with radish cytoplasm present positive correlative relationship with parental characters except that number of leaves shows very loose negative correlative relation. Dry matter and soluable solid substance content of blade and petiole and chlorophyll content per unit area of the F_1 hybrids have significant relation with those of parental parents. The correlation coefficient of fresh leaf weight per unit area was close to the significant level at 5%. Among the F_1 hybrid characters, fresh leaf weight per unit area correlated significantly with plant weight (the genetic correlation coefficient was 0.9004 and the phenotype coefficient 0.7820**), the correlation coefficient of chlorophyll content per unit area approached the significant level at 5%. Parents with fine aggregate characters, large fresh leaf weight per unit area and high chlorophyll content are preferential to the breeding of F_1 hybrids with radish cytoplasm.

(2) The genetic variation of F_1 hybrids with radish cytoplasm in dry matter and soluable solid substance content in fresh blade and petiote, chlorophyll content and fresh leaf weight in per unit area and specific leaf weight is

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mainly controlled by additional genetic effects and vitamin C content in fresh blade and petiole mainly governed by non-additional effects. These are similar to the situation with non-heading Chinese cabbage cytoplasm. In plant weight and weight ratio of blade / petiole, the situation is different. The specific combining ability of plant weight with radish cytoplasm was 71.55, mainly presenting non-additional effects, but that with non-heading Chinese cabbage cytoplasm was 54.02, showing nearly equal additional and non-additional effects. The general combining ability in weight ratio of blade / petiole with non-heading Chinese cabbage cytoplasm was 70.28, mainly indicating additional effects, that with radish cytoplasm was 52.40, showing equal additional and non-additional effects. The correlation coefficient between F_1 hybrids with radish cytoplasm and parents in plant weight and weight ratio of blade / petiole was less than that with non-heading Chinese cabbage cytoplasm by 0.2 and did not attained the significant level at 5% (the correlation coefficient with non-heading Chinese cabbage cytoplasm in plant weight was 0.6489*. in weight ratio of blade / petiole was 0.7713**). Extensive expansion of parental nuclear genetic background and determination of combining ability in early backcrossing generation in the breeding of F_1 hybrids with redish cytoplasm are necessary.

EVALUATION OF RADISH-DERIVED CYTOPLASMIC MALE STERILITY FOR USE IN TROPICAL HEADING CHINESE CABBAGE

Jin-Young Yoon, Romeo T. Opeña and Lien-Chung Chang

In an attempt to develop an alternative scheme to the self-incompatibility system in F1 hybrid production of Brassica crops, nuclei of B. oleracea and B. napus were substituted (Bannerot et. al. 1974) into the cytoplasmic male sterile (cms) radish, Raphanus sativus, described by Ogura (1968) Cytosterile. B. campestris was later generated by repeated backcrosses of B. campestris to the cms B. napus (Williams and Heyn 1981). The cms B. campestris with partially restored nectaries (Leung et. al. 1983) was then repeatedly backcrossed to self-compatible tropical heading Chinese cabbages, B. campestris ssp. pekinensis, at AVRDC. Five consecutive backcrosses with intense selection in each generation for improved nectary and reduced chlorosis did not lead to appreciable selection gains. Netary was still minimal or absent in most plants. Varying degrees of chlorosis was commonly observed in the terminal part of the newly developing flower stalks in all three backcross families. The cms backcross derivatives, on the other hand, had already become similar to the recurrent parents with respect to head production when compared during the 1986 autumn/winter growing season (Anon. 1988).

In the 1987 summer season, three cms backcross lines and their F1 hybrids were compared with their respective male fertile recurrent parents and their corresponding hybrids. Other AVRDC inbreds were used as pollen parents of the F1 hybrids. The field design was randomized complete block with three replications.

The performance of cms lines per se and as parents of F1 hybrids were found to be much poorer than that of their corresponding male-fertile recurrent parents (Table 1). In general, they were weak, slow-growing and matured late. None of the F1's which were developed from cms maternal parents produced marketable heads until the termination of the field experiment on the 50th day after transplanting, whereas all hybrids from male fertile parents showed 100% heading rate. The poor field performance described above, together with poor nectary development and persistant chlorosis even after five backcrosses, in which over 98% of the nuclear substances have been theoretically substituted, implies that incompatibility exists between the radish cytoplasm and the Chinese cabbage nucleus. The difference in performance between the cms-backcross derivatives and their recurrent parents was far greater in the summer trial than would have been expected from previous observations during the cool seasons. This may indicate that the cytoplasm-nucleus incompatibility was accentuated under the adverse hot and humid conditions of the summer season.

In conclusion, the radish-derived cms does not seem to be a useful germplasm resource for the tropical heading Chinese cabbage programunless efficient means can be employed to overcome the detrimental interaction between the cytoplasmic factors of radish and the Chinese cabbage nucleus.

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Table 1. Means of cms lines and F1 hybrids developed therefrom, compared to their male-fertile recurrent parents and corresponding F1 hybrids.

Recurrent parent	Entry groups	No. entries	Largest leaf length (cm)	Mean growth (g)	Maturity[z] (DAT)	Heading[y] rate (%)
CT1-27	CMS parent (p)	1	14.2	130	(>50)	0
	MF recurrent p.	1	23.7	539	35	100
	Difference[x]		**	**		
	F1's from CMS	4	19.0	282	(>50)	0
	F1's from MF	5	28.2	1,179	34	100
	Difference[x]		***	***		
CT1-32	CMS parent	1	18.9	204	(>50)	0
	MF recurrent p.	1	27.8	678	50	100
	Difference[x]		***	***		
	F1's from CMS p.	10	17.4	179	(>50)	0
	F1's from MF p.	5	28.0	1,274	35	100
	Difference[x]		***	***		
7252-1	F1's from CMS p.	8	18.2	341	(>50)	0
	F1's from MF p.	4	29.3	1,466	35	100
	Difference[x]		***	***		

[z]Number of days from transplanting to marketable maturity.
[y](Number of marketable heads harvested/number of plants grown) x 100.
[x]Single d.f. group contrast was employed. ** and *** denote the significance of the difference at .01 and .001 probability levels, respectively.

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STABLE AND EFFECTIVE RESTORER LINES DEVELOPED IN INDIAN MUSTARD BRASSICA JUNCEA (L.) CZERN

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Cytoplasmic male sterility in B.juncea was discovered by Anand and Rawat in 1978. Pollen fertility restoring genes for the CMS B.juncea were found in a Pakistani strain of B.nigra and an Indian strain of B.campestris. In order to transfer these genes into B.juncea, the restorer plants were separately crossed to B.juncea and through recurrent back crossings, plants similar to B.juncea in gross morphological features but with restorer genes from B.nigra (RN) and B.campestris (RC) were obtained. These were assigned code numbers and promising ones were further crossed to CMS plants and also selfed to confirm their stability and to understand the genetics of restoration. Among the restorer lines evaluated, RC-11 and RC-13 from B.campestris source and RN-26 from B.nigra source were found to produce 90-98% fertile pollen but were not consistent in performance.

Earlier studies of Anand et al. (1985) had indicated that two genes were involved in the inheritance of fertility restoration for each of the sources of restoration and the restoration process is possibly controlled by complementary genes.

A further analysis has also shown the restorer genes of B.nigra and B.campestris to be of independent origin, borne on different genomes (Angadi 1987). Since B.juncea is a digenomic species involving the genomes of B. campestris and B. nigra, bringing together the two independent sources could be expected to yield a more effective restorer line. Hence with the objective of bringing together the independent lcomplementary genes, the two promising RCs and RN-26 were intercrossed. Both the combinations viz., RC-11 x RN-26 and RC-13 x RN-26 were found to produce higher amounts of pollen than their component parents. Pollen viability ranged from 91 to 93% (Angadi 1987). From these families, single plants were selected and selfed to obtain a stable line, and two lines showing complete pollen fertility restoration have been isolated.

As RC X RN combinations showing full restoration must have restorer genes in the S cytoplasm, restorer genes were transfered into desirable back ground. For this, 22 dpromising B.juncea strains were individually crossed with the restorers using the latter as females and recurrent back crossing was done. This led to the

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development of promising strains with effective and stable restorer genes which have been used in the development of first ever B. juncea hybrids on a commercial scale. These hybrids in the first year of evaluation, have recorded 25% higher yields over the best ruling variety.

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RESTORATION OF MALE FERTILITY IN CMS-B. NAPUS WITH RADISH CYTOPLASM

F W HEYN

As outlined in a previous note (C N 1, 15, 1976) restorer genes had been introduced from a Raphanobrassica originating from CHOPINET. Restoration of male fertility was linked to the also dominant characteristic white flower colour. By successive backcrosses to euploid cms-B. napus superfluous radish chromosomes had been eliminated. Full seed fertility of the restorer plants could not be obtained. The best single plant reached a seed set of 28 seeds per siliqua on open pollination. This level could not be stabilized in a strain. The majority of restorer plants had a lower seed set. Seed set produced on euploid yellow ms-plants was always higher than that on the restorer plants - both crossing parents showing always some inviable embryos in the siliquae. It is highly probable that the white flowered restorers carry an extra radish chromosome which explains the always imperfect seed fertility. Such alien addition lines show a wide variation in the transmission rate of the extra chromosome through the male gametes (SERNYK 1983). - The white flowered restorers also showed a wide variation in their segregating progeny resulting from open pollination among each other and from backcrosses produced on the yellow male sterile euploid plants.

Since several years partially and fully male fertile yellow flowering plants were detected. Part of them resulted from dormant seeds of F-plasm rape grown for comparison in the plot. Testcross and selfing progenies were grown for verification. Backcross segregation for fully/partially male fertility : male sterility showed a wide variation of segregation. The percentage of restorers ranged from 8, 14, 23, 43, to 50. A clear cut classification was impossible because the material was grown as an annual form in spring outside in a garden. At its flowering time there is a very heavy infestation of all the common insect pests which are mass-multiplied in the winter rape fields. Especially the pollen beetle poses a great problem. Therefore only a few selected progenies were grown 1988 in a glasshouse protected by screens against insects.
Progeny TR 793 segregated 6 male fertile restorers with normally developed anthers and 6 male sterile plants. The exceptional finding was the complete seed set on TR 793-9 yellow ms pollinated by TR 793-8 yellow male fertile. This was the first case that a cms-plant gave a perfect seed set - without any dead or shrunken seed - from pollination by a restorer plant, which itself produced just about 16 seeds per siliqua after selfing. Future progenies will show whether a cytologically stable restorer with yellow flowers can be produced.

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VALIDITY STUDY OF MICROSPORE CULTURE METHOD IN BRASSICA CROPS

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Regeneration of plants from microspores by isolated microspore culture method was first reported by Lichter (1982) in Brassica napus L.. This method is really effective in B. napus and Fan et al (1988) got over 14 % of embryos per cultured microspores by this mehod with some modifications. In other Brassica crops, regeneration of B. carinata (Choung and Beversdorf 1985) and B. campestris (Miyoshi and Shiga 1987, Sato et al 1987) were reported. All of them used basically similar method to Lichter's. We tested the validity of this method (Keller et al 1988)in B.juncea and B. campestris.

In B. juncea, 10.9 embryos per 5 x 10^4 microspores were obtained after two-weeks-culture at 25 °C with first-three-days-incubationat at 32.5 °C (Table 1). 5.8 embryos were obtained from 5 x 10^4 microspores of B. campestris with 35 °C one day/32.5 °C one day treatment. Most of these embryos developed to normal plants.

Up to this time, regenerated plants were obtained from the microspores of all amphidiploid Brassica species and of a diploid species by this method. Validity study of the method in B. oleracea and B. nigra is in progress.

Table 1. Effect of high temperature treatment on the yield of microspore-derived embryos of B. juncea and B. campestris.

Microspore donor	Temperature treatment	Relative embryo yield*
B. juncea		
RLM 514	35°C, 4 days	3.17
	35°C, 2 days	2.34
	35°C, 1 day/32.5°C, 2 days	3.17
	32.5°C, 3 days	10.90
B. campestris		
Aso No. 1	35°C, 4 days	0
	35°C, 2 days	2.57
	35°C, 1 day/32.5°C, 1 day	5.82
Summer harvest	35°C, 4 days	0
	35°C, 2 days	0.29
	35°C, 1 day/32.5°C, 1 day	1.64
Takakei No. 26	32.5°C, 5 days	0.13
	32.5°C, 3 days	0.56
	32.5°C, 2 days	1.75

* Expected number of embryos from 5 x 10^4 microspores.

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DIFFERENTIAL ANDROGENIC RESPONSE IN BRASSICA JUNCEA (L.) CZERN AND COSS.

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Since the production of haploids through anther culture in Datura innoxia by Guha and Maheshwari (1964), a large number of haploids have been produced in many crop species. Haploids are useful for producing homozygous diploids from microspores containing new gene combinations and for mutation breeding adopting microbiological methods. In the present study, six cultivars of Brassica juncea i.e. PR-15, RLM-514, RS-64, RH-30 and their hybrids with disease resistant source RC-781 and aphid resistant source T-6342 were used. Anthers with uninucleate microspores in 2-3mm size buds were selected for culture. Cold pretreatments were given for 1,3 and 5 days before culturing. It was followed by incubation at 37°C for 2 days. Out of the three media tried, Nitsch (1974) and Gamborg's (1968) media did not produce any callus, however anthers burst open to produce androgenic callus on Keller et al. (1975) medium. Per cent response ranged from 0.2 to 20.3 per cent in Brassica juncea at 25°C without any temperature treatment. It was observed that cold pretreatment to flower buds for 5 days alongwith elevated temperature treatments for two days, was most suitable for inducing callusing in different genotypes. Disease resistant source RC-781 showed maximum response (27%). The hybrids showed more percent response than the parental genotypes. From the results, it can be concluded that there are enormous differneces among genotypes within species. The better response of hybrids over parents is indicative of heterotic effect for androgenesis. This is in conformity with the observation of Keller and Stringam (1978). Cold pretreatment followed by incubation at elevated temperature (37°C) increases the frequency of androgenic callusing in Brassica juncea also.

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HIGH FREQUENCY INDUCTION OF ANDROGENESIS IN ETHIOPIAN MUSTARD (*Brassica carinata* A. Br.)

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Conventional as well as recent biotechnological techniques require appropriate sources of desirable genes to be exploited for a successful crop improvement programme. *Brassica carinata* is endowed with genes for resistance to white rust and tolerance to *Alternaria* blight and drought. These useful attributes of *B. carinata* can be utilized in the improvement of Rapeseed & mustard. Anther culture technique provides an efficient method to obtain homozygous lines in a very short period in comparison to conventional methods. It also increases the selection efficiency and early expression of the recessive genes. With this view, studies on *in vitro* anther culture response and regeneration of androgenic calli were undertaken in two cultivars of *B. carinata* viz., BCID-1 and HC-1.

Anthers from unopened flower buds, of the two cultivars, with uninucleate microspores were cultured on four media namely,

R1 =Keller *et al.* (1975) medium.
R2 =Keller *et al.* (1975) modified with 100 mg serine.
R3 =KB5 modified with organics of B5(Gamborg *et al.*1968)
R4 =N6 medium (Chu *et al.* 1975) modified with organics of B5.

The anthers were cultured in dark at 25±2°C for 4 weeks for the callus initiation. There was no anther response on R1 and R2 media, whereas, the frequency of anther response was very high on R3 and R4 media. Anther response was more on R4 medium as compared to R3 medium. The highest anther response (84.70%) was observed in cultivar BCID-1 on R4 medium, where anthers burst open and produced embryogenic callus. The frequency of response on the R3 medium was 36.70%. Cultivar HC-1 also had a similar anther response on R3 medium but on R4 medium, it had lower anther response (42.0%) as compared to BCID-1. This shows that composition of media and genotype play important role in androgenesis in *B. carinata*.

