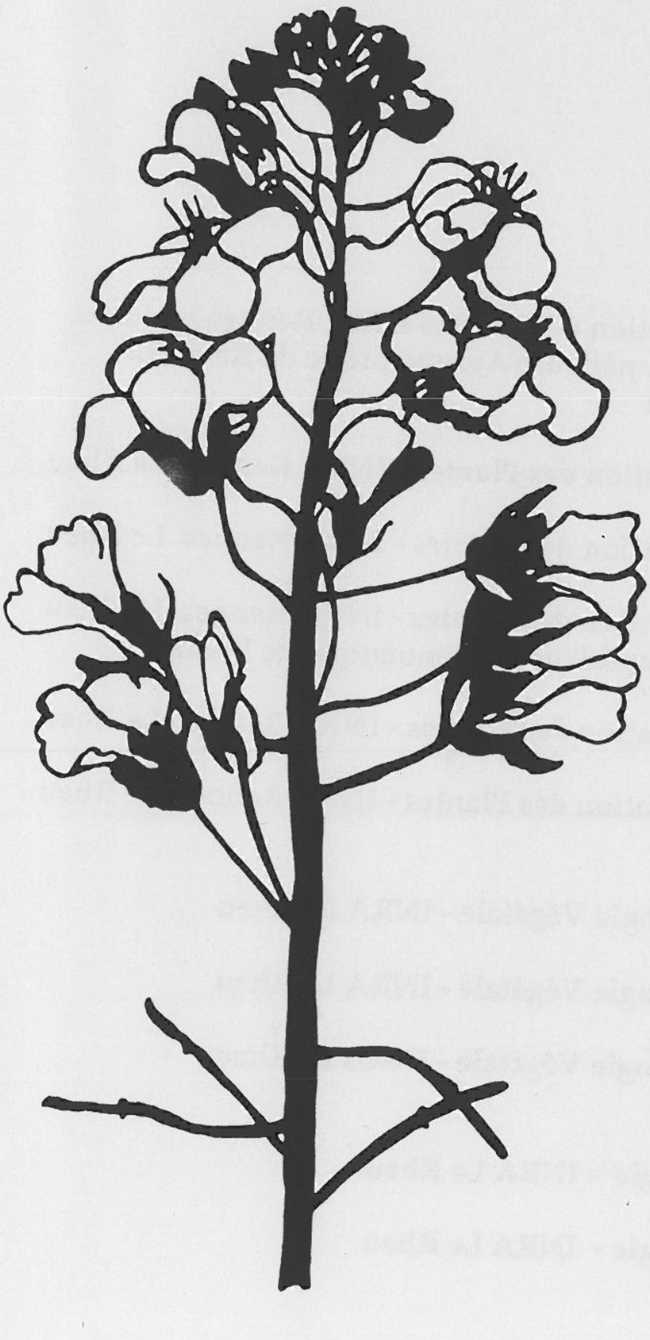
CRUCIFERAE NEWSLETTER



Nr. 17

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EDITORIAL

We are pleased to present you with the second issue of Cruciferae Newsletter coming from our new editing team and which is Cruciferae Newsletter Nr. 17. I think we have now reached our editing rhythm and hope to produce from now onwards, one issue every ten to twelve months.

However, please, remember that this Newsletter is yours and that we can't publish it without your help.

First, we are already looking forward to new papers for the next Cruciferae Newsletter Nr. 18 (deadlines January 31.1996)

Secondly, we also need your financial support and we want to thank here all of you who have financially contributed to the edition during 1994. You will find herewith a financial support form for 1995 for those who want to help and make Cruciferae Newsletter a real and regular publication.

With many thanks again.

Grégoire THOMAS
Coordinator

The Editing Committee and all members of Cruciferae Newsletter are very grateful to all the companies and institutes which have brought financial support to the edition.

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Camelina sativa: Old face - new prospects

A. Schuster & W. Friedt, Institute of Crop Science and Plant Breeding, Justus-Liebig-University, Ludwigstr. 23, D-35390 Giessen, Germany

INTRODUCTION

Camelina sativa (L.) Crtz. (false flax, gold-of-pleasure) was the most important oil crop in Europe during bronze and iron age - even older than poppy and linseed. It is still not clear why people did hardly grow any *Camelina sativa* in the Middle Age and thereafter, although this crop is extremely well adjusted to the European climate (KROLL, 1994). Looking out for regenerable energy resources *Camelina sativa* has been re-discovered recently (BRAMM, 1993).

The oil of false flax can be used for producing soap or colour. In fact, using it for vegetable-oil engines is being considered. According to the current directions on animal feedstuffs it is not allowed to feed *Camelina sativa* to animals. It is not known, however, why this prohibition does still exist, since new research work (BÖHME & DAENICKE, 1994) shows that it is possible to use shred of flase flax for animal nutrition.