MS media with different concentrations of growth regulators were tried and the ones supplemented with IAA (0.5 mg/l) + BA (0.5 mg/l) or NAA (0.2 mg/l) + Kn (2 mg/l) were found suitable for regeneration. The callus was kept at 25±2°C in bright light (6000 lux) with 16 h. photoperiod. It turned green within 15 days and few plants were regenerated which we plan to grow in the pot house during the coming season. Their ploidy level will be confirmed and the information gathered utilized in the interspecific hybridization programme through anther culture to fix the characters in F_1 generation.

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Transfer of male sterile cybrid cytoplasms into winter rape cultivars of Brassica napus by protoplast fusion

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One objective of the rapeseed breeding programmes is the creation of hybrid varieties. Several cytoplasmic male sterility systems (cms) are under study to facilitate the production of hybrid seeds. One of these cms systems originates from the japanese cms Ogura radish and was introduced into rapeseed by backcrossing (1). This cms Ogu system was modified by protoplast fusion with a fertile spring cultivar of rapeseed (2). Resulting cybrids have several characteristics which make some of them more attractive for breeding than the original cms, besides the fact that their defects were corrected and that they have a simplified nuclear restoration scheme.

In France, the majority of rapeseed cultivars are winter lines. The problem is therefore to transfer the cms cybrid into these lines. As backcrosses are extremely time consuming, alternative methods are being investigated. One of these is the fusion of protoplasts between a winter cultivar and the cms donor. A similar approach with a cms Polima donor has been reported (3).

In this study, we chose to tranfer the cytoplasm of the cms cybrid 58, Brutor spring cv (2) into the Bienvenu winter cv. After fusion treatment and plant regeneration, we looked for a cybrid containing the cybrid cms of the spring donor and the nucleus of the winter cv.

The method used was that previously described (4) except for the following points:

- The screening was only done at the flowering stage. To facilitate the experiment, we irradiated the leaves of the cms donor at 15krad, prior to protoplast isolation to prevent this genome from regenerating.
- To regenerate plants from protoplasts of the winter cv, several modifications were made to the culture media composition. In particular addition of ammonium nitrate (10 mM) to B and C media and of cytokinin (BA 2 mg/l) to D medium. 4% of colonies on E medium were able to regenerate.
- All regenerated plants were submitted to a vernalisation treatment once they had achieved the required growth in a greenhouse.
- As overall morphology doesn't allow a precise distinction between the two parental genotypes, we developed isozyme analysis on leaf extracts, and performed assays for APS, LAP and Perox.

After a fusion experiment, we obtained 45 completely male sterile plants among 285 regenerated ones. Most of them had the morphology of the cms donor. Isozyme analyses showed that 15 plants out of 20 have effectively the cms parental patterns, indicating that they escaped the irradiation treatment. Four plants have bands of the two parents and one (n°51) has the winter cv patterns. Plant n°51 has only the diploid chromosome number (38) with almost normal pairing (two univalents are present in some cells). Mitochondrial DNA analysis showed that this plant has the SalI mt DNA patterns of the cms donor, except for one band modification. This may be the result of recombination of the mtDNA.

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The plant has also been crossed by the winter cv and by the restorer genotype to verify that the male sterility is cytoplasmic and stable and also that it kept the same restoration segregation scheme in the progeny.

This simple experiment produced a cybrid plant having the winter cv nucleus in the male sterile cytoplasm of the cybrid donor in about two years. But it is worth noting that this method can induce new mt DNA recombinations. It is therefore important to regenerate enough plants to be able to select the unmodified cms cybrids. Progress is being made in improving the regeneration rate of the "00" lines of winter rapeseed and new fusion experiments are underway.

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IMPROVEMENT OF A CAULIFLOWER INBRED THROUGH PROTOPLAST REGENERATION

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The inbred cauliflower line NY 7642B (Dickson, 1985) carries the persistent white trait, forming white curds even in direct sunlight. We describe here the development of an improved line regenerated from leaf protoplasts of 7642B. This line has the good horticultural characteristics of 7642B, but matures earlier and has better seed set.

Protoplasts from 7642B leaves were isolated and cultured according to Robertson and Earle, 1986. In 1986, seedlings from 87 self-pollinated regenerants and 8 self-pollinated seed-grown 7642B plants were transplanted along with commercial cultivars in two separate locations in central New York. Most of the protoplast-derived lines formed curd earlier than the seed plant counterparts; some of the early protoplast-derived lines also formed curd with acceptable size, color, shape and firmness. 19 plants from the protoplast-derived lines were selected for further evaluation the following year: these varied in earliness and were selected for curd color, size and solidity. Some seed plant counterparts were also selected as controls.

Although unfavorable conditions during the summer of 1987 caused all of the plants to mature later than in 1986, the progeny of the early 1986 selections were also earlier than 7642B. One line showed a particularly promising combination of earliness, good horticultural characteristics, and good seed set (the latter is poor in the original 7642B stock). Several plants from this row, 3317, were self-pollinated.

Seed from one of these plants (3317-1) was planted in 1988 along with the original 7642 population. Each type was planted on two dates. Prolonged heat during the summer of 1988 delayed maturity, but, like their parent, the 3317-1 progeny were earlier than 7642B. Among plants seeded June 9, 7642B formed mature heads an average of 104 days after seeding, while 3317-1 progeny did so only 87 days after seeding. This difference was statistically significant ($P<<0.01$, $df=58$). Firmness, color, head shape and overall quality of the 3317 progeny were at least as good as for 7642B. We are still in the process of taking data from the second planting, but preliminary results are similar to those from the first planting. Data on seed set will be collected from plants brought into the greenhouse and pollinated by bees in an enclosure.

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PRODUCTION OF ATRAZINE RESISTANT BROCCOLI BY PROTOPLAST FUSION WITH *BRASSICA CAMPESTRIS*.

M.C. Christey and E.D. Earle.

Cytoplasmic male sterile 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 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.

The cytoplasmic characters of atrazine resistance and male sterility can only be combined by protoplast fusion. In an attempt to combine these traits mesophyll protoplasts from a BN line were fused with etiolated hypocotyl protoplasts from atrazine resistant *B. campestris* (Candle). Using the plate fusion procedure of Jourdan (1988), 16 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 1 colony, were resistant to atrazine as determined by their ability to grow, survive and root in the presence of 25µM 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 have been potted up to determine whether they still exhibit petaloid sterility.

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A COMPARISON OF OVARY, OVULE AND EMBRYO CULTURE IN PRODUCING HYBRIDS FROM WIDE CROSSES AMONG RAPID CYCLING BRASSICA SPECIES AND RAPHANUS

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This study was undertaken to examine the most efficient ways of deriving hybrid plants from recovered hybrid embryos following interspecific and intergeneric crosses of rapid cycling crucifers from the Crucifer Genetics Cooperative (CrGC) collection. Rapid cycling base populations of B. nigra RCBr (CrGC 2-1), B. oleracea RCBo (CrGC 3-1) and Raphanus sativus RCRs (CrGC 7-1) were used as male parents. The male sterile Brassica rapa, RCBr, (CrGC 1-17) was used as the female parent. Ovaries, ovules and embryos were placed on various media at 2, 6, 8, 10, 13, 15 days after pollination (DAP). Media used for ovary culture were: W-3 based on WHITE's, B5-1 and B5-2 based on B5 medium; for ovule culture W-1 and W-2 based on WHITE's, MS-2 based on MS medium; and for embryo culture, MS-1 and MS-2, both based on MS medium.

Ovary culture: With respect to the most suitable ages for embryo growth different genome combinations varied on different media. The optimum ages of culture for RCBr x RCBn was 15 DAP; for RCBr x RCRs it was 8-10 DAP. With RCBr x RCBo between 6 and 15 DAP was satisfactory. W-3 medium was the most suitable for RCBr x RCBn and was usable for RCBr x RCBo and RCBr x RCRs. B5-1 medium was similar to W-3 for RCBr x RCBo. The 3 media were similar for RCBr x RCRs.

Ovule and Embryo Culture: Thirteen DAP ovules of RCBr x RCBo, RCBr x RCRs, and 15 DAP ovules of RCBr x RCBn gave the highest numbers of hybrid seed in ovule culture respectively. Media W-1, W-2 were suitable for RCBr x RCBo, and W-2 was best for RCBr x RCBn and RCBr x RCRs. Thirteen DAP embryos of RCBr x RCBo, RCBr x RCBn and 10 DAP embryos of RCBr x RCRs gave the best yield of hybrids in embryo culture. Although we got the same numbers of hybrid seedlings from MS-1 and MS-2 media, most of the seedlings from MS-2, which contained higher hormone concentrations than MS-1, were unable to develop into normal plants.

The progenies form RCBr x RCBn, RCBr x RCBo and RCBr x RCRs exhibits hybrid chromosome numbers of 18, 19 and 19 respectively.

In these experiments, we found that ovary culture combined with later embryo rescue was the best way to recover hybrids from the crosses of RCBr x RCBn and RCBr x RCRs, whereas embryo culture was most effective for RCBr x RCBo hybrids.

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PRODUCTION OF ERUCA BRASSICA HYBRIDS BY EMBRYO RESCUE AND DNA ANAYSIS OF THE HYBRIDS

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Some of the wild allies of crop Brassica have potential value as donors of useful nuclear/organelle genes. Interspecific and intergeneric hybridization can be used as an important tool for broadening the genetic base of cultivated species and introgression of desirable genes from wild species to the crop type. Eruca is resistant to drought and to some diseases and pests. Intergeneric hybridization of Eruca with Brassica sp. by ovary culture has been reported by Matsuzawa and Sarashima (1986). This report deals with the production of Eruca-Brassica hybrid by embryo rescue and morphological, cytological and DNA analyses of the hybrids.

Eruca sativa (n=11) and Brassica campestris (n=10) (ssp. oleifera var. brown sarson) plants were grown in pots and emasculated flower buds of Eruca sativa were pollinated with freshly collected pollen of B. campestris. Ovaries were excised 4 - 5 days after pollination and surface sterilized with 0.05% mercuric chloride. They were then cultured on Murashige and Skoog medium (1962) supplemented with 1.0 ppm kinetin, 0.1 ppm napthalene acetic acid, 1.0 ppm gibberellic acid and 10.0 ppm casein hydrolysate (medium A). After two weeks of subculture the ovaries were dissected and globular ovules were cultured on the same medium. After four weeks of subculture, out of 34 ovules one produced a viable embryo. The embryo formed callus at the base after a week.

It was transferred to MS basal medium with 3% sucrose and after further growth of callus for two weeks transferred back to medium A. Several embryos were produced on this medium within two weeks. The callus was subcultured every 3-4 weeks on the same medium and maintained at 25 $\pm$ 2°C under 16 hour light (2000 lux) and 8 hour dark period. It continued to form more embryos up to 7-8 subcultures.

The embryos were grown on liquid MS basal medium containing 3% sucrose on a slow shaker (60 rpm) for 3-4 days amd then on solidified MS basal medium containing 1% sucrose for further development. The embryos developed normally to form plantlets with good root and shoot system.

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When the plantlets were about 4" tall, they were transferred to autoclaved sand and watered daily with half strength MS basal without sucrose or hormones. After a week they were planted in pots containing soil and peatmoss (3:1) and grown to maturity under natural conditions.

Acetocarmine staining of hybrids showed a chromosome number of 2n=42 indicating that they were amphidiploids produced by doubling of chromosomes. The pollen fertility was 86% and the plants were self-fertile.

These hybrids were intermediate between Eruca and Brassica in general morphological characteristics and growth pattern. The leaves were petiolate and smaller in size like Eruca. Inflorescence was characteristic of Brassica with flowers at 45°. Petals were yellow like Brassica but flowers had small style completely enclosed by anthers, a character from Eruca. Siliqua were intermediate between two parents in respect of length of the valve and beak, and arrangement of pods on the axis. Hybrids had only one row of seeds as in Brassica but seeds resemble Eruca as they are flat and brownish in colour.

Total DNA of Brassica campestris, Eruca sativa and the hybrids was prepared from freeze dried leaf material and analyzed using 18s ribosomal DNA and B. campestris satellite DNA probes. The B. campestris satellite DNA hybridizes to B. campestris and the hybrids but not to Eruca sativa clearly indicating the presence of B. campestris genome. The 18s ribosomal DNA hybridization pattern shows that Eruca sativa has specific bands at 0.8 kb and 1.8 kb and B. campestris has a specific band at 2.6 kb. The hybrids have 0.8, 1.8 and 2.6 kb bands, showing the presence of both parental genomes.

These hybrids have been crossed with B. juncea, B. campestris and B. nigra and also selfed and further breeding work is in progress to screen for new Brassicas with useful agronomical traits.

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86 PRODUCTION OF F_1 INTERSPECIFIC HYBRIDS BETWEEN BRASSICA OLERACEA AND B. CAMPESTRIS THROUGH IN VITRO EMBRYO RESCUE

J. Řepkova and M. Smolíkova

Interspecific hybridization within the genus Brassica is aimed at transferring agronomically useful traits such as disease, drought and frost resistance. Various in vitro techniques have been employed in order to overcome the crossability barriers and rescue inviable hybrids. Interspecific hybrids between B. campestris x B. oleracea (Mutsuzawa 1978), B. napus x B. juncea (Bajaj et al. 1986) and B. napus x B. oleracea (Ayotte et al. 1987, Quazi 1988) were successfully obtained by embryo rescue.

The objective of our study was to investigate the efficiency of in vitro embryo culture and to produce interspecific hybrids between B. oleracea, B. campestris and B. napus. Our work has been aimed at wider adaptability of hybrids to frost and their use in breeding practice.

The following material was used in the crosses: B. oleracea L. var. acephala DC. - 14 varieties, B. campestris L. var. oleifera DC. - 2 varieties and B. napus L. var. napus - 2 varieties. Reciprocal pollinations were carried out 2 - 3 days before the onset of flowering and after emasculation. Embryos were cultivated in tubes on medium L2 (Phillips and Collins 1979) and M (Monnier 1973) with 25 - 125 g/l of sucrose and 8 g/l of agar 20 - 35 days after pollination.

Altogether 327 pollinations were made between B. oleracea, B. campestris and B. napus. No siliques developed in the crosses of B. oleracea x B. napus. 27 cross combinations were made between B. oleracea and B. campestris, but only embryos of 7 combinations were rescued (Tab. 1). Silique development ranged from 20% to 100% with the mean value of 75,3%. 21 embryos at various stages were dissected. Embryos at hart, torpedo stage and full-grown embryos were cultivated on media with 125, 75 and 50 to 25 g/l of sucrose, alternatively. L2 basal medium was better for

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embryos cultivation due to their faster development.

18 grown-up plants were obtained. Some of them seemed to be intermediate as to morphological characteristics (leaf shapes, colour of petioles). Cytogenetic study, fertility and hybrid vigour will be reported in a further publication.

Table 1: Results of interspecific hybridization between B. oleracea and B. campestris by embryo culture

Cross combination	Number of pollin. flowers	develop. siliques	DAP	No.of cultiv. embryos hart stage	torpedo stage	full-grown	No. of hybrids obtained
2 x 20	24	14	36	-	-	1	1
5 x 22	5	1	35	-	-	1	1
6 x 20	12	9	24	4	3	-	5
9 x 20	5	3	20	1	2	-	3
12 x 20	8	7	22	-	-	2	2
13 x 22	5	3	34	-	-	4	4
14 x 20	5	5	23	-	1	2	2
Total	69	52		5	6	10	18

DAP - days after pollination, 2-14 B. oleracea: 2 -PULAWSKA ZIELONA, 5 -MAPRO, 6 -POLYCAUL, 9 -FURCHENKOHL, 12 -MARROW STEM, 13 -MASEC, 14 -VV 11; 20, 22 B. campestris: 20 -REX, 22 -P 3/86

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ISOLATION OF PETAL PROTOPLASTS OF BRASSICA NAPUS L.

S. Millam, A.T.H. Burns, T.J. Hocking

Protoplast isolation and culture has been reported from most tissues of Brassica napus (for review see Schenck and Hoffman, 1984) but not previously from petals. The isolation of protoplasts from petals of Nicotiana was reported by Flick and Evans, 1983. The applications of Brassica petal protoplasts may include somatic hybridisation experiments where the distinctiveness of such protoplasts could be useful in visual heterokaryon selection. In this communication we report a simple method for the isolation of petal protoplasts from both field-grown and glasshouse grown plants.

Petals of Brassica napus cultivar Bienvenu were removed from the plants at a stage immediately prior to full extension. The petals were surface-sterilised in several ways. It was found that a simple 1 minute soak in 70% alcohol plus 0.01% Tween 80 was the most effective method and also that the release of protoplasts was enhanced by such a treatment compared with either non-sterilisation or the use of hypochlorite-based methods. The medium used for protoplast isolation was based on that of Murashige and Skoog. It was found that a range of osmotica could be used but yields per gram fresh weight of tissue were optimum in 13% sucrose (6.8 x 10'6/g). Several enzyme combinations were tested for maximum protoplast release, a combination of 1% Onozuka Cellulase and 0.2% Macerozyme gave a higher yield than the enzyme combination used by Flick and Evans (Onozuka 1.0%: Macerozyme 0.5% and Driselase 0.25%) the figures being 6.2×10^6/g against 3.9×10^6/g.

The optimum incubation time was found to be 6 hours at 25°C. The protoplast/enzyme suspension was filtered through a 64 µm mesh and the protoplasts purified by centrifugation-resuspension methods. Several culture media were investigated, but no development beyond a single division was observed except in the protoplast regeneration medium of Millam et al. (1988) where the protoplasts survived for up to 28 days. Initial fusion experiments using petal protoplasts will be reported at a later date.

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HIGH FREQUENCY IN VITRO MORPHOGENESIS IN BRASSICA CARINATA A.Br.

Surya-Parkash, D R Sharma and J B Chowdhury

Genetic variability is the base of plant breeding. Somaclonal variation has been found to be a good source of genetic variation and thus provides an increased genetic base for breeding new value-added varieties. This is only possible when reproducible protocols for high frequency regeneration are available in desired crop species.

To get high frequency of regeneration in B.carinata cv. HC-2 different explants such as cotyledonary leaves, petioles, stem segments, leaf pieces and regenerated young plantlets were cultured on two different modifications of MS medium (Murashige and Skoog, 1962), namely Y medium (MS+ 0.05 mg/l NAA) and Z medium (MS+0.2 mg/l NAA+2mg/l Kinetin). Among the different explants used young plantlets showed high frequency regeneration on Z medium (212.66%) and Y medium (126.03%).

The young plantlets were raised under in vitro conditions from terminal buds. The plantlets were cut from the base and cultured on Y and Z media. Callus was formed at the cut ends. From this callus multiple shoots (1 to 7) were formed. While the callus obtained from other explants showed very poor or no regeneration. This may be due to the reason that the young plantlet is providing required harmones to the callus for plant regeneration, which otherwise may not be present in the medium.

Most of the regenerated plants were bearing roots. Those without roots were subcultured on Y medium after 20 days of regeneration. A slight cut in regenerated shoot, above the medium level was found congenial for root development. The regenerated plants showed morphological as well as cytological variation (Surya-Parkash, 1987).

Above described method provides excellent mean to induce genetic variability in this crop and makes it feasible to produce large number of regenerants and their possible use in crop improvement.

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GENOMIC INTERACTION FOR SHOOT REGENERATION IN BRASSICA

S.B. Narasimhulu, Shyam Prakash, R. Rishi and V.L. Chopra

In continuation of the previous studies on shoot regeneration responses of diploid and natural amphidiploid Brassicas, we have investigated regeneration responses of known diploid Brassica species and their synthetic combinations for establishing parent product relationship for regeneration potential. Two experiments have been conducted. The first experiment was directed to identifying the growth regulator combination that gives the best average regeneration response for the species under test so that a specific growth regulator supplementation could be used in the subsequent study. Cotyledons excised from seven genotypes representing each of the six species including reciprocally different B. carinata's were inoculated in ten different growth regulator combination supplemented MS or B_5 basal media. Regeneration responses were ranked across the media for each of the species on a scale of 1 to 10 with the most effective medium given the rank of 1. MS basal medium with the growth regulator combinations IAA and BAP at 11.4 and 17.7 uM respectively was identified as a superior medium on the basis of lowest cumulative rank score.

In the second experiment regeneration responses of seventeen different genotypes; five parental diploid species representing the A, B and C genomes, four synthetic amphidiploids, two natural amphidiploids, five trigenomic combinations and one tetragenomic combinations were assessed on the above medium with three replications. The data was analysed in a randomized block design and the error mean squares are used for computing significance of differences.

The study indicated that among parental diploid species B. campestris (AA) regenerated with a low frequency while B. nigra (BB) is a high regenerator. Among amphidiploids, synthetic as well as natural, which served as female parents for trigenomic combinations, regeneration frequency was high with synthetic B. carinata derived from B. oleracea as the female parent and it was least in the natural amphidiploid B. carinata. The low regeneration responses of synthetic B. juncea and B. napus in relation to their superior B and C parents indicates that A genome has a negative influence on shoot regeneration. The inhibition also reflects negative interaction of B. campestris cytoplasm with the alien genome. Cytoplasmic differences for shoot regeneration are evident with synthetic B. carinata because C cytoplasm regenerated with a high frequency compared to its reciprocal. Among the trigenomic combination, the regeneration response of BBC was significantly greater than the additivity of the combining genomes, the low regenerating natural B. carinata and the highly regenerating diploid B. nigra. The trigenomic combination AAC obtained by crossing synthetic B. napus with B. campestris showed a response superior over that of its best parent indicating that intra-genomic interaction (between doses of A genome) are different from inter-genomic interaction (between A and C genomes) particularly in the context of unequal genome dosage. The three genomes A, B and C in the trigenomic combination ABC obtained by crossing either synthetic B. napus with B. nigra or natural B. juncea with B. oleracea showed very low regeneration responses indicating a negative interaction resulting in a depressed shoot regeneration.

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Negative interaction of genomes A, B and C evident in the trigenomic combination ABC is not observed in the tetragenomic combination ABBC obtained by crossing the two natural amphidiploids B. juncea and B. carinata. This could be due to an extra dose of B genome. The possibility of this combination being a metromorph can also not be ruled out in view of the similarities of its response with the female parent.

VARIATION FOR SALT TOLERANCE IN SOMATIC EMBRYOIDS IN MUSTARD

P.B. Kirti, Seema Dargan and V.L. Chopra

A simple single step method for producing somatic embryoids in hypocotyl explant culture of mustard has been reported earlier (Kirti et al., 1987; Kirti and Chopra, 1988). Since incorporation of tolerance to salt in an acceptable agronomic background is an advantage, we have used induced somatic embryogenesis for making selection for tolerance to salt stress in mustard, B. juncea. The importance of embryoids for variant selection lies in the fact that they originate from single cells.

Somatic embryoids were subjected to different levels of salt stress. Embryoids were transferred onto MS medium containing 2% sucrose, 0.2 mg/l BAP, and two different levels of salt; (a) 1% made up of 0.5% sodium chloride and 0.5% potassium chloride, (b) 1.5% made up of 0.75% sodium chloride and 0.75% potassium chloride. Some embryoids could survive the salt stress and produced shoots. Others turned white and died within a week of culture. Plants have been raised from surviving embryoids in growth chamber. Stem explants from resistant plants were taken to study the callusing potential on salt medium (MS + 2%, sucrose + 0.5 mg/l, 2,4D + 0.5 mg/l, BAP + 0.5 g/l, NaCl + 0.5 g/l KCl) in comparison with control plants. Whereas all control explants died on the salt medium, explants from tolerant plants callused profusely.

These studies indicate that variation exists for salt tolerance among embryoids developed in somatic cultures and they can be profitably utilized in selection experiments. Further studies on the characterization of salt tolerant plants are in progress.

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PLANTLET REGENERATION BY SOMATIC EMBRYOGENESIS IN BRASSICA NIGRA

Vibha Gupta, Abha Agnihotri & V. Jagannathan

Brassica nigra (black mustard) is used mainly as a spice. Also, it can be hybridised with B. campestris to synthesise B. juncea, which is an important oil seed crop in India. There are a few reports on the regeneration of viable plantlets from B. nigra callus or protoplasts. Dietert et.al (1982) reported organogenesis in B. nigra from hypocotyl explants. Schenck and Hoffman (1979) and Glimelius (1984) studied protoplast cultures but did not obtain plant regeneration. Klimaszewska and Keller (1986) described somatic embryogenesis in cell suspension and protoplast cultures of B. nigra. However, none of the embryos derived from protoplasts developed into plantlets. Most of the embryoids derived from cell suspensions also failed to develop into plantlets and the six which survived gave rise only to sterile plants.

This report deals with the formation of embryoids and secondary embryoids from callus of B. nigra cv. IC 257 and regeneration of viable plants from them.

Callus formation and Embryogenesis

Seeds were surface sterilized with 0.2% mercuric chloride and grown aseptically. Hypocotyl explants from 10 day-old seedlings were transferred to MS medium (Murashige and Skoog 1962) containing different supplements. All the subcultures were made every 3-4 weeks and maintained at 25±2° C under 16 hour light (2000 lux illumination) and 8 hour dark period. Callus growth as a pale yellow globular mass was obtained on MS medium with 3% sucrose and 1 ppm each of kinetin (Kn) and 2, 4-dichlorophenoxyacetic acid (2,4- D). The callus was maintained by subcultures on the same medium.

It was transferred to media containing different combinations of auxins and cytokinins for the induction of morphogenesis. Torpedo shaped somatic embryos were observed on media containing 1 ppm Kn and 0.05 ppm 2,4-D or 0.1 ppm naphthalene acetic acid (NAA). Media without auxin failed to induce embryogenesis. NAA gave better results than 2,4-D. A marked increase in embryogenic response was observed by the addition of 1 ppm of gibberelic acid (GA). This Kn - NAA -

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GA_3 medium was used for further work. The production of embryoids was observed even after 6-7 subcultures on this medium. The embryos gave rise only to roots if transferred to callusing medium.

Secondary Embryogenesis and Plantlet Formation

The embryos produced on Kn - NAA - GA_3 medium, when subcultured in solidified MS basal medium without any hormones, gave rise to secondary embryos. Each embryo enlarged and gave rise to 6-7 secondary embryos from the hypocotyl and cotyledonary region. These secondary embryos were similar in all respects to the primary embryos during subsequent growth and development into plantlets.

Both primary and secondary embryos readily developed distinct roots and shoots upon transfer to liquid MS basal medium with no hormones. After a week these plantlets were transferred to solidified MS basal medium with 1% sucrose for plantlet development. These plantlets were then grown for a week on moist sand under aseptic conditions to harden them and subsequently transplanted into peat moss-soil in pots and allowed to develop to maturity under natural conditions. Ninety percent of the embryos survived and developed into plantlets. About 90% of these plantlets flowered and set seeds. These seeds germinated normally and produced viable plants.

The profuse and continuous production of embryos from *B. nigra* by the method described here offers opportunities for using this species for somatic cell hybridization and genetic transformation.

We thank Dr. Shyam Prakash, Department of Genetics, Indian Agriculture Research Institute. New Delhi for seeds of *B. nigra* cv.IC 257.

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USE OF PEDUNCLE EXPLANTS FOR HIGH EFFICIENCY REGENERATION OF *BRASSICA OLERACEA* L. - POTENTIAL FOR TRANSFORMATION.

M.C. Christey and E.D. Earle.

The use of peduncle explants for regeneration of *Brassica* was reported in *B. napus* by Stringam (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*. To determine if regeneration from peduncle explants was possible in *B. campestris* a survey was undertaken of 3 rapid cycling lines. Culture on over 50 different hormone combinations has only yielded rare shoots. In contrast, peduncle explants from broccoli, cabbage and chinese broccoli have been found to readily regenerate shoots *in vitro*. Regeneration rates of over 80% are reproducibly obtained with multiple shoots per explant.

PROCEDURE

Peduncles were removed from plants in the process of bolting and flower buds and pedicels discarded. Young fleshy peduncles were chosen that had unopen flower buds at the top and few, if any, open buds further down. Only the top 5-7 cm was used to avoid the harder woody tissue present further down the peduncle. Whole peduncles were surface sterilized and cut into several 5mm explants. These were placed horizontally onto Linsmaier-Skoog medium containing 3% sucrose and 1mg/l benzyladenine and solidified with 0.25% Gelrite (Scott Laboratories Inc.). Care was taken to note the orientation of each explant. After 3-4 weeks the entire regenerating ends were excised and transferred to hormone-free medium for further shoot enlargement. Individual shoots were excised and placed on this medium for rooting prior to transfer to soil.

RESULTS

The first effect noted on culture was a swelling of the entire explant, followed by further swelling of the basal ends of explants. After 7 days both ends were covered in a small amount of white or pale green callus but the surface area of the basal end was approximately 2 times that of the apical end. Shoot regeneration was first noted after 7-10 days and after 3 weeks numerous buds were present often covering the surface of the explant but usually concentrated on the lower half closest to the medium. Multiple (1-25) vegetative buds were produced on each explant.

With cabbage, 82% of 97 explants from 10 peduncles had regenerated after 2 weeks. Of those explants regenerating, 68% had regenerated from the basal end only, 4% from the apical end only and 28% from both ends. 47% of the explants regenerating from the basal end had over 10 buds present, whereas 15% of the apical ends had over 10 present. With lines

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derived from Green Comet broccoli (Harris Moran Seed Co.) regeneration rates of over 85% are reproducibly obtained with a similiar polarity of regeneration noted. With Chinese broccoli (Guy Lon, Fredonia Seeds) regeneration rates of 88% were obtained from 115 explants. 53% of the regenerating explants produced buds on both ends of the explants but buds were larger and more numerous on the basal end. When cloth tape(Carolina Biologicals) rather than Parafilm was used to close petri dishes regeneration rates were similar but more buds were present per explant.

TRANSFORMATION

This high and reproducible regeneration rate with the rapid production of numerous buds makes this a procedure with potential for the transformation of *B. oleracea* via cocultivation with *Agrobacterium tumefaciens*. However, numerous experiments with Green Comet peduncle explants have only yielded 1 putative transformant. This was obtained with *A. tumefaciens* strain LBA4404 carrying genes specifying kanamycin resistance and ß-glucoronidase 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 (50µg/ml). Leaf explants have regenerated further kanamycin-resistant shoots on medium E of Pelletier (1983) containing 50µg/ml kanamycin. Experiments are in progress to assess the potential of this kanamycin resistant plant as a marker for protoplast fusion experiments. Protoplasts from this plant, isolated according to the procedure of Robertson and Earle (1986), form green colonies on medium E containing 25µg/ml kanamycin. Southern analysis has not yet been conducted.