Camelina sativa belongs to the familiy of *Brassicaceae* and similar to rapeseeds we know winter- and summer-forms. Regarding our results of research it is not effective to grow winter-forms, because the productivity of this type cannot with compete the results of winter rapeseed. Recent results of the summer-forms are most promising though.

PRODUCTION

The results of trials throughout several years (FRIEDT et al., 1994, GRAF & VETTER, 1994) have shown, that a seed yield of 2.5 - 3 t/ha can be reached easily with spring cultivars of *Camelina sativa*. In our trials we could prove that an intensity of sowing of 400 vigorous seeds/m², a distance of the rows of about 12 cm and an amount of about 130 kg/ha nitrogen will be most effective. There are no problems to be expected with weed control as the distance between the rows is rather small. Additionally *Camelina sativa* is highly competitive because of its rosette-form. Good results could be achieved in a one-year-trial with pre-sowing herbicide treatment („Elancolan" = Trifluralin, „Butisan S" = Metazachlor). Apart from this false flax shows promising qualities with its good resistance against pests and diseases (ANDERSSON & OLSSON, 1950), its resistance against drought (MAKOWSKI & KLOSTERMANN, 1995) and frost as well as ist very short vegetation period of 100-110 days (RÜTHER, 1957). The fairly low thousand-seed-weight of about 1 g, however, could cause some problems so that an increase of the seed weight would be highly beneficial.

INGREDIENTS

a) Fat content

The analysis of a collection of *Camelina sativa* varieties via nuclear magnetic resonance spectrometry (MADSEN, 1976) showed a fat content of 40.3 - 47.4 % at 100 % dry matter.

b) Fatty acid composition

A comparatively large number of fatty acids can be found in the oil of false flax. This can cause problems for an industrial usage of the crop. Therefore, breeding work including mutation induction (FRIEDT et al. 1994) has been initiated to change the fatty acid composition. Table 1 shows the variation of a *Camelina sativa* collection, here the composition was analysed by gas-chromatography (MARQUARD, 1987).

Table 1: Variation in the fatty acid compostition of *Camelina sativa*

Fatty acid	% of total fatty acids
Palmitic (16:0)	5.9-7.5
Stearic (18:0)	2.3-3.2
Oleic (18:1)	15.2-20.7
Linoleic (18:2)	14.8-19.2
Linolenic (18:3)	30.4-35.9
Eicosenioc (20:0)	15.4-18.3
Erucic (22:1)	2.7-3.9

c) Glucosinolates

Camelina sativa seed was prepared according to a method described by DEMES (1989) and analysed via High-Pressure-Liquid-Chromatography (HPLC). First results showed a total glycosinolate content of 19 - 32 µmol/g in the air-dried seed.

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Preliminary study about an endangered wild *Brassica* population in Albania

Lufter XHUVELI
Agricultural University of Tirana, Albania
Karl HAMMER and Thomas GLADIS
IPK-Genebank, Gatersleben, Germany

According to the Flora of Albania, there are existing two wild *Brassica* species in the Southern coast of Albania, *B. incana* TEN. and *B. oleracea* L. (PAPARISTO et al. 1988, pp. 368-369). The biennial (!) *B. incana* is only known from Himara, near to the Ionian Sea while the wild bi- or perennial *B. oleracea* is distributed through the coastal rocks from Vlorë up to Sarandë (see the map). The authors of the Flora differentiate the two species by presence or absence of hairs, the more or less woody basis of the stem and some fruit characters which are known to be very variable. *B. oleracea*, of course, occurs in cultivation also. In the elder Flora of Albania by DEMIRI (1983, p. 220), both species are differentiated as follows:

B. oleracea: leaves glabrous or sparsely hairy, up to 1.20m tall, **cultivated**
B. incana: basal leaves with hairs, upper part of the plant glabrous, about 1.00m tall, perennial, **wild**

In April 1994, L. XHUVELI detected one small population of wild *Brassica* plants from the *B. oleracea* group through the rocks, 10-40m far from the coast of Adriatic sea, 6km South of Vlorë (fig. 1). This population contains some dozens of plants. Many of them were in different development phases, i.e. some elder were vegetative, without flowers or pods, other with flowers and further plants showed half mature pods. There was collected a small seed sample and part of it was brought to the Gatersleben genebank. Three of them germinated immediately and gave completely glabrous plants (fig. 2, 3). Because these plants are very young, there can not be given a definite classification of this accession. Perhaps it might be a stabilized introgression between wild and cultivated forms because up to now, the presence of more than one wild *Brassica* species in Albania is neglected in the international literature. But this material might be of importance and great interest because of the northern frontier of distribution is corresponding with related populations of *B. incana* in Italy and postulated introgressions there (see SNOGERUP et al. 1990).