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REGENERATION THROUGH SHOOT ORGANOGENESIS AND SOMATIC EMBRYOGENESIS IN HYPOCOTYL PROTOPLAST CULTURE OF MUSTARD BRASSICA JUNCEA (L) CZERN AND COSS

P.B. Kirti and V.L. Chopra

Mesophyll and stem cortex protoplasts have been cultured and regenerated with low efficiency (Chatterjee et al., 1985 and Keller, 1986). Following the observation of Glimelius (1984) and Barsby et al. (1986) that hypocotyl protoplasts of rapeseed regenerate with a high efficiency, we undertook a study to evaluate the response of hypocotyl protoplasts of B. juncea to culture and regeneration. As a result, a simple protocol has been developed for culturing hypocotyl protoplasts of mustard.

Hypocotyl protoplasts were isolated and cultured following the method described by Glimelius (1984) with some modification. Seeds were germinated on half strength MS medium (Murashige and Skoog, 1962). Hypocotyls from six day old aetiolated seedlings were used for protoplast isolation. Hypocotyl segments were plasmolysed for one hour in protoplast culture medium. Enzymes cellulase R-10 (Onozuka) 1.0% and Pectolyase Y-23 (Seishin) 0.1% were dissolved directly in the protoplast culture medium. Protoplasts were cultured in 8 p medium (Kao and Michayluk, 1975), as described by Glimelius (1984) with 68.4 g/litre glucose, 1.0 mg/l 2,4D, 0.1 mg/l NAA, 0.5 mg/l zeatin riboside at a density of 5×10^4/ml of medium; Two ml of protoplast suspension was dispensed in 5 cm glass petriplates and incubated in dark for the first three days of culture at 24 $\pm$ 2°C. On the fourth day, cultures were exposed to light (Ca 2500 lux) in a 16/8h light-dark photoperiod at 25 $\pm$ 1°C. On the eighth day, cultures were diluted with 0.5 ml of 8p medium with 3.42% sucrose, 0.1 mg/l 2,4D and 1.0 mg/l BAP. A second dilution was made on eleventh day. By fifteenth day, large cell colonies were obtained, which were plated on K_3 medium (Nagy and Maliga, 1976) containing 0.1 mg/l 2,4D, 1.0 mg/l BAP and 3.42% sucrose. After another two weeks, small calli of 3-5 mm diameter were plated on MS medium with 1.1% sucrose, 0.1 mg/l IAA, 2.0 mg/l zeatin riboside and 2.0 mg/l BAP. Out of 792 calli plased on this medium, 340 calli produced multiple shoots giving a regeneration frequency of about 44%. Calli with regenerated shoots were transferred to MS medium with 1% sucrose for shoot elongation. Elongated shoots were maintained on 1/2 MS with 1.0 mg/l IBA for rooting.

Somatic embryogenesis was also observed in primary cultures (direct embryogenesis) occurring at a frequency of 0.0001 - 0.0002%. Some embryoids also developed after first plating on solid medium. A higher and repeatable frequency of somatic embryogenesis was obtained when small calli were plated on MS medium with 2% sucrose, 0-50 mg/l 1,4D, 0.50 mg/l NAA and 0.50 mg/l BAP-riboside. About 10% calli produced embryoids. The development of embryoids followed the same path as described for somatic embryoids in hypocotyl explant culture (Kirti and Chopra, 1987, 1988).

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THE RELATIONSHIP BETWEEN TUMOR FORMATION AND IN VITRO DIFFERENTIATION TYPE OF RADISH INBRED LINES

L.A.Lutova, I.S.Buzovkina, S.O.Shishkova

The genetic collection of inbred lines of radish Raphanus sativus var radicola Pers. was used for differentiation and morfogenesis process investigation. The lines used were obtained by inbreeding from few plants of "saxa" and "Virovsky bely" varieties (Narbut, 1966).

After 20 inbred generations lines with spontaneous root-crop tumor formation were reveald among the non-tumorigenic ones. In vitro seedling cotyledon cultures of these lines differed in their ability to form roots, calli and buds. Genetic analysis of these features showed that callus formation ability is dominant and monogenically inherited. Root forming ability is under the control of two complementary genes, as it was shown. The genes are referred to as C and R_1, R_2, respectivly. They are inherited independently.

The relationship between type morphogenesis of intact plants and isolated parts cultivated in vitro was investigated. Cotyledons of most tumorigenic lines formed large calli, but do not formed roots. They are referred to as "tumor" type calli and are able to grow on media without phytohormones, or, in other words, "tumor type" calli were phytohormone independent.

Non-tumorigenic lines formed prominent roots and compact hormone dependent calli. Thus it is tempting to speculate about the linkage between tumor and callus generation mechanisms. However, among tumorigenic lines were hormone dependent ones. Besides this, for intact plant tumorigenity is recessive, while hormone independence in vitro is dominant. So it was concluded that these characters are controlled with different genes.

We tried to suppress the in vitro differentiation phenotypically by exogenous phytohormones. Kinetin restored root formation of rootlesslines and auxins (IAA, NAA, 2,4-D) restored callus formation of lines unable to form it.

Sensitive and resistant to phytohormones lines were revealed. All non-tumorigenic lines were resistant, while tumorigenic ones could be resistant or sensitive. In vitro cotyledons of hormone sensitive lines are characterized by necrosis process. Differences between tumorigenic lines in hormone dependence and sensivity supposes different nature of tumorigenity among them.

Thus we showed that relationship between tumorigenity and regeneration exists and that hormone independence is not obligatiry for tumorigenic phenotype.

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ESTABLISHMENT OF CALLUS CULTURES FROM BRUSSELS SPROUTS

J Williams D A C Pink and N L Biddington

This work is part of a project to develop methods for *in vitro* selection for resistance in Brussels sprout to *Alternaria brassicae*. The criterion for a suitable tissue culture system to be used in the project was that it should provide a large number of healthy plant cells which could be subcultured several times and could be regenerated into whole plants.

We decided to use callus cultures as a basis for this system. However, initial work with Brussels sprout cultures proved very disappointing. Callus could be initiated from leaf disc and hypocotyl explants on NA medium following the methods of Dietert *et al* (1982). However, this callus turned brown in colour, failed to grow further and could not be maintained beyond a couple of subcultures.

Several experiments were performed using various recommended media and a range of hormone concentrations in an attempt to improve callus growth. None of these treatments proved successful for the long term culture of callus. However, work carried out at Wellesbourne (Biddington *et al* 1987) has identified the ethylene inhibitor silver nitrate ($AgNO_3$) as an enhancer of embryogenesis in brassica anther culture. We decided to examine whether $AgNO_3$ had any effect on callus initiation and maintenance when incorporated into NA medium at 0,1,3, and 10 mg/l (24°C/darkness).

$AgNO_3$ had little effect on initiation of callus under these conditions although significant ($P<0.05$) differences occurred between genotypes. However, when subcultured onto identical media and transferred to light conditions (22°C/16 hr day) more dramatic results were obtained. Leaf disc callus grew significantly better in the presence of all concentrations of $AgNO_3$ as compared to basic NA medium. Significant differences between genotypes were also noted. The growth of callus derived from the F_1 hybrid cv. Tornado was greater than that of callus obtained from the inbred line 118 at all concentrations of $AgNO_3$.

Maintenance of hypocotyl callus derived from 3 F_1 hybrid cultivars was significantly better in the presence of 1 and 3 mg/l $AgNO_3$ but no overall difference between the 3 cultivars was observed. Healthy callus from these experiments has been subcultured every 4-6 weeks. It has now been maintained through 5 subcultures. Cultures on NA media without $AgNO_3$ failed to grow but media with $AgNO_3$ has supported the growth of large quantities of green callus for use in further experiments.

Another approach to producing callus cultures of Brussels sprouts was to use B5 medium containing low levels of kinetin and 2,4D to produce differentiating callus (R. Cresswell). This medium has the advantage that regenerants could spontaneously arise from the

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surface of the callus. Hypocotyl explants from 3 F_1 hybrid cultivars produced good differentiating callus producing roots and in some cases shoots. Regeneration of shoots using this medium has also been obtained from hypocotyl callus transferred from NA medium (+ $AgNO_3$).

Shoot production was cultivar dependent. The callus derived from the F_1 hybrid cv. Aeries produced a large number of shoots whilst cvs Tornado and Gower produced few. Interestingly, callus from the male inbred parent of Aeries also appeared to regenerate shoots readily but the female parent produced very poor callus and rapidly died. The ability to regenerate shoots from callus cultures of cv. Aeries appears to have a dominant genetic component. Further attempts to increase shoot production are still in progress but so far do not indicate any improvements.

In general hybrid material has been used in these experiments. However, we are now attempting to produce callus cultures from a range of inbred lines previously assessed in the field in 1985 and 1986 for their reaction to Alternaria brassicae (Pink et al 1987). Use of inbred material will increase the probability of detecting somaclonal variation among the progeny of regenerants.

The next step in this work is to attempt in vitro selection for disease resistance. We intend to use an ethyl acetate extract from the crude culture filtrate from Alternaria brassicae (Williams & Pink 1987) to select for resistance to dark leaf spot.

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ISOLATION OF ISOZYMES FROM PLASMODIOPHORA BRASSICAE WOR.

E Liehr and J Grunewaldt

Physiologic races of Plasmodiophora brassicae are usually identified with a set of differential hosts, for example the European Clubroot Differential set (Buczacki et al. 1975). As the efficiency of tester sets is limited (Toxopeus et al. 1986), the identification may be more distinct when genetically controlled markers, for example isozymes could be used. A prerequisite is the linkage between the virulence loci and the isozymes investigated.

A method to isolate isozymes out of two samples with different pathogenicity is described. Sample 1 was collected from Chinese cabbage, Sample 2 from turnip. The isozymes were extracted from resting spores, stored at -22°C within the galls.

All preparations from homogenization to electrophoresis were performed at 0 to 5°C. For each sample the homogenate was prepared from about 1,000 g clubs according to Buczacki et al. (1975). The solid components were concentrated by centrifugation at 17,000 g and 4°C for 10 minutes, re-suspended in distilled water and again centrifuged. The resting spores were mechanically separated from the other fractions, washed four times with distilled water by repeated centrifugation at 12,000 g and 4°C for 15 minutes. The spore concentration was then adjusted with H_2O bidest. to 10^{10} spores per ml. After adding 5% (w/v) sucrose portions of 5 ml of the spore suspensions were incubated at -30°C for about 16 hours. The frozen spore paste was then forced through a nozzle with 2.5 mm diameter of an X-Press, type X 25 (AB Biox Nacka, Sweden) by a hydraulic press with 200-300 bar. After repeating this three to five times, nearly all spores were cracked. The obtained homogenate was centrifuged at 35,000 g and 4°C for 10 minutes. The supernatant containing soluble enzymes was tacken for electrophoresis.

The following enzyme systems were analyzed: diaphorase (DIA, EC 1.8.1.4), phosphoglucomutase (PGM, EC 5.4.2.2), aconitate hydratase (ACO, EC 4.2.1.3), isocitratdehydrogenase (ICD, EC 1.1.1.42) and phosphoglucoisomerase (PGI, EC 5.3.1.9). After isoelectric focusing DIA were stained according to Wehling (1986). PGM staining was modified from Kahl and Stegemann (1973) and ACO from Shaw and Prasad (1970). For these enzymes vertical PAGE according to Wehling (1986) was performed. Horizontal starch gel electrophoresis according to Wehling (1986) was carried out and this gels were stained for ICD modified from Henriksen and Jelnes (1980) and for PGI modified from Nielsen (1980).

Differences between the two spore samples investigated were observed in DIA, PGM, ACO and PGI zymograms. No differences were detected in the ICD patterns. Both samples showed three homologeous DIA bands and Sample 1 in addition three other bands. Three identical PGM bands were detected

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in both spore extracts but a fourth one in Sample 2. Two homologeous ACO bands were recognized in both spore samples. Sample 2 showed in addition two other bands. PGI stained gel showed three diffuse bands for Sample 1, but only one band was detected for Sample 2.

Analyzing other than resting spore extracts, for example extracts from non-infected Chinese cabbage or turnip, cell fragments, and bacteria, the characteristic bands found in spore extracts could not be detected.

Using the described technique of enzyme extraction and subsequent electrophoresis, differences in isozyme phenotypes among the two samples could be found. If these patterns are correlated with specific virulence loci has to be confirmed.

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102 NEW INOCULATION METHOD FOR THE TESTING OF BRASSICACEAE FOR RESISTANCE TO PLASMODIOPHORA BRASSICAE WOR.

J. Rod

The introduction of a suitable rapid inoculation method which is easy to use and gives reliable and repeatable results appears to be one of the most important preconditions for successful plant breeding for resistance. As far as clubroot disease is concerned several inoculation techniques have been already developed. The slurry method (Toxopeus and Jansen, 1975) and the dip method (Johnston, 1968), or their various modifications, are widely used in plant breeding and research. Comparative studies on the influence of different inoculation procedures indicated substantial differences between the results obtained (Lewis and Brokenshire, 1977; Schoeller and Grunewaldt, 1987).

Both of these basic methods have been tested at our Research Institute. With use of the dip method, however, a lower percentage of infection as well as lower disease index (DI) were found in most cases. Although the application of the slurry method resulted in more acceptable results, it is rather laborious to carry out under our conditions. Over a period we have developed the following method which has been successfully employed at our Institute for some time now. Using this new method clubroot infection in control plants (Brassica oleracea var. pekinensis cv. 'Granaat') has always been 100-per-cent, and DI approaches or equals 100.

38-cell-module trays (each cell-module being 100 ml in volume) are filled with a mixture comprising equal volumes of perlite, soil and peat previously sterilised with dazomet. The substrate is then pressed gently down so that its surface is 1 cm below the upper edge of the cells. The filled trays are placed into shallow plastic dishes in which 1-2 cm depth of water is kept throughout the test. After even and thorough moistening of the substrate, three seeds of the crop to be tested are sown onto the substrate surface of each cell. If poor seed germination is taken

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into account, and a sufficient number of seeds is available, each cell is sown with 6 seeds (3 pairs) thinned to 3 seedlings just after their emergence. Thus, 114 plantlets per tray are tested in total. Once sown each seed, or pair of seeds, is immediately treated by micropipetting onto it 0.5 ml of suspension containing P. brassicae resting spores at the concentration 10^6 spores/ml. No differences were found in clubroot infection with the concentration applied ranging between 10^5 - 10^6. Following inoculation the seeds are covered with a layer of perlite up to the edge of the cells. The sown and inoculated trays are then maintained in a glasshouse environment at 22 - 25°C for 6 weeks. After this period the pathogenicity of the clubroot population is evaluated (Dobson et al. 1983). The method described is easy and rapid to use with the further advantage that it is possible to evaluate 550 plantlets per 1 m^2/glasshouse (or phytotron), equivalent to 4800 plantlets/year. Besides the testing for resistance this method (or partly modified) can be employed in a number of other experiments.

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FOLIAR INFECTION OF *BRASSICA* PLANTS BY *ALTERNARIA BRASSICICOLA* IN RELATION TO LEAF SURFACE CHARACTERISTICS

L A Berry and J H Lennard

Variations in the infection responses of different cruciferous hosts to inoculation with *Alternaria brassicicola* (Schw.) Wiltshire have been associated with differences in leaf surface wax levels, and rubbing leaves to remove epicuticular wax before inoculation has been shown to increase the infection rate (Prasanna, 1984). The role of surface waxes in reducing infection may be attributed to a physical or chemical barrier effect, an influence on leaf wettability or a combination of these factors (Royle, 1976). The aims of the present study were to assess the effects of plant host and leaf age on the infection rates of *A. brassicicola* and to relate the results to leaf surface wax levels and leaf surface wettability.

The investigations were carried out on three *Brassica* hosts, Swede (*Brassica napus* ssp. *rapifera* , cv. Doon Major), Oilseed rape (*Brassica napus* ssp. *oleifera* , cv. Jet Neuf), and Brussels sprout (*Brassica oleracea* var. *gemmifera* , cv. Cambridge No 5). Plants were raised in a glasshouse for 8 weeks then transferred out doors for 2 weeks, when leaf samples were taken from the apical middle and basal stem positions. Assessments of leaf wax weight per unit area were based on the methods of Silva Fernandez (1965) and Rawlinson et al (1978) and of wettability on the methods of Silva Fernandez (1965). For infection studies leaf discs, 14 mm in diameter, were taken from leaves in apical, middle and basal positions on the stems of the respective hosts and placed on water agar with 80 ppm. benzimidazole, in petri dishes. Leaf discs were then inoculated with a spore suspension of *A. brassicicola* (10,000 spores/ml), using an airbrush, then incubated at 20°C with a 12 hour day length for 36 hours, when the discs were decolourised in ethanol and numbers of lesions counted using a stereoscan microscope.

The quantities of wax per unit area of leaf were similar for comparable leaves of the three hosts but varied appreciably with leaf position, lower leaves, ie older leaves, possessing less wax (Table 1). With respect to surface wettability, leaves of Brussels sprout tended to be more water repellant then leaves of rape and swede at equivalent positions, except in the case of young leaves. Leaves towards the middle position for all plants showed a reduced wettability in comparison with leaves towards the base or apex. Wettability is thought to be a function of wax morphology rather than wax amount (Martin and Juniper, 1970) and the reduced wettability of leaves from the apex may be attributed to a transition from precursor to mature crystals, immature waxes having a different structure. According to Martin and Juniper (1970), water repellancy is greatest when the wax has a crystalline structure in the form of projecting rods or tubes: Holloway et al (1977) reported that these are the predominant conformations on the leaves of *Brassica* species. The increased wettability of basal leaves may be due to weathering of the wax structure as leaves age.

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In considering the infection rate of *A. brassicicola* on leaves of the three hosts, lesion number per leaf disc increased with leaf age for swede and oilseed rape but was relatively high on younger, apical leaves as well as on older, basal leaves for Brussels sprout (Table 1). There appeared to be a direct relationship between leaf susceptibility and wettability in the case of Brussels sprout. However, with swede and oilseed rape there was an increase in infection rate from the apical to the basal leaf position. Thus, from the middle leaf to the basal leaf position a greater infection rate was again associated with increasing wettability but the reduced infection on young leaves seemed to be linked with the increased wax present. Further work is in progress, where a more detailed examination of leaf age effects on infection is being made and where several surface characteristics are being modified by the use of surfactants.

Table 1. Leaf wax level (mg/cm^2), wettability (reciprocal of % triton-x-100 concentration) and infection rate of *Alternaria brassicicola* (lesion number per leaf disc) in relation to host and leaf position.

Host	Leaf position	Wax level	Triton-x-100 (%)	Infection rate
Brussels sprout	Apical	0.252	0.018	820
	Middle	0.086	0.078	325
	Basal	0.042	0.048	778
Oilseed rape	Apical	0.202	0.030	515
	Middle	0.084	0.054	764
	Basal	0.052	0.030	1161
Swede	Apical	0.104	0.034	362
	Middle	0.066	0.044	680
	Basal	0.050	0.030	1356
SED	±	0.042	0.006	160
(DF)		(32)	(32)	(55)

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TESTING CULTIVARS OF RADISH ON RESISTANCE TO RHIZOCTONIA SOLANI (KUEHN)

M. Nieuwhof

1. Introduction

Rhizoctonia solani (Kuehn) can cause severe losses in radish crops by damping off of young plants. For this reason in 1986 a research project was started at the IVT to find resistance to this pathogen. Parts of this project are:

- development of a screening method for resistance to Rhizoctonia
- research on sensitivity of radish for the different anastomosis groups of Rhizoctonia
- testing of an international collection of radish cultivars
- propagation by selfing of plants which remain healthy after testing and testing the progenies.

A short report is presented of the results of testing a collection of 355 radish varieties.

2. Materials and methods

From 1986 to 1988 355 cultivars were tested in 11 experiments. In each experiment per cultivar 100 seeds were sown out. The experiments were carried out at 22°C in a soil heavily infected with Rhizoctonia (mixture of 50 parts of soil and 1 part of a heavily infected medium). For testing a Rhizoctonia isolate of the anastomosis group AG4 was used.

3. Results

3.1. General

Two experiments failed by loss of virulence of the Rhizoctonia isolate. In the experiments with a severe attack by Rhizoctonia most seedlings were killed already before emergence and most of the few emerged plants collapsed shortly after emergence. At the end of the experiments, about a month after sowing out, the roots of most remaining plants were attacked. In experiments with a less severe attack more plants emerged, but most died off and at the end of the experiments again very limited numbers of completely healthy plants remained.

3.2. Factors influencing attack of radish cultivars by Rhizoctonia

Most received seed samples had been already disinfected. It can be concluded from Table 1 that seedlings of cultivars with disinfected seeds were less heavily attacked by Rhizoctonia than cultivars with untreated seeds. Also when the seeds were washed to remove the fungicides, as was done in the last 6 experiments, still an aftereffect of disinfection of the seeds remained.

In two germination trials it was found that the germination energy of a number of the seed samples was rather low, though most seeds of these samples still emerged in a disease free soil. Table 1 shows that cultivars with a low germination energy were more heavily attacked by the fungus than cultivars with seeds with a good germination energy.

To get a good idea of the sensitivity of radish cultivars to Rhizoctonia, cultivars can only be compared when seed samples are used with the same germination energy, which are not disinfected or which are disinfected in the same way. After correction for disinfection and germination energy of the seeds, small differences in susceptibility to Rhizoctonia seemed still to be present. These differences were not clearly correlated with differences in types of the radish cultivars. However, no cultivars were found with a clear degree of resistance. Also when the seeds were heavily

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disinfected and infection of the plants was retarded, at the end of the experiments all or almost all plants of all cultivars showed clear symptoms.

3.3. Selection response

In 1986 and 1987 from the spring experiments a number of plants which were still completely healthy at the end of the test, were propagated by selfing. All plants of the progenies which were produced were attacked in a heavily infected soil (dilution soil / medium 50:1). To test if a low degree of resistance might be present, in 1988 the progenies were also screened in a lightly infected soil (dilution soil / medium 200:1 and 1000:1), but also under these circumstances practically all plants were attacked.

4. Discussion and summary

It is the aim of this project to find a form of high resistance to Rhizoctonia. Among the 355 radish cultivars tested until now this was not found. Perhaps at a low level of partial resistance differences between cultivars occur.

A detailed report of this research is published as IVT report 250, which can be obtained on request (Nieuwhof, M. and S. Giezen. Testing cultivars of radish on resistance to Rhizoctonia solani. Dutch with Engl.summ. and Engl.text of the tables).

Table 1. Effect of disinfection and germination energy of seeds on sensitivity of radish cultivars to Rhizoctonia. Distribution (%'s) over emergence classes. Seed disinfection: 0=not disinfected, 1=normal disinfected, 2=heavily disinfected.

		Number of cultivars	Percentage of emerged plants[1]				
			<11	11 - 20	21 - 32	32 - 42	43 - 77
Disinfection	0	77	13	42	47		
	1	147	6	37	44	11	1
	2	131	1	18	40	31	11
Germination energy	normal	290	1	25	49	19	6
	low	65	23	60	15	2	

[1] Average of 9 experiments.

INFLUENCE OF HOST RESISTANCE ON COLONIZATION AND INCUBATION PERIOD OF ALBUGO CANDIDA IN MUSTARD

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White rust caused by Albugo candida (Lev.) Kuntze causes serious damage to mustard in India. Although disease appears on all above ground plant parts but its symptoms on leaves and inflorescence result into local as well as systemic infection are more pronounced and destructive.

Materials and Methods. To observe colonization and incubation period of Albugo candida, leaves of RC 781,RH 8541, (resistant), RH 30, RLM 1357 and Kranti (susceptable) varieties were drop inoculated in screen house by sporangial suspension of A.candida. The site of inoculation was marked by 1 mm diameter cork borer and cut from inoculated host leaves at different intervals. These host bits were made transparent by leaf cleaning technique (Ram and Nayyar, 1978) and examined microscopically for colonization of A.candida in leaf tissue of resistant (RC 781 and RH 8541) and susceptible (RH 30, RLM 1357 and Kranti) varieties. The incubation period was measured from time of inoculation to the appearance of just visible pustule(s).

Results and Discussion. It was clear from Table 1 that in host varieties like RH 30, RLM 1357, Kranti and RC 781, the pathogen could colonize with in a period of 76.8,74.4, 79.2 and 204.0 hours and showed incubation periods of 98.4, 91.2,124.8 and 283.2 hours respectively. In RC 781 exceptionally long period required for colonization and expression of symptoms was due to inherent resistant character of the variety. The pathogen could not establish in the host tissues of variety RH 8541 due to its high degree of resistance. The susceptible cultivars like RH 30, RLM 1357 and Kranti required shorter time for colonization and incubation periods in comparison to resistant (RC 781) and highly resistant (RH 8541) varieties. Our studies have proved that the decision between compatible (susceptible) and incompatible (resistant) reactions of Albugo-Brassica juncea system can be made with in 80 hours of inoculation. Colonization in all the susceptible leaf tissues was observed in 80 hours of inoculation when observed under compound microscope, while in resistant cultivar, RC 781 colonization took place in 204 hours after inoculation. Verma et al.(1975) found that up to the formation of haustoria there is no difference in infection process in resistant and susceptible hosts of Brassica juncea, B.campestris, B.nigra and B.napus. However, Napper (1933) found that A.candida entered through stomata of a resistant host as readily as those of susceptible host.

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Our observations proved that delay in colonization and incubation periods of resistant host formed a practical base to identify host resistance in field as well as laboratory conditions. Besides this, age of plant, age of leaf involved, prevailing environmental conditions and nutrients applied may influence the incubation periods and thereby host resistance. Coffey (1975) observed incubation period of 192 hours in cabbage cotyledons under favourable conditions (Rh= high; Temp.= 15°C) when zoospore suspension was inoculated to establish infection. Large amount of variations in the incubation period of the varieties tested here indicated the level of resistance against Albugo, more the period of incubation higher is the level of resistance in the host variety. Therefore, growing of resistant varieties in the endemic area of white rust will be useful in curbing the infection rate of the disease and thereby slowing down the epidemic development of white rust under field conditions.

Table 1 Colonization and incubation period of Albugo candida in resistant and susceptible varieties of mustard

Varieties		Colonization in host tissues (hours)	SD	Incubation period (hours)	SD
RH 30	(S)	76.8	4.6	94.4	4.8
RLM 1357	(S)	74.4	3.8	91.2	3.6
Kranti	(S)	79.2	5.8	124.8	5.8
RC 781	(R)	204.0	5.7	283.2	6.5
RH 8541	(R)	-	-	-	-

R = Resistant to white rust SD= Standard deviation
S = Susceptible to white rust - = No infection

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PERFORMANCE OF WHITE RUST (Albugo candida) RESISTANCE GENOTYPES DEVELOPED FROM INTERSPECIFIC CROSSES OF B.juncea L. x B.carinata L.

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Indian mustard (B.juncea (L) Czern & Coss) is a predominantly cultivated Brassica species in India. Although, highly adapted to varying agro-climatic conditions and cropping patterns, Indian mustard is highly susceptible to white rust (Albugo candida Kunze) disease. This disease causes appreciable losses in seed yield upto a tune of 50 per cent under late sown conditions (Saharan et al, 1984). Contrary to it, Ethiopian mustard (B.carinata L.)possess a very high degree of resistance to this disease although this species is not very well adapted to our conditions. An attempt was, therefore, made at this centre to transfer the resistance to white rust from Ethiopian mustard to the Indian mustard. It has also been reported that the resistant gene is located in 'C' genome(B.oleracea L.) of B.carinata which is an amphidiploid of B.oleracea L. x B. nigra L. (Singh and Singh, 1988). B.juncea L. which is susceptible to white rust is an amphidiploid of B. campestris L. x B.nigra L.

The results of evaluation of 15 advanced generation (F_6) progenies of 2 interspecific crosses of B.juncea L.x B.carinata L. in respect of resistance to white rust and seed yield alongwith their maturity duration, 1000 seed weight and oil content are given in Table 1. The data indicated significant differences among the progenies in respect of resistance reaction to white rust disease, seed yield and maturity duration. Differences among progenies for 1000 seed weight and oil content were, however, marginal. The disease resistance score varied from 8.4 per cent in ISBH 7 A progeny to 71.8 per cent in susceptible national check variety, Kranti. It was evident that the transfer of resistance in progenies was of higher order. So much so that all the hybrid progenies showed resistant reaction to this disease. It was clear further that the high level of resistance of B.carinata L. soruce has shown dilution of resistance in the interspecific cross progenies. Nevertheless, two of the progenies namely ISBH 7 A and ISBH3 A maintained high level of resistance to white rust transfered from B. carinata. These two progenies surpassed the national check variety of mustard, kranti in seed yield with a significant margin. These were at par with Kranti in respect of maturity, 1000 seed weight and oil content. The results of interspecific hybridization between B.juncea L. and B.carinata L.envisaged easy transfer of white rust resistance into high seed yield back ground through pedigree selection from an unadapted species B.carinata L. to the adapted species B.juncea L.

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Table 1: Performance of F_6 progenies of Brassica juncea L.Czern & Coss x Brassica carinata L. Interspecific crosses in respect of seed yield, white rust resistance and othe desirable characters.

S.No.	Progeny	Seed yield (kg/ha)	White rust incidence (%)	Days of maturity (days)	1000 seed weight (g)	Oil content (%)
1.	ISBH3A	2914	9.6	148	3.8	41.5
2.	ISBH7A	2682	8.4	143	4.1	35.6
3.	ISBH14B	2222	22.6	142	2.9	39.7
4.	ISBH9A	2048	11.6	146	2.6	38.2
5.	ISBH3B	1921	14.2	140	3.4	38.7
6.	ISBH6B	1921	15.8	141	2.6	36.8
7.	ISBH6A	1905	23.4	141	3.1	41.5
8.	ISBH16	1809	16.0	150	2.9	40.5
9.	ISBH1A	1764	15.0	142	3.0	39.6
10.	ISBH15B	1730	21.2	152	2.9	42.9
11.	ISBH8A	1651	16.6	146	2.7	40.2
12.	ISBH17A	1508	13.5	151	2.4	37.5
13.	ISBH4	1476	16.6	144	3.6	40.9
14.	ISBH178	1365	18.1	148	2.9	37.0
15.	ISBH14A	1301	16.2	151	3.2	41.3
16.	Kranti(National check variety of B.juncea spp.)	2143	71.8	141	3.7	39.2
	C.D. at 5%	153.37	6.30	-	-	-
	C.V. %	12.14	15.28	-	-	-

112 SPECIFICITY OF ALBUGO CANDIDA AND PERONOSPORA PARASITICA PATHOTYPES TOWARD RAPID-CYCLING CRUCIFERS

C B Hill, I R Crute, C Sherriff and P H Williams

Six races of Albugo candida (AC) and 11 isolates of Peronospora parasitica (PP) were tested on rapid-cycling populations (Williams and Hill, 1986) of Brassica rapa (aa, CrGC 1-1), B. nigra (bb, CrGC 2-1), B. oleracea (cc, CrGC 3-1), B. juncea (aabb, CrGC 4-1), B. napus (aacc, CrGC 5-1), B. carinata (bbcc, CrGC 6-1) and Raphanus sativus (rr, CrGC 7-1). Eighty plants of each population plus appropriate susceptible control plants were inoculated and handled as described in CrGC Information Documents DSAC-05-13-85-WILPAU and DSPP-05-13-85-WILPAU (Williams, 1985).