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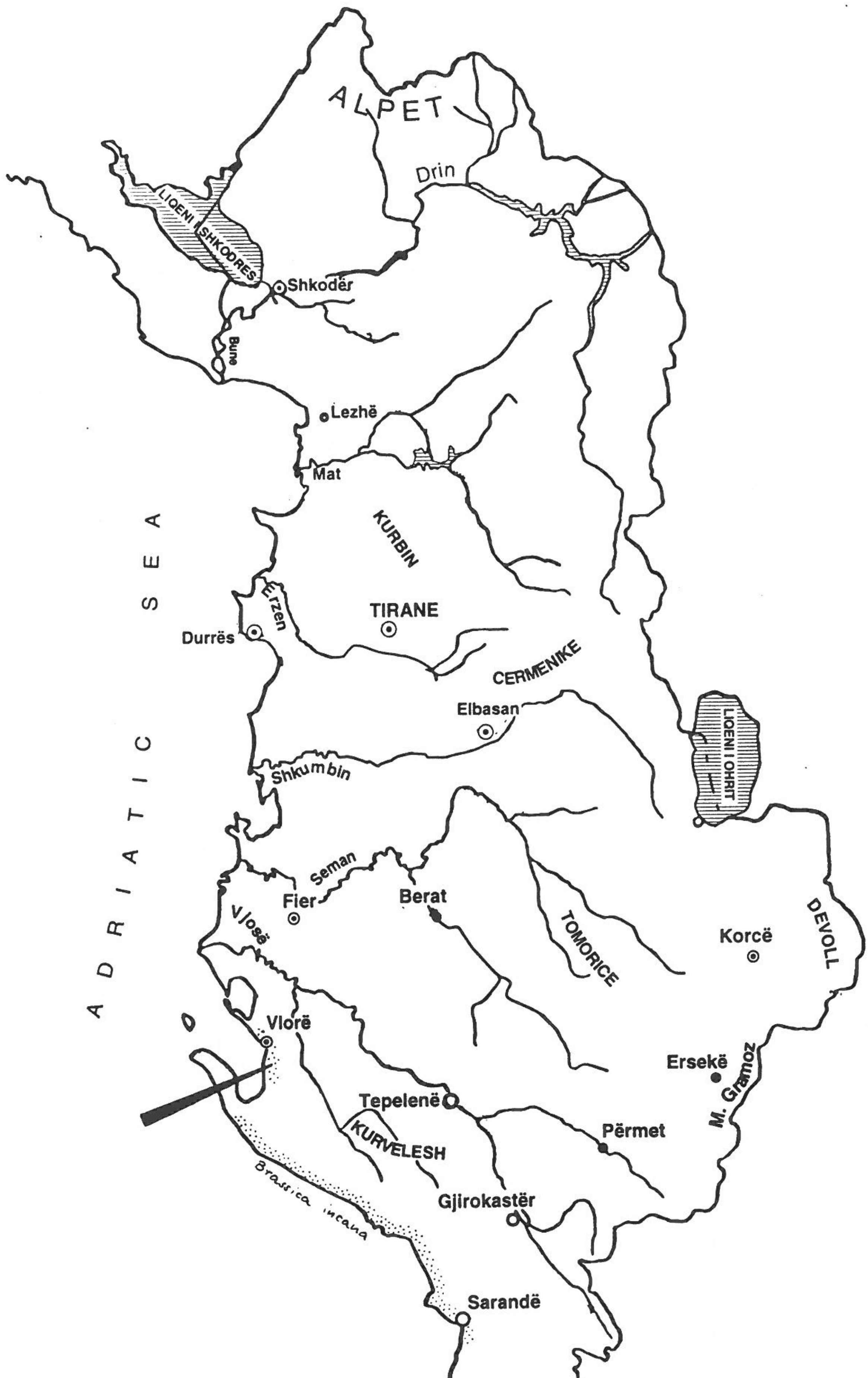

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Elbasan
LIQENI I OHRIT
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Berat
Vjosë
TOMORICE
Korcë
DEVOLL
Vlorë
Ersekë
M. Gramoz
Tepelenë
KURVELESH
Përmet
Brassica incana
Gjirokastër
Sarandë

EVALUATION OF WILD ALLIES OF BRASSICA UNDER NATURAL CONDITIONS

S.K.GUPTA, T.R.SHARMA AND H.S.CHIB
Dryland Research Station, Dhiansar, Jammu-181133, INDIA

In crop improvement, it is important to expand the genetic resources by selective introgression of alien gene in a good agronomic base (Inomata, 1995). If the existing variability in the breeding material is limited, then it becomes necessary to evaluate allies of Brassica under natural conditions. Keeping in view the importance of wild germplasm, 15 different wild allies of Brassica were grown during Rabi 1994-95 at SKUAST, Dryland Research Station, Dhiansar Bari Brahmana, Jammu to identify the sources possessing resistance against various diseases viz. Alternaria blight, White rust, Downey and Powdery mildew in the available wild germplasm. The observation recorded on various morphological and physiological traits indicated a lot of variation in the different species with special reference to growth habit, plant type, branching pattern, pod size, beak length, flower colour, sepal area, petal area, leaf area and maturity period.