Results are summarized in the table below. Presence of sporulation on host plants did not distinguish AC7 from AC8 but disease indices (D.I.) indicate the host species adaptation. PP isolates #827 and #829, both from England, had identical specificity based on presence of sporulation but D.I.'s helped to distinguish them.

Specificity toward particular genotypes within each rapid-cycling population also exists. Highly resistant and susceptible plants have been selected within each population and genetic studies of host specificity are in progress.

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RESPONSES OF SEVEN RAPID-CYCLING POPULATIONS INOCULATED WITH SIX RACES OF ALBUGO CANDIDA (Ac) AND 11 ISOLATES OF PERONOSPORA PARASITICA (Pp)

Pathogen	Homologous Host Genome	CrGC Collection #	CrGC Stock 1		2		3		4		5		6		7	
			S[a]	DI[b]	S	DI	S	DI	S	DI	S	DI	S	DI	S	DI
Ac1[c]	rr	1083	-	1	+	1	-	0	+	1	-	0	+	1	+	1
Ac2	aabb	1084	+	1	+	3	-	0	+	9	-	0	-	0	-	1
Ac7	aa	767	+	7	+	1	+	1	+	5	-	0	-	0	-	0
Ac8	bb	1195	+	1	+	9	+	1	+	9	-	0	-	0	-	0
Ac9	cc	1104	+	1	+	1	+	9	+	1	+	5	+	1	-	0
Ac10	ss	1196	+	1	+	1	-	0	+	1	+	3	-	0	-	0
Pp	aa	558	+	3	+	1	-	0	-	1	+	3	+	1	-	1
Pp	aa	640	+	3	+	1	-	1	+	1	+	3	-	1	+	1
Pp	aa	641	+	1	-	1	-	1	+	1	+	3	-	1	-	1
Pp	aa	829	+	3	+	1	+	1	-	1	+	3	+	1	-	0
Pp	aa	830	-	0	-	0	-	0	-	0	+	5	-	0	-	1
Pp	aabb	642	+	1	+	3	-	1	+	5	-	1	-	1	-	1
Pp	cc	643	+	1	-	1	+	9	-	0	-	1	+	3	-	0
Pp	cc	715	-	1	+	1	+	9	+	1	+	3	+	1	-	1
Pp	cc	828	-	0	-	0	+	9	-	0	-	0	+	1	-	0
Pp	cc	831	-	1	-	0	+	9	-	1	+	1	+	1	-	1
Pp	aacc	827	+	1	+	1	+	5	-	1	+	7	+	1	-	0

[a] Sporulation: + = present on some individuals in the population, - = not present

[b] Disease Index based on 0, 1, 3, 5, 7, 9 scale where 0 = immunity and 9 = full susceptibility (see Williams, 1985)

[c] Ac1 = race 1 from rr, Ac2 = race 2 from aabb, Ac7 = race 7 from aa (Pound and Williams, 1963). Ac8, 9, 10 are tentative classifications; race 8 is from bb, race 9 from cc and race 10 from Sinapis arvensis (ss).

Requirements for analysis of host-species specificity in Peronospora parasitica (Downy Mildew)

Nigel A. Moss, I.R. Crute, J.A. Lucas, P.L. Gordon

The Oomycete fungus Peronospora parasitica causes downy mildew of many host species within the family Cruciferae. The pathogen is widespread and common on cultivated Brassica species including oilseed rape and the valuable horticultural forms of B. oleracea and B. campestris. Isolates of P. parasitica from Brassica are, in general, specifically adapted to their species of origin (Sherriff and Lucas, 1987). To identify genes controlling this host-species specificity, genetic characterisation of host and pathogen is being undertaken in the relationship between B. campestris, B. napus and B. oleracea and isolates of P. parasitica adapted to each.

First it was necessary to identify suitable genetic markers in both partners; studies to date have sought differential resistance in host accessions including rapid cycling Brassica genotypes (Williams and Hill, 1986), and characterised fungal isolates for virulence phenotype. Using these markers, the intention is to characterise hybrid progeny from crosses between heterologous isolates and examine the inheritance of species specificity.

Differential host resistance to homologous isolates of P. parasitica has been identified in B. campestris, B. napus and B. oleracea. In B. napus resistance located in the oilseed rape cultivar Cresor is controlled by a single dominant allele (Lucas et al., 1988). In B. oleracea, differential resistance has been located in a 'land-race' cauliflower "Palermo Green". A model based on 2 or possibly 3 major genes is proposed to account for the reaction patterns of individual plants to selected fungal isolates within a host population (Table 1). In B. campestris, both rapid cycling and commercial genotypes have been examined for differential resistance (Table 2). Four homologous isolates have identified a range of differential host responses and it should be possible in time to develop further a differential host series within this species.

Table 1. Palermo Green Model

			Resistance phenotype				
			-	R1	-	R1	(approx) % resistant
Isolate	A1	A2	-	-	R2	R2	seedlings observed
P005	1	2	+	-	-	-	95
P015	1	-	+	-	+	-	80
P018	-	2	+	+	-	-	80
P006	-	-	+	+	+	+	0
% phenotype in seed stock			5	15	15	65	

KEY : A = AVIRULENCE GENE R = RESISTANCE GENE
+ = SUSCEPTIBILITY - = RESISTANCE

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Table 2 Differential virulence of four P. parasitica isolates from B. campestris on six hosts lines

HOST	P007	P008	P013	P014
CA88014*[a]	+	+	+	+
JADE PAGODA	+	-	+	+
CA87063*	-	-	+	+
SNOWBALL	-	+	-	-
CA87068*	-	-	-	+
CA87065*	-	-	-	-

\+ = Susceptible
\- = Resistant
a = Universally susceptible
* = Rapid cycling lines

In addition to specific virulence, pathogen isolates have been characterised in relation to sexual compatibility type (SCT) and response to phenylamide fungicide.

P. parasitica is predominantly heterothallic, existing as two SCT's referred to as P1 and P2 (Sherriff and Lucas, 1989). Determination of SCT of an unknown isolate is achieved by mixing conidia in a 1:1 ratio with isolates of known SCT and inoculating to a common host. In heterothallic isolates, oospores may form in combination with isolates of opposite SCT.

Insensitivity of P. parasitica from B. oleracea to phenylamide fungicides was identified in the UK in 1983 (Crute et al., 1985). A differential degree of insensitivity to two related phenylamide fungicides has been demonstrated (Table 3). Metalaxyl is more active against a sensitive isolate (P005) than Cyprofuram but the converse is true with an insensitive isolate (P006). It remains to be seen if this situation is observed for all phenylamide insensitive isolates of P. parasitica. Future work will investigate this further and examine the inheritance of fungicide insensitivity.

Table 3. Responses of phenylamide sensitive and insensitive isolates of P. parasitica to phenylamide fungicides

Compound	Isolate	[Fungicide] ug/ml 0.05	0.5	5.0	50	Factor of Insensitivity
Metalaxyl	P005	32[a]	0	0	0	x1000
	P006	94	89	100	83	
Cyprofuram	P005	84	79	0	0	x10
	P006	100	94	78	3	

[a] Figures are reciprocals of mean latent periods (time from inoculation to sporulation expressed as a percentage of the untreated control). Crute et al, 1987).

An important step in the project is the recovery of hybrid progeny from crosses between heterologous isolates. Inoculation of selected isolates of opposite SCT to a specially chosen common susceptible host, generates oospores. After a period of maturation, rehydrated oospores have resulted in infection of host seedlings. Genetic characterisation of such progeny, using the markers described to identify true hybrids, will demonstrate the potential for genetic exchange between heterologous isolates and provide data on the control of species specificity.

Isoenzyme markers are particularly important in discriminating between self and true hybrid progeny. Using PAGE, the enzymes PGI and MDH have shown major band differences between B. oleracea type isolates and between heterologous isolates of P. parasitica.

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USE OF ULTRAWEAK BIOCHEMILUMINESCENCE TO STUDIES OF FROST RESISTANCE OF THE RAPE COTYLEDONS

E.Grabikowski

Destructive action of temperature below 0°C is mainly connected with crystallization of water in plant tissues. The extent of damages and their final effect depend above all on the site in which the crystallization take place. The formation of ice in cytoplasma leads to blockade of metabolic processes and in consequence causes a death of cell. On the other hand the crystallization of water in the intercellular space may be reversible, and not very harmful, depending on the level of dehydratation of the cellular inside. An overstep of certain level of dehydratation may lead to irreversible structural changes of cellular organic compounds.

The disturbance of metabolic processes in cells take place also during the ice thawing in plant tissues. The decisive influence in this case plays the rate of phase transition of ice to water in intercellular space. Too fast process of thawing may lead to irreversible mechanical damage of cell membranes caused by violent hydratation of intracellular space,

Above mentioned effects, occuring under the influence of temperature, are reflected by the change of the intensity of ultraweak biochemiluminescence (UBCL) of plant tissues.

An effect of a freeze-thaw in the cotyledons of green rape on their UBCL was investigated by means of a sensitive quantometric device. Two varieties of rape (Górczański and Jantar) of different frost resistance constituted the objects of investigation. Frost resistance of Górczański variety was higher than Jantar one.

It has been stated that the shapes of kinetic curves and the rate of UBCL intensity increase of the cotyledons of green rape during thawing (the rate of UBCL increase is higher for Jantar rape cotyledons than for Górczański ones) can be useful in estimation of the relative degree of thermical destruction of plant tissues of the investigated rape varieties.

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USE OF THE DELAYED LUMINESCENCE METHOD FOR EVALUATION OF FROST RESISTANCE IN BRASSICA PLANTS

A. Brzóstowicz

The influence of natural frost hardening conditions on the frost resistance of three cultivars of Brassica napus L. (Siberian, Quinta, Bishop) and three cultivars of Brassica campestris L. (Szczeciński, Perko, Pluto) was studied.

Frost resistance was measured in October and in January by the delayed luminescence method (Brzóstowicz et al.1985). The temperature (t_m) at which the maximum of delayed luminescence intensity occurs was chosen as index of frost resistance.

Parallelly an electrical conductivity test (Dexter et al. 1932) was performed for comparison. As index of frost resistance in this test served the temperature (t_{k50}) at which 50% of plant tissues is damaged.

For all cultivars of unhardened plants (in October) as well t_m as t_{k50} was similar. In hardened (in January) plants diversification of t_m and t_{k50} values was observed. It was found that the temperature t_m correlates with t_{k50}. The investigations showed the usefulness of the luminescence method for evaluation of the frost resistance of various Brassica cultivars and for observation of their resistance change during hardening to cold.

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COMPARISON OF THE DELAYED LUMINESCENCE IN TRIAZINE SUSCEPTIBLE AND RESISTANT BIOTYPES OF Brassica napus

Antoni Murkowski

Triazine herbicides are potent inhibitors of photosynthesis /1/. Their phytotoxic action is mainly manifested in interference with electron transport and in consequence causes changes in the delayed luminescence /DL/ decay recorded in the time longer than 1 s after switching off the exciting light /2/.DL is measured by means of a photomultiplier tube and amplifier-recording system /3/.

Influence of simazine on two varieties of Brassica napus plants was investigated: Jet Neuf-susceptible and Triton - resistant. The plants were grown in pots with sand and Hoagland nutrient. Lighting was 250 $\mu E \cdot s^{-1} m^{-2}$/PhAR/, photoperiod: 12h/12h, temperature: 16°C/14°C /day/night/. After 4 weeks leaf discs /16 mm in diameter/ were cut off and put on surface of simazine solution /10 μM/ or water /control/.After 1 hour, decay kinetics of the DL of leaves were measured as described in /4/. For comparison measurements of fluorescence intensity of these leaves according to /1/ were done.All results shown in the table are averages from 5 measurements. Least signicant differences /LSD/ were calculated by means of analysis of variance on the confidence level 0,99.

DL decay parameter				Fluorescence intensity			
Jet Neuf		Triton		Jet Neuf		Triton	
Contr.	Simaz.	Contr.	Simaz.	Contr.	Simaz.	Contr.	Simaz.
4,18	24,0	10,2	14,6	2,32	7,80	3,04	2,88
100%	574%	100%	143%	100%	336%	100%	95%
$LSD_{99}=\pm$ 5,82		$LSD_{99}=\pm$ 4,30		$LSD_{99}=\pm$ 1,60		$LSD_{99}=\pm$3,23	

The results of this experiment show that delayed luminescence test is more sensitive than the fluorescence one.

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DIFFERENTIAL DEPOSITION OF STORAGE PROTEIN IN DEVELOPING BRASSICA JUNCEA SEEDS

Kamal Dhawan, Himmat S.Nainawatee and Parkash Kumar

Besides oil, mustard seeds are also rich in proteins. A general awareness for the use of mustard seed protein as a food and feed has recently begun (Bears- Rogers, 1978). Brassica seed proteins have been poorly characterised and understood. B. napus seed proteins are reported to be composed of two major proteins corresponding to 12 and 1.7 sedimentation values (Crouch and Sussex, 1981). B. juncea is an important mustard crop of India and its seeds have been found to contain three major storage proteins having 112, 26 and 10 kD molecular weights (Dhawan and Nainawatee, unpublished). In this communication the pattern of accumulation of different seed-storage proteins of B. juncea cv. Prakash is reported. The mustard crop was raised under field conditions following standard agronomic practices. Individual flower buds were tagged on the plants. Pods were sampled at three days interval from 10DAF (days after flowering) stage untill maturity. Seeds were removed from the pods and used for the extraction of proteins. Seeds were ground and defatted using hexane : carbon tetrachloride (1:1 v/v). The defatted material was repeatedly extracted with water and the residue was extracted with 0.05 M phosphate buffer (pH 6.8) containing 0.3M sodium chloride and 0.01% sodium azide. The suspension was centrifuged at 10,000 g for 30 min. The supernetant was used for separation of proteins by HPLC (high performance liquid chromatography) using Shimadzu LC 4A system. Separation of proteins was done on Shodex WS-803 gel filteration column at 1 ml/min. flow rate of 0.05M phosphate buffer (pH 6.8) containing 0.3M NaCl and 0.01% sodium azide as eluant. The protein profile was monitored using SPD-2AS UV detector and data computed by CR2A x system. Calibration for molecular weights was done using bovine serum albumin (67 kD), ovalbumin (45 kD), chymotrypsinogen A (25 kD) and lysozyme (14.3 kD).