Secondly, all these species were also evaluated for resistance against the above mentioned diseases. It was also observed that three species of Diplotaxis viz. erucoides, siifolia and virgata have been found to possess resistance against Alternaria blight, White rust, Downey and Powder mildew whereas other species namely B. spinescens, D. muralis, D. vescaria, D. desnottesi, B. teunifolia and H. incana found to have moderate resistance (Table 1). Although, the above study is preliminary one yet it provides lots of information to the breeders who are actively engaged in Brassica research. It is also worthwhile to mention that all these species are being studied thoroughly under field and laboratory conditions to confirm the resistance for Alternaria blight and other important diseases in the available wild germplasm.

Table 1: **Screening of wild allies of Brassica for Ab, Wr, Dm and Pm under natural conditions using 0-5 scale**

Name of wild allies	Origin	Fld*			
		Ab	Wr	Dm	Pm
B. tournefortii Gouan	Australia	3	0	0	0
B. spinescens Pomel	Algeria	3	0	0	0
B. arvensis L.	Morocco	2	0	0	0
H. incana	Kula-Guri	2	0	0	0
D. erucoides DC	India	1	0	0	0
D. vescarica (L) Cav.	Morocco	2	0	0	0
B. maurorum	Spain	3	0	0	0
D. siifolia G. Kunze	Morocco	1	0	0	0
B. desnottesii	-	2	0	0	0
B. fruticulasa cyrillo	Madrid Spain	5	0	0	0

B. tenuifolia (L) DC	-	2	0	0	0
Erucastrum cardaminoides	-	3	0	0	0
D. virgata DC	Madrid Spain	1	0	0	0
D. muralis DC	Spain	2	0	0	0
B. napus (00)	Canada	2	0	0	0

Fld* : Field observation. Score based on scale of 0-5, where 0 = resistance and 5 = dead

Ab = Alternaria blight
Wr = White rust
Dm = Downey mildew, and
Pd = Powdery mildew

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EFFECT OF HYDRAZINE AND EMS ON CHROMOCENTRE FREQUENCY AND MEIOTIC ABERRATIONS IN *RAPHANUS SATIVUS* L.

Nandjee Kumar

Chemical mutagens have widely been used in inducing mutation in several crop plants e.g. *Brassica* (Fowler and Stefansson,1972), Barley (Grover and Tyagi,1980 b), Rice (Roy & Jana,1973) and Wheet (Bhatia and Swaminathan, 1962). Here, the paper reports the study of effect of hydrate-hydrazine (HZ) and ethyl methane sulphonate (EMS) on the number and distribution of chromocentres representing pericentric constitutive hetrochromatin in stigmatic papillae cells and meiotic aberrations in *Raphanus sativus* L

In the present study, 100 seeds were treated with different concentrations of HZ 0.01%, 0.10%) and EMS (0.001%, 0.005%) separately for different durations (8,16,24 hrs.). Some seeds were left untreated (Control). The treated seeds along with the control were sown in the experimental plots under identical conditions. The usual squash technique was adopted for cytological studies (Darlington and LaCour, 1960). The chromocentre frequency was studied at interphase stage while meiotic aberrations at metaphase and anaphase stages of cell cycle by scoring 100 cells in each case.

Analysis of variance revealed that the mean chromocentre frequency in HZ and EMS treated plants decreased significantly ($P < 0.05$) in comparison to the control. A considerable variation in the distribution of chromocentres in the resting nuclei was also noted in the treated plants. The percent meiotic aberrations (univalents, multivalents, bridges and laggards) increased significantly ($P < 0.05$) with increasing concentrations of HZ and EMS. Similar effect has earlier been reported on treatment with Vincristine (Panicker and Dayal, 1985), γ-rays (mehta and Dayal, 1986) and Colchicine (Ahmad and Dayal, 1987).

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NATURAL FATTY ACID VARIATION IN THE GENUS *BRASSICA* AND ITS EXPLOITATION THROUGH RESYNTHESIS

W. LÜHS and W. FRIEDT, Institute of Crop Science and Plant Breeding, Justus-Liebig-University, Ludwigstr. 23, D-35390 Giessen, Germany

INTRODUCTION

Vegetable oils with one major fatty acid component are important raw materials for various applications in oleochemistry (1). In the course of a breeding program for high-erucic acid rapeseed (HEAR) we have investigated the fatty acid composition of available rapeseed and *Brassica* accessions. Natural variability of oilseed rape (*B. napus*) is limited regarding high percent erucic acid (C22:1), since most of the traditional and newly bred cultivars do not exceed an average of about 50% (2-3).