Seeds obtained from pods upto 21 DAF stage did not show any protein. Proteins were detected from 24 DAF stage onwards. At this stage protein of only very low molecular weight (10 kD) was present (Fig.1a), whereas in mature seeds (45 DAF), proteins of 112, 26 and 10 kD were present (Fig.1b). The pattern of appearance of different proteins during seed development is shown in Fig.2. At 24 DAF stage major protein present in the seed was of 10 kD molecular weight. Its amount decreased abruptly upto 33 DAF stage, then it again increased. Proportion of 10 kD protein in mature seed was 35.6 per cent. The pattern of accumulation of 26 kD protein was opposite to 10 kD protein. At 24 DAF stage the proportion of 26 kD protein was very low. It increased at 27 DAF stage and after 30 DAF stage it again decreased. In mature seeds its proportion was 24.2 per cent. The high molecular weight 112 protein showed appearance at 27 DAF stage and then onwards it continously increased upto seed maturity. In mature seeds, its

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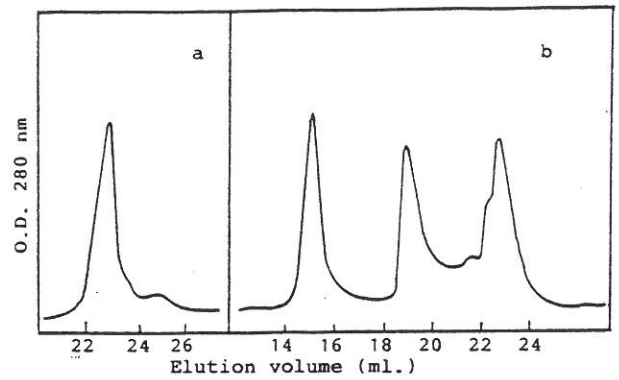

Fig.1. HPLC pattern of proteins at (a) 24 days (b) 45 days after flowering

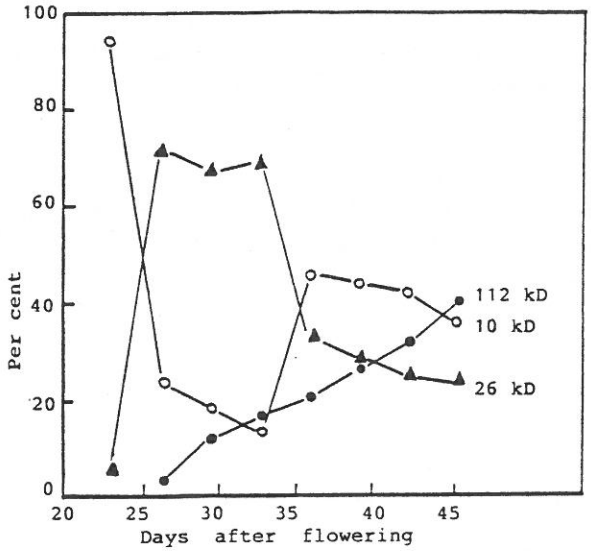

Fig.2. Deposition of different proteins during seed development.

proportion was highest (40.2 per cent). The overall pattern of accumulation showed differential expression of storage protein gene activity. Such differential accumulation of seed-storage protein as a consequence of differential gene expression is known in B. napus (Crouch and Sussex, 1981) and soybean (Goldberg et al., 1981). This information can be useful in studying regulation and manipulation of seed-storage protein genes.

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THE USE OF SODIUM CHLORIDE SOLUTION TO OVERCOME SELF-INCOMPATIBILITY IN BRASSICA CAMPESTRIS

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A new method of selfing SI brassica, recently reported by Tao and Yang (2), showed that sodium chloride was effective in overcoming SI in Chinese cabbage when flowers were sprayed 0.5 to 1.0 hours after pollination with a 3% NaCl solution. This method is interesting because it is inexpensive and easily applied. To obtain more information about effectiveness of salt the following experiment was done.

Rapid cycling B. rapa plants form the Crucifer Genetics Cooperative stock CrGC 1-1 were grown in pots in a greenhouse at 22±2°C and 24h photoperiod. Each plant was pruned to three inflorescences, each of which received a different treatment so that each plant was considered a replication with about 10 flowers per inflorescence. Five plants were used per experiment. Pollinations were done with beesticks (3). The number of seeds per silique was counted 7 days after pollination when the seeds were easily visible inside the silique. The salt solution was applied on the stigma with a cotton swab 5-10 minutes before self-pollination with the following treatments: (a) distilled water; (b) 15 g/l NaCl solution; (c) regular cross-pollination with compatible pollen. Simultaneously five extra plants were treated in the same manner as described above and used for microscopic observation of the pistils. Flowers for microscopic examination were removed 24h after pollination and the pistils fixed in ethanol:acetic acid (3:1) for 3 h, cleared in 8N NaOH for 2h and observed by the decolorized aniline blue fluorescent method (1). The following events were recorded: (i) number of pollen grains adhering to the stigma; (ii) number of pollen grains germinating on the stigma; (iii) number of papillae with callose.

In previous experiments using similar procedures as described above (unpublished data) 15.9, 1.6, and 0.1 seeds per silique were obtained on cross-pollinated flowers, self-pollinated with 5 g/l of sodium chloride applied 2h after pollination, and self-pollinated control, respectively. When salt was applied 2 h before pollination 12.5, 1.6, and 0.4 seeds/silique were recorded respectively for cross-pollinated, salt treated flowers and control. In these two tests only the cross-pollinated plants had a significantly higher number of seeds per silique in comparison with the control. Salt applied 15 minutes before pollination produced significantly higher seed set (5.7 seeds/silique) than salt applied 15 minutes after (2.6 seeds/silique). Therefore the best timing for salt application seems to be a few minutes before pollination. Increasing the concentration of NaCl from 5 to 15 g/l, resulted in an increase of seed set from 5.6 to 8.2 seeds/silique.

In the present research the results from the previous experiments are confirmed. Self-pollinated salt-treated flowers set 7.2 seeds/silique compared with 17.1 on cross-pollinated and only 0.7 on self-pollinated water treated control flowers (Table 1). Microscopic

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observation of stigmas showed that salt treated flowers had intermediate levels of pollen adhesion, pollen germination and callose formation between cross-pollinated and self-pollinated control flowers (Table 2). Sodium chloride treatment of the stigma is a very effective method for self pollinating SI B. rapa plants. Salt application is simple and fast. Ten to 15 flowers can be treated per minute and the amount o seed produced is sufficient for most breeding purposes.

Table 1. Effect of 15 g/l NaCl solution applied to the stigma of B. rapa plants on the number of seeds/silique in comparison with cross-pollinated flowers and the water treated self-pollinated control flowers

Treatments	No. seeds/ silique[z]	No. flowers treated
Cross-pollinted	17.1 a	51
Selfed (15 g/l NaCl)	7.2 b	52
Selfed (Water)	0.7 c	50
F test	147.9***	

[z] Means followed by the same letter are not significantly different by LSD (P = 5%).

Table 2. Effect of sodium chloride applied to the stigmas of self-pollinated B. rapa on pollen adhesion, pollen germination and callose formation in comparison with cross-pollinated flowers and water treated self-pollinated control flowers[z]

	Pollen adhesion[y]			Pollen germination[x]			Callose formation[w]		
Treatments	<20	20-50	>50	<5	5-25	>25	<10	10-30	>30
Cross-pollinated	1	9	40	0	7	43	31	17	2
Selfed (15 g/l NaCl)	13	15	22	13	18	19	20	23	7
Selfed (water)	44	5	1	47	2	1	0	13	37

[z]50 flowers from 5 different plants were observed
[y]Number of stigmas with <20; 20-50; and >50 adhering pollen grains
[x]Number of stigmas with <5; 5-20; and >20 germinating pollen grains
[w]Number of stigmas with <10; 10-30; and >30 papillae with callose

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MONITORING VIABILITY IN THE GENUS BRASSICA

J J Baladrón and A Ordás

One of the main problems that breeders must face is maintaining their collections in good conditions of viability. The optimum would be to have facilities for long-term storage (Cromarty et al., 1982), but unfortunately in most cases the available conditions fit into the type known as active collections in which medium-term conservation is the best that can be obtained.

In the Misión Biológica de Galicia a program to collect landraces of brassica crops from Galicia (northwestern Spain) was started in 1985 (Ordás and Baladrón, 1985). The 345 samples collected so far are kept in cold storage at 0-2 °C and at about 50% of relative humidity.

To check the decay in viability 25 samples of the several species of the genus Brassica grown in Galicia (B. oleracea, B. napus and B. campestris) were tested for viability in 1985 and 1988. As most samples were of small size only 50 seeds (two replications of 25 seeds each) per entry were used. The viability was estimated by germination tests placing the seeds on petri dishes with two layers of filter paper. The dishes were kept continuously moistened and placed in an incubator at 25 °C without illumination. Counting of germinated seeds were made after the third and fifth days. The statistical analysis of data was carried out following Wilcoxon's signed rank test (Steel and Torrie, 1981, p.539). The value of the T statistic (T=136) obtained from the results shown in Table 1 shows that, for the time being, there is no decay in viability.

Obviously the ideal would be to regenerate a sample as soon as possible after it is collected. As the available funds are usually limited it seems that, from the results shown above, the efforts can still be devoted for a few more years to finish the collection of landraces from the northwest of Spain, an urgent task in view of the rapidity with which genetic erosion is taking place.

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Table 1. Differences in viability among 25 samples of cultivated brassicas according to Wilcoxon's signed rank test.

Entry	14	24	26	27	28	29	30	31	33
Diff.	+1	-2	+5	-16	+1	+15	-16	+1	+1
Rank	+6	-10	+14	-23	+6	+21	-24	+6	+6

Entry	34	35	36	37	38	39	40	41	42
Diff.	+6	+7	=	=	-1	+9	-5	+7	-12
Rank	+16	+17	+1½	-1½	-6	+19	-14	+18	-20

Entry	43	44	45	46	47	48	49
Diff.	+15	+38	-5	-1	-1	+4	-4
Rank	+22	+25	-14	-6	-6	+11½	-11½

YIELD IMPROVEMENT IN RAYA (*BRASSICA JUNCEA* L. CZERN & COSS) BY THE USE OF PHENOLS

Rajinder Sharma and Lakhvir Singh

Raya (*Brassica juncea* L. Czern & Coss) an important oilseed crop was sprayed with aqueous solution of Salicylic acid (20 ppm), Caffeic acid (50 ppm) and Tannic acid (50 ppm), at the time of anthesis to study their effect on yield and its components. The experiment was conducted in the field keeping three replications for each treatment in a randomized block design. Yield contributing parameters viz. number of siliqua per plant, length of siliqua, number of seeds per siliqua, 1000-seed weight, Harvest Index (HI) and yield per plant were studied in a sample of five plants selected at random from each plot. Total yield per plot was also recorded after harvesting.

All the phenolic treatments increased the yield and yield contributing parameters significantly over control (Table 1). Phenols are known to act as analogues of growth hormones. The increased yield in the present case may be due to enhanced mobilization of photosynthates to the developing siliquae, thus improving the yield components. There is ample evidence that the effect of cytokinins is mediated through an increase in the endogenous level of phenols (Filnova, 1983) and Cytokinins are known to cause directed transport of photosynthates from source to sink (Mothes and Engelbrecht, 1961). So the use of phenols at the time of anthesis can go a long way in improving the yield of raya and it should be tried on other crops also.

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Table 1. Effect of some phenolic compounds on yield and its components in Raya CV. RLM-198

Treatment (ppm)	No. of siliqua/ plant	Length of siliqua (cm)	No. of seeds/ siliqua	Seed Yield/ plant (g)	Harvest index (%)	1000-seed wt.(g)	Yield/ha (kg)
C (0)	302	4.1	12.5	5.93	15.02	1.850	772.227
SA(20)	367	4.5	13.9	7.00	12.14	1.990	791.164
CA (50)	381	4.4	13.6	8.09	16.13	2.010	799.636
TA (50)	402	4.3	13.6	9.43	16.88	2.153	854.752
C.D.at 5% level	54.1	0.2	1.1	3.17	N.S.	0.057	15.561

N.S. = Non Significant

C = Control, SA = Salicylic acid, CA = Caffeic acid, TA = Tannic acid

BOLTING CHARACTERS OF SLOW BOLTING BRASSICA CAMPESTRIS VARIETIES UNDER NONVERNALIZED CONDITION

S. Yui and H. Yoshikawa

Flower bud initiation and bolting of Brassica campestris are mainly governed by low temperature requirement. The higher low temperature requirement of a variety is, the slower the bolting is. Long day and high temperature requirement are said to be secondary factors for bolting characters. However, two slow bolting varieties reported here might be exceptional cases because they flowered without any vernalization treatment.

In Experiment 1, three B.campestris varieties were sown in a greenhouse (temperature 10-30°C, natural day length). After 4 days, they were transferred to a growth chamber, i.e. 16 hours day length, 25°C constant temperature. As shown in Fig.1, some individuals of two slow bolting varieties bolted. Those two are 'Osaka Shirona Bansei (chinensis)' and 'Maruba Mibuna (japonica)', both originated in Japan. Seeds of flowered plants of each variety were obtained.

In Experiment 2, the progenies of bolted 'Osaka Shirona Bansei' in Experiment 1 were cultivated under 8 different environmental conditions shown in Table 1. In all temperature conditions with 12 hours day length, no bolting was observed. High percentage of bolting was observed with 16 and 20 day length treatments. Then it is certain that a main factor to induce flower bud initiation and bolting of the progenies is long day length (16 hours or longer).

It is suggestive that slow bolting character of 'Osaka Shirona Bansei' is said to be introduced from a late bolting Pak-Choi (B.campestris, chinensis) of China. On the other hand, 'Maruba Mibuna' is said to have genetic relation with B.juncea, which has no low temperature requirement for its flowering.

Now we are examining the progenies of 'Osaka Shirona Bansei' to get more information about their bolting characters. We are also trying to breed a slow bolting B.campestris variety which is sensitive to long day length and has no low temperature requirement for its bolting.

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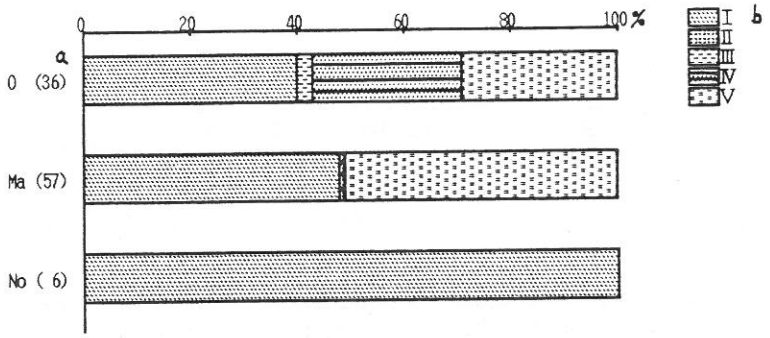

[a] Variety symbol (number of plants)
0 : Osaka Shirona Bansei (chinensis)
Ma: Maruba Mibuna (japonica)
No: Nozaki No.2 (pekinensis)

[b] I : vegetative growth II : bud appearance
III : shoot elongation IV : bud app.and shoot elong.
V : flowering

Fig.1. Bud appearance, shoot elongation and flowering of B.campestris varieties under controled environment (25°C,16hours day length, no vernalization treat.)

Table 1. Bolting percentage of the progenies of flowered 'Osaka Shirona Bansei' under controled environment (no vernalization treatment)

day length	temperature (day-night, °C) 25-25	25-20	20-20	15-8
12	0[a](0/9)[b]	0 (0/5)	0 (0/5)	0 (0/5)
16	73(8/11)	60(3/5)	80(4/5)	----[c]
20	57(4/7)	----	----	----

a : percentage of flowered plants
b : numbers of plants flowered and examined, respectively
c : not examined

PRELIMINARY RESULTS ON THE RELATIONS BETWEEN CULTIVATION METHODS, FUNGICIDE APPLICATION AND DISEASE INCIDENCE IN THE SEED PRODUCTION OF EARLY CAULIFLOWER VARIETY (cv.Brio)

D.Esiyok N.Cetinkaya E.Onogur B.Eser

In the seed production of early cauliflower varieties, curds are under the harmfull effects of various external factors which cause rotting of curds subsequently limit seed yield. Low temperature and heavy rain are the most effective factors and Alternaria spp. which causes dry lesions on curds follows them (Esiyok 1986, Onogur et al. 1986 a,b.). In order to control the negative effects of these factors, some experiments were maintained. In the one of these experiments three different cultivation methods and three fungicides were tested.