MATERIAL AND METHODS

Seed samples of accessions of rapeseed and other *Brassica* species were part of own stocks or were purchased at local seed stores. A large number of *Brassica* accessions was gratefully provided by the gene bank section of the *Institut für Pflanzengenetik und Kulturpflanzenforschung* (Gatersleben, Germany). Fatty acid composition was examined following a gas chromatography method as described earlier (3).

RESULTS AND DISCUSSION

Progress in breeding for modified fatty acid composition in *B. napus* using classical breeding methods depends on sufficient variation towards the property of interest. The results of our screening program (cf. Table 1) indicate that oilseed rape is limited in having seed oils with a C22:1 proportion above 60%. The swedes as rapiferous form were found to contain only an average of about 34% C22:1, that makes them unsuitable as a source of variation for increasing the erucic content of oilseed rape.

B. napus is a natural amphidiploid species which has originated from spontaneous crossing between *B. rapa* and *B. oleracea*. Hence, an effective route to increase genetic variability of rapeseed is its resynthesis, i.e., the very rare events which have happened in nature are replayed by experimental hybridization of the original diploid progenitors. Actually, this approach can be successful only if desirable variability really exists in the parental species, so we had to screen *Brassica* accessions for fatty acid composition in their seed oil. The species *B. oleracea* and *B. rapa* are both highly polymorphic, since they include important vegetable, oilseed and fodder crops. A similar degree of variation was also found for high erucic acid content in the seed oil ranging from 30.1 to 61.4% in *B. rapa* and from 28.2 to 63.4% in the *B. oleracea* cytodeme (including also wild relatives), respectively. With regard to the total number of *Brassica* accessions (n=606) analyzed in this study the cauliflower seed samples (n=108) showed the highest proportions of erucic acid ranging from 46.6 to 63.4% with a mean (±S.E.) of 57.9±0.3% (comprehensive data not shown in Table 1).

Consequently, cauliflower and 'Yellow sarson' were the prominent candidates for interspecific crosses regarding our resynthesis experiments described previously (3-4). The latter were interesting as parental *B. rapa* due to their comparatively high erucic content, but also because of an important practical reason. As compared to Chinese cabbages this oleiferous *B. rapa* form more likely was a suitable parent to obtain a high-erucic *B. napus* with oil seeds - and not a newly synthesized vegetable such as 'Hakuran' (Chinese cabbage x cabbage).

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Table 1. Natural variation of major fatty acids (as percent of total fatty acids) in the seed oil of *Brassica napus* and the ancestral diploid species, *B. rapa* and *B. oleracea*

Species	Common name	n	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1	C24:1	Statistics
B. rapa ssp. *oleifera*	turnip rape	12	1.9±0.1	13.7±0.5	12.6±0.3	6.8±0.4	9.0±0.3	50.1±0.7	1.6±0.1	Mean±S.E.
			1.5-2.3	10.1-16.8	10.8-13.9	4.5-9.2	6.9-10.9	46.7-55.4	1.3-1.8	Range
B. rapa ssp. *rapa*	turnip	10	2.3±0.1	13.7±1.2	13.2±0.6	8.1±0.5	8.7±0.5	48.6±2.0	1.5±0.1	Mean±S.E.
			1.9-2.8	9.4-21.6	10.6-17.4	5.8-10.6	5.6-10.7	33.5-56.0	1.2-2.0	Range
B. rapa ssp. *chinensis*	Chinese cabbage (pak-choi)	17	1.7±0.1	14.8±1.2	11.3±0.4	6.7±0.4	7.9±0.3	51.6±1.6	1.7±0.1	Mean±S.E.
			1.4-2.6	9.5-29.2	7.0-15.5	4.3-9.5	4.9-10.7	30.1-60.3	1.1-2.3	Range
B. rapa ssp. *pekinensis*	Chinese cabbage (pe-tsai)	90	1.7±0.1	12.8±0.4	11.1±0.1	7.0±0.1	7.5±0.2	53.5±0.5	2.0±0.1	Mean±S.E.
			1.2-2.5	7.5-31.2	8.0-14.5	4.4-10.2	3.7-12.5	33.0-61.4	1.2-2.8	Range
B. rapa ssp. *trilocularis*	Yellow sarson	15	2.0±0.2	12.5±0.6	10.8±0.5	7.8±0.5	7.5±0.7	53.5±1.2	1.3±0.1	Mean±S.E.
			1.3-3.4	10.0-17.6	7.1-13.2	5.0-10.8	4.1-12.4	45.3-60.4	1.0-1.9	Range
B. rapa ssp. *dichotoma*	Brown sarson/ toria	27	2.0±0.1	12.1±0.5	12.3±0.1	8.8±0.3	8.4±0.2	50.8±0.4	1.2±0.1	Mean±S.E.
			1.4-2.6	6.8-18.4	11.0-13.4	6.2-12.0	6.4-10.3	46.3-54.3	0.7-2.0	Range
B. rapa	**group (total)**	**171**	**1.8±0.1**	**13.0±0.3**	**11.5±0.1**	**7.3±0.1**	**7.9±0.1**	**52.4±0.4**	**1.7±0.1**	**Mean±S.E.**
			1.2-3.4	**6.8-31.2**	**7.0-17.4**	**4.3-12.0**	**3.7-12.5**	**30.1-61.4**	**0.7-2.8**	**Range**
B. oleracea conv. *acephala*	curled kitchen kale/ marrow-stem kale	15	3.4±0.1	14.6±0.8	13.4±0.6	8.7±0.3	9.6±0.4	45.8±1.2	1.2±0.1	Mean±S.E.
			2.1-4.3	10.2-21.1	9.4-17.8	6.7-11.1	7.6-13.8	37.6-55.4	0.8-1.7	Range
B. oleracea conv. *fructicosa*	Brussels sprouts	8	3.9±0.2	14.5±1.1	12.9±0.5	8.4±0.9	8.8±0.6	46.1±1.2	1.6±0.2	Mean±S.E.
			3.0-4.5	9.7-18.2	11.8-15.1	5.5-12.2	6.4-11.3	42.9-53.4	1.1-2.5	Range
B. oleracea conv. *capitata*	cabbage/ Savoy cabbage	43	3.7±0.1	14.2±0.5	12.6±0.3	8.3±0.2	8.7±0.2	47.6±0.6	1.5±0.1	Mean±S.E.
			2.7-4.7	9.4-31.6	8.8-17.3	5.3-12.4	6.0-12.4	28.2-55.0	0.8-2.6	Range
B. oleracea conv. *caulorapa*	kohlrabi	18	3.5±0.1	14.5±0.7	12.6±0.3	8.3±0.4	8.8±0.4	47.9±1.2	1.3±0.1	Mean±S.E.
			2.9-4.0	9.7-20.9	10.5-14.6	5.7-12.4	5.2-10.8	36.5-58.1	0.5-1.9	Range
B. oleracea conv. *botrytis*	cauliflower/ broccoli	158	3.3±0.1	9.9±0.2	11.6±0.1	7.8±0.1	5.4±0.1	56.1±0.3	2.0±0.1	Mean±S.E.
			1.8-5.1	5.8-16.1	7.5-18.0	5.1-12.5	2.8-11.0	43.9-63.4	0.7-3.0	Range
B. oleracea ssp. *oleracea*	(wild kale)	22	3.1±0.1	13.3±0.6	11.5±0.3	6.9±0.3	7.3±0.3	52.2±0.8	2.0±0.1	Mean±S.E.
B. montana, *B. incana*, *B. alboglabra*			2.4-4.0	7.3-18.8	9.5-14.6	4.8-9.2	3.8-10.0	43.4-60.6	1.2-3.0	Range
B. oleracea	**group (total)**	**264**	**3.4±0.1**	**11.6±0.2**	**12.0±0.1**	**7.9±0.1**	**6.6±0.1**	**53.0±0.4**	**1.8±0.1**	**Mean±S.E.**
			1.8-5.1	**5.8-31.6**	**7.5-18.0**	**4.8-12.5**	**2.8-13.8**	**28.2-63.4**	**0.5-3.0**	**Range**
B. napus ssp. *oleifera*	oilseed rape	67	2.8±0.1	12.2±0.2	11.7±0.1	6.8±0.1	9.2±0.2	52.6±0.4	1.1±0.1	Mean±S.E.
			2.3-3.7	7.8-16.0	9.6-13.8	4.8-8.4	6.1-13.9	45.1-59.1	0.5-2.0	Range
B. napus ssp. *rapifera*	swede, rutabaga	11	3.3±0.1	23.2±1.5	13.1±0.4	8.4±0.6	13.7±0.6	34.3±2.4	1.0±0.1	Mean±S.E.
			2.8-4.3	15.3-29.9	11.7-16.4	5.9-10.7	9.9-16.2	25.0-48.6	0.4-1.5	Range
B. napus	**group (total)**	**78**	**2.9±0.1**	**13.7±0.5**	**11.9±0.1**	**7.0±0.1**	**9.8±0.3**	**50.0±0.9**	**1.1±0.1**	**Mean±S.E.**
			2.3-4.3	**7.8-29.9**	**9.6-16.4**	**4.8-10.7**	**6.1-16.2**	**25.0-59.1**	**0.4-2.0**	**Range**

Intergeneric hybrids between *Moricandia arvensis* and *Raphanus sativus*

BANG S. W., Y. KANEKO and Y. MATSUZAWA
Lab. of Plant Breeding, Fac. of Agriculture, Utsunomiya Univ. Utsunomiya 321, Japan.

Introduction

Moricandia arvensis (L.)DC. which is classified as a member of the genus *Moricandia* was reported to be a C_3-C_4 intermediate species (Apel et al.,1978). Intergeneric hybridizations between *M. arvensis* and *Brassica* species were carried out for the introduction of this traits into *Brassica* crops (Apel et al. 1984 , Takahata 1990 a,b).