In all cultivation methods, plants were grown in open field conditions. When the plants reached curd maturation stage for market, they were divided as three groups. In the first group of plant (CM1) nothing was done and they were accepted as a control. In the second group of plants (CM2) some lobes were cut out, at the central part of curds, which was about % 10-15 of total curd area. As a third cultivation methods (CM3), polyethylene (PE) sheet were covered over the plants forming a tunnel structure of which both sides were open along the growing season. In all cultivation methods, three different fungicides, Propineb (Antracol 70 WP), Mancozeb (Dithane M-45 S) and Captan (Koruma Captan 50 WP) were sprayed onto the curds, totaly six times, between the curd maturation stage for market and formation of flower bud stage.

Effectiveness of fungicides against Alternaria spp. were assessed based on the percentage of infested area on the curds.Other symptoms caused by Peronospora brassicae and bacteria began to be seen on the curds after bolting stage and the infection rates of them were assessed separetely. The results including the seed yield/plant were given in table 1. As it is seen on table 1, CM3 plants gave the best result among the cultivation methods tested against Alternaria spp. On the other hand all the fungicides improved the best effect of CM3. The positive effects of fungicides against Alternaria spp. were also seen in CM1 and CM2 plants.

When we look at the infection rates caused by Peronospora brassicae and bacteria, it is seen, there are no positive effects of fungicides, but the infection rates of them are decreased in CM3 plants. This feature are clearly seen from table 1. Total infection area values are generally lower in CM3 plants than the others. It was about % 19.33-28.92 in CM3 plants,although they were % 34.24-59.00 and % 41.33-58.57 in CM2 and CM1 plants respectively. In the light of these findings, it is seen, bacteria (identification of bacteria is going on) and Peronospora brassicae do not effect the seed yield values significantly (on a large scale), although they reach big infection rate values. But, Alternaria spp. may affect the seed yield significantly. Hence, this pathogen should be absolutely controlled before the period when bolting began.

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Table 1. Infested area of curds (%), average seed yield of plants treated with 3 fungicides, in all cultivation methods.

FACTORS		INFESTED AREA CAUSED BY				
Cultivation methods	Fungicides	Alternaria spp. (%)	P.brassicae (%)	Bacteria (%)	Total infested area (%)	Average seed yield g/plant
CM1	Captan	9.33	18.33	21.33	48.99	31.33
	Mancozeb	11.92	14.23	21.92	48.07	24.77
	Propineb	11.00	15.33	15.00	41.33	21.33
	Control	22.50	16.07	20.00	58.57	21.33
CM2	Captan	9.00	15.67	20.00	44.67	31.00
	Mancozeb	8.85	11.54	13.85	34.24	40.83
	Propineb	10.38	18.85	23.85	53.08	26.43
	Control	20.50	19.50	19.00	59.00	20.63
CM3	Captan	2.67	8.33	8.33	19.33	71.47
	Mancozeb	1.79	9.64	12.14	23.57	57.37
	Propineb	3.21	8.21	12.14	23.56	61.10
	Control	9.64	8.21	11.07	28.92	29.77

When the seed yield values compared, it is seen, there is no big difference between the plants which fungicides were not sprayed on, in all cultivation methods. But it is also seen that seed yields can be improved significantly by fungicides, particularly in CM3 plants. As it is mentioned above, all the fungicides can not control the bacteria and Peronospora brassicae. Hence, it is likely that all the seed yield improvements obtained by fungicides is related with the high effectiveness of fungicides against Alternaria spp.

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POLLINATION OF COLE CROPS BY HONEY BEES OR BLOW FLIES?

I.W. Boukema, F.D. Dotinga and E.C. de Groot.

Introduction

The Centre for Genetic Resources, the Netherlands (CGN) holds a collection of brassicas. Regeneration of *Brassica oleracea* is carried out at the Institute for Horticultural Plant Breeding (IVT) in isolation rooms in an unheated glasshouse. In the past the IVT utilized honey bees and blow flies for pollination. Keeping honey bees is relatively labour and time consuming, whereas fly pupae can be ordered easily from specialized fly breeding firms. By several authors blow flies are preferred to honey bees for regeneration of cross pollinating crops, because flies pollinate more randomly. A disadvantage of flies is that they escape more easily from isolation cages than honey bees, which stick to their hive. In order to make a decision if in the future only flies can be used for regeneration, a small experiment was set up to compare pollination by flies and bees.

Materials and Methods

Autumn 1986 red and white cabbage plants from OP-selections (storage cabbage types) were uprooted from the field, their heads cut off and potted and overwintered in a greenhouse at circa 10°C.

March 1987 the cabbage plants were placed in two adjacent isolation rooms in a green house. One red cabbage plant was placed in the middle, and around this plant three circles with respectively 6, 12 and 24 white cabbage plants. Each circle was divided in four sections. The plant distances were approximately 45 cm between the plants within the circles as well as between the circles. On the 1st of May 1987 bees respectively flies were put in the isolation rooms for pollination.

At the beginning of July 1987 seeds were harvested. The white cabbage plants were harvested per section of a circle. In total 26 portions of seeds were obtained from both rooms (two of red cabbage plants and 24 of white cabbage plants).

In March 1988 at least 60 seeds per portion were sown to score the progenies from the white cabbages on anthocyanin coloration of the hypocotyl. The hypocotyls of white cabbage seedlings contained no or just a little anthocyanin. Seedlings of red cabbage and of hybrids from red and white cabbage were clearly red.

Results and Discussion

The mean seed production per plant after pollination by flies and by bees was the same, namely 8 grams per plant. However, the seed production of the red cabbage plant in the room with flies was higher than of the one in the room with bees (14.5 grams and 8.6 grams respectively). This may be caused by the fact that the red cabbage plant in the room

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with flies had produced more flowers. As a consequence this heavier plant also could have produced more pollen than the red cabbage plant in the room with bees.

Results from the anthocyanin score on the seedlings are shown in the Table. Progenies originating from white cabbages pollinated by flies contain more hybrids between white and red cabbage than those pollinated by bees. This can result partly from the fact that in the room with flies the amount of pollen of the red cabbage in the total amount of pollen was higher. For the other part it may result from the more random pollination of flies.

Distances between the plants play a very important role, as can be seen from the results: the inner circle of white cabbage plants delivers a considerable higher amount of hybrid plants in the progeny than the outer circles. This is more pronounced with bees as pollinating insect than with flies.

It is not clear why the amount of hybrid seedlings is so high in section 3 of circle 1 in both isolation rooms. Probably the location of the isolation rooms caused this effect, e.g. more hours of sun on this particular site.

Summarizing it can be concluded that pollination with flies compared to that with bees may be as good or even better for regenerating populations of cole crops in the IVT-isolation rooms.

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Table: Percentages of seedlings with anthocyanin per insect, per circle and per sector.

	insect							
	flies				bees			
	sector				sector			
circle	1	2	3	4	1	2	3	4
1	9	3	24	2	9	9	20	1
2	7	1	6	8	1	5	0	3
3	7	3	5	6	0	1	1	2

MECHANISMS OF RESISTANCE TO DIAMONDBACK MOTH IN CABBAGE

S. D. Eigenbrode, A.M. Shelton, M.H. Dickson

As part of our efforts to develop cabbage varieties resistant to diamondback moth (*Plutella xylostella* L.), we are investigating the mechanisms producing resistance in some of the most promising breeding lines. These include glossy cabbages derived from an Australian cauliflower, PI 234599 (Lin et al., 1983) as well as nonglossy types both with and without this PI in their parentage (Table 1).

We are using two approaches. 1) Cabbage extracts are prepared using hot ethanol. Fractions of this extract are incorporated into an artificial diet used routinely for rearing our laboratory cultures of diamondback moth. Larval survivorship is then measured on the extract supplemented diets and pure diet controls. 2) Larval behavior is monitored on resistant and susceptible plants to determine causes of mortality and plant characteristics producing resistance.

Extracts

Polar fractions have produced the most promising results with this bioassay for resistance. These are prepared by evaporating ethanol extracts, at reduced pressure, to dryness. After elution with hexanes, the extract is redissolved in water, concentrated and added to diet. Of the lines investigated, 131 and 2503, nonglossy types with intermediate resistance, have produced the most consistently active extracts (Table 1). Current efforts, in collaboration with chemists at Boyce Thompson Institute at Cornell, are directed towards improving the activity of these extracts and identifying the compounds involved.

Behavioral Observations

Movement rates of individual neonate diamondback larvae are much greater on glossy resistant plants descended from the PI than on susceptible controls or nonglossy resistant types (Table 2). This increased movement is associated with decreased feeding by larvae on these resistant plants. Twenty four hours after inoculation with 500 larvae resistant plants have a mean of 120 feeding lesions/plant and susceptibles have 990 lesions/plant. Present efforts, in our laboratory, are directed towards determining the specific characteristics of the resistant leaf which produce the increased movement rates and reduced feeding. This is being accomplished with the use of video recordings of larvae under controlled conditions and interactive computer assisted measurement of larval behaviors.

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Table 1: Percent survivorship (±SEM) of Plutella larvae on plants, leaf disks or diet+extract from cabbage lines or controls in field and laboratory experiments (See text for description of experiments.).

Experiment	Susceptible Line	Resistant Lines				
	Rup	2506 nonglossy	2503 nonglossy	131 nonglossy	2518 glossy	control
Whole Plant [1]	30.15±5.8a	30.81±1.7a	21.44±3.1ab	15.11±1.8b	0.11±.073c	-
Polar [2] extract	54.4±2.8ab	50.5±3.6bc	45.5±2.5c	46.5±2.8c	53.4±2.9ab	58.7±3.4a

Values in the same row with the same letter are not significantly different (P≤.05) based on Fishers Protected LSD 1: N=9 plants/line. Plants were inoculated with 200 eggs and percent survivorship determined at pupation. 2: Combined data from 7 separate bioassays; N=60-80 diet cups/line.

Table 2: Movement rates of diamondback larvae on resistant and susceptible plants ±SEM.

Experiment	Susceptible Line	Resistant Lines			
	Rup	2506	2503	2535	2518(glossy)
Larval Movement (cm/min) in field §	0.561±.069	- -	- -	- -	1.564±0.225
Leaving Rate ¶	.0061±.0015a	.0065±.0014a	.0103±.0023a	.0065±.0010a	.0209±.0061b

§ N = 44 for Rup and 34 for 2518, P<.0001, Student's t. Larvae were observed for one hour.

¶ proportion of larvae leaving a 10 cm diam. circle on a leaf in the field/minute. N for five cabbage types, in order: 70, 41, 40, 38, 90. Values in the same row with same letter are not statistically different using ANOVA and Fisher's protected LSD , P≤ .05.

EFFECT OF TEMPERATURE ON FERTILITY OF OILSEED RAPE (*BRASSICA NAPUS*)

M.J. MORRISON

Researchers have determined that the reduction in fertility of certain crops exposed to high temperatures was likely due to pollen inviability, or ovule abortion. A conscious effort has been made in these crops to select for a reproductive system that will operate at higher temperatures. In oilseed rape, seed yield decreases when flowering occurs during a hot period of the growing season. To improve high temperature tolerance in oilseed rape and thereby increase seed yield, it is necessary to determine the temperature where fertility is eliminated.

Spring oilseed rape (*B. napus* cv. Westar) was grown from seed to maturity in growth cabinets set at mean daily temperatures of 10, 13.5, 15, 17, 20, 22 and 25°C. Temperatures regimes were established by setting the minimum and maximum temperatures 5°C higher and lower than the mean and alternatively increasing and decreasing the temperature 1°C per hour in a stepwise manner. Westar plants were grown in a soilless potting mixture, watered to field capacity daily and fertilized at each growth stage. Observations on phenological development were made daily on 150 plants per cabinet.

Plants grown at the 22 and 25°C mean temperature regimes did not produce viable pods or seeds, wheareas fertility was normal at temperatures up to and including the 20°C regime. No seed was produced from over 100 plants in either the 22 and 25°C temperature regimes. The sterile pods produced on the continualy flowering plants, remained small and did not elongate or fill. Mean temperatures of 20, 22 and 25°C were established with temperature ranges of 15-25, 17-27 and 20-30°C, respectively. Therefore, it appeared that cabinet maximum temperatures greater than 25°C resulted in sterility. Vegetative development prior to flowering was not affected by the high temperatures and the plants received sufficient water to maintain leaf turgidity. Plants from the 25°C cabinet were transferred at various stages of floral development to the 20°C cabinet. When the plants were transferred after receiving high temperatures in the bud stage, the first flowers produced resulted in sterile pods. The condition of sterility remained for about 10 days, after which the flowers produced fertile pods. Similar results occurred in plants transferred at later stages of floral development. Thus, it appears that temperatures in excess of 25°C cause sterility early in the bud phase of development. The cellular mechanism of floral sterility was not determined. Fan and Stefansson (1986) observed that temperatures greater than 26°C operated on buds to promote normal stamen development of some cytoplasmic male sterile (CMS) rapeseed plants. It is interesting to note that sterility and its reversion to fertility in sterile plants, are caused by similar high temperatures. Currently, there is insufficient evidence to link the two mechanisms.

Work is currently being undertaken at the Plant Research Centre to further characterize the effect of high temperatures on oilseed rape. If successful, and high temperature tolerant lines can be selected economic yields can be improved when flowering occurs during high temperatures.

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QUANTITATIVE LEVELS OF GLUCOSINOLATES IN RAPESEED POLLEN

S G Dungey

The recent finding of glucosinolates in rapeseed and Indian mustard pollen (1) suggested that these compounds may be accessible to pollen selection (2). To establish a quantitative basis for pollen selection it was necessary to determine whether the relative amounts of glucosinolates in pollen could be correlated with the relative levels of glucosinolates in seed. This report presents some preliminary quantitative data on glucosinolates in pollen and seed of two rapeseed cultivars Midas and Tatyoon.

Pollen and seed from phytotron-grown plants were extracted and analysed for glucosinolates according to Dungey et al. (1). Pollen samples were further concentrated by freeze drying to attain adequate sensitivity for HPLC detection.

The results (Table 1) indicated that the glucosinolate content of pollen generally reflected that of seed with respect to the Canola glucosinolates. The Indole glucosinolates were either absent from pollen or present only in trace amounts. The results also indicated that quantitative differences observable at the seed level were also evident at the pollen level. These very promising results, if extended to other cultivars, would provide the quantitative basis for the pollen selection of low glucosinolate cultivars.

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Table 1. Glucosinolate content in pollen and seed from Midas and Tatyoon (μmoles/g)

Glucosinolate		Cultivar: Midas Seed	Midas Pollen	Tatyoon Seed	Tatyoon Pollen
2.0H Butenyl		71.90	0.95	28.80	0.66
2.0H Pentenyl		4.10	0.21	1.10	0.15
3. Butenyl		19.20	0.04	9.70	0.04
4.0H Indolyl		5.60	-	5.30	-
Indolyl		0.60	-	1.60	-
4. Pentenyl		5.10	0.13	2.10	0.04
2. Pnenylethyl		1.90	0.13	1.30	0.05
4. Methoxyindolyl		0.20	-	0.13	-
1. Methoxyindolyl		0.20	-	0.24	-
Totals	*x	110.80	1.35	50.50	0.94
	sd	7.10	0.19	6.70	0.28
	cv%	6.4%	14%	13%	29%

* Analysis of 3 replicates, each replicate contained the combined seed or pollen from 4-8 plants. Levels are expressed in μmoles/g of meal, for seed and μmoles/g fresh material for pollen.

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ERRATUM

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to replace p.119 in Cruciferae Newsletter No. 12 (1987)

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