This work was programmed to produce alloplasmic *R.sativus* with *M.arvensis* cytoplasm in 1993. In this present study, we describe that F_1 hybrids in intergeneric cross *M.arvensis* × *R.sativus* were obtained through ovary culture and that BC_1 plant derivatives were produced by the backcrossing of amphidiploid with *R.sativus* fllowed by ovary culture.

Materials and Methods

M.arvensis (L.)DC. (2n=28, MaMa), one of an accession of Crucifearae genetic stocks in Laboratory of Plant Breeding, Tohuku University, Japan and kindly provided by Prof. Dr. Hinata, and 7 accessions of *R.sativus* L.(2n=18, RR) were grown in vinyl house. Flower buds emasculated one day before anthesis were immediately pollinated with fresh pollen and were bagged. Ovaries were excised 7 days after pollination and sterilized. After washing in sterile distilled water, the ovaries were cultured on MS medium (Murashige & Skoog 1962) containing 500mg/L casein hydrolysate, 3% sucrose and 1.1% agar at 25°C with 16 hours photoperiod.

The hybrid embryos germinating during the ovary culture were subcultured on the White medium (1963) containing 500mg/L casein hydrolysate, 3% sucrose and 0.8% agar at 15°C with 16 hours photoperiod.

The production diagram of F_1 and BC_1 plants between *M.arvensis* and *R.sativus* is shown in Fig. 1.

To determine the number of chromosomes, root tips of hybrid plants were pretreated, fixed and stained with fuchsin, and then chromosome numbers were counted using the 1% acetocarmin squash method. The meiotic observation for PMCs was examined by staining 1% acetic orcein. Pollen fertility was determined using acetocarmin staining method.

Results and Discussion

Table 1 presents the results that the F_1 hybrids in intergeneric cross, *M.arvensis* × *R.sativus* were produced by ovary culture. In this cross, 12 hybrids were obtained from 157 cultured ovaries. F_1 hybrids showed the chromosome paring of $1_{III} + (5\text{-}0)_{II} + (13\text{-}23)_{I}$ and pollen sterility. Amphidiploid hybrids that were induced by colchicine treatment showed 62% bivalent chromosome paring without higher paring and 27 - 92% pollen fertility.

For producing BC_1 plant, amphidiploid hybrid was backcrossed with *R.sativus* followed by ovary culture(Table 2). As a result, 6 BC_1 plants were obtained. Two plants of them were sesquidiploids with 2n=32 suggesting MaRR genome constitusion. Other 4 plants were hyperploid (2n=55) with MaMaRRR genome that seems to arise from the combination of an unreduced BC_1 gamete (n=46, MaMaRR) and a normal *R.sativus* gamete (n=9, R). This phenomenon is known to occur with a relatively high frequency in Brassiceae (Namai 1976 , Ripley and Arnison 1990).

Now, we are producing alloplasmic *R.sativus* with *M.arvensis* cytoplasm by successive backcrossing of BC_1 hybrids (2n=32) with *R.sativus* and new amphidiploid plant.

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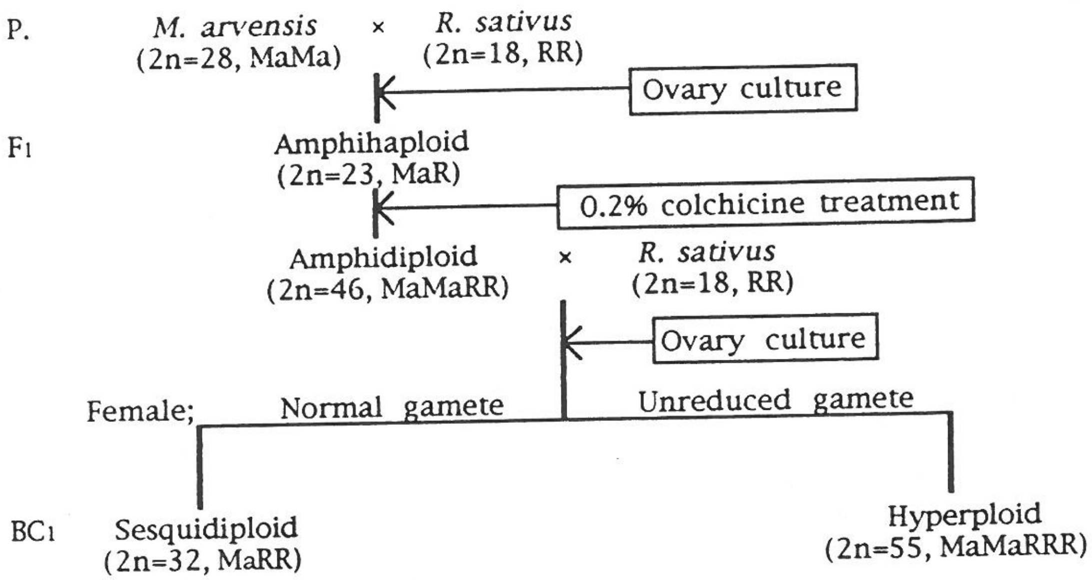

Fig. 1. Schematic diagram showing the formation of F1 and BC1 plants between *M.arvensis* and *R.sativus*

Table 1. Production of F1 hybrids in intergeneric cross, *Moricandia arvensis* × *Raphanus sativus* through ovary culture

Cross combination	No. of ovaries cultured	No. of embryos cultured	No. of F1 hybrid obtained
M.arvensis × *R. sativus* *	157	65	12

* Seven cultivars were used in this cross

Table 2. BC1 plants obtained by backcrossing of F1 hybrid (2n = 46, MaMaRR) with *R. sativus* (2n= 18, RR) through ovary culture, their chromosome number and genome constitution

Cross combination	No. of ovaries cultured	No. of embryos cultured	BC1 plants obtained		
			Numbers	2n	Genomes
F1 hybrid × *R.sativus* *	61	14	2 4	32 55	MaRR MaMaRRR

* Cultivar used in this cross is ' Pink Ball' which was introduced from Korea

Interspecific hybrids of *Brassica maurorum* with *Brassica* crops, and their cytology

J. S. Bijral, T. R. Sharma, B. B. Gupta and Kuldip Singh
SKUAST, Regional Agricultural Research Station
R. S. Pura - 181102 - INDIA

Many of the wild species in the tribe Brassiceae have potential value as donors of useful agronomic traits such as resistance to diseases and insect and nematode pests; cytoplasmic/nuclear male sterility; intermediate C_3 - C_4 photosynthetic activity and tolerance to abiotic (drought, cold, salt, etc.) stresses which could be incorporated into *Brassica* breeding programmes. Possibilities of gene transfer from wild species to *Brassica* crops were investigated in some wide hybrids (Bijral *et al.*, 1991, 1993; Bijral & Sharma, 1994). In order to broaden the gene pool of *Brassica* crops still further, interspecific hybrids of *Brassica juncea* and *Brassica napus* with *Brassica maurorum* Dur. (2n=16), a wild species, were produced and their cytology is reported in the present communication.

The materials used in the present study comprised *B. juncea*, *B. napus* and *B. maurorum.* Bud pollination (about 3 to 4 days prior to flowering) was carried out by conventional method after emasculation of the flower buds of *B. juncea* and *B. napus*. Chromosome pairing at the first meiotic division was examined by 2.0 per cent acetocarmine.

B. juncea X *B. maurorum* hybrids had 26 chromosomes and the mean meiotic chromosome pairing was 9.6 I + 7.2 II (ring + rod) + 0.1 III per meiocyte. Of the bivalents, 92.0% were ring and 8.0% rod configurations. A maximum of 10.0 bivalents (range 5-10) per cell was recorded. *B. napus* X *B. maurorum* hybrids, on the other hand, showed 27 chromosomes and the mean chromosome associations were 9.36 I + 6.8 II (ring + rod) + 0.8 III + 0.1 IV per microsporcyte. Of the bivalents, 87.6% were ring and 12.4% rod configurations. Multivalent associations were recorded in 69.2% of the cells analysed, the most frequent being trivalents (91.7%). The ranges of univalents, bivalents, trivalents and quadrivalents were 6-15, 5-9, 0-2 and 0-1, respectively. Presence of 8 and 6 ring bivalents in most of the cells in the *B. juncea* X *B. maurorum* and *B. napus* X *B. maurorum* hybrids, respectively indicates the existence of partial homology between the genomes of the parent species. Reasonable seed set in the interspecific hybrids further suggests the possibility of transfer of useful gene(s) from *B. maurorum* to *B. juncea* and *B. napus*.

Acknowledgement

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USEFUL END PRODUCTS FROM BRASSICA JUNCEA X B. CARINATA AND BRASSICA JUNCEA X B. CAMPESTRIS CROSSES

R.K. Katiyar and R. Chamola

National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi-110 012,India

Within the family Cruciferae, the tribe Brassiceae is most important from economic point of view, since it includes the genus Brassica with several crop species providing edible roots, stems, leaves, buds, flowers, seeds, and oilseeds. It is also a reservoir of large number of cultivated and wild genera and species providing good sources of biotic and abiotic stresses resistance and other important economic traits(Warwick,1993). Ease in sexual crossability among a few genera and number of species provided the way of crop improvement by polyploidy,recombination and introgression breeding(Namai et al.1980). The recent progress in overcoming the cross incompatability barriers by the application of biotechnology, ovary culture, embryo rescue and protoplast fusion(Hinata and Konno,1979; Kirti et al.,1991; Nand Kumar and Shivanna,1993 and Narasimhulu et al.,1994),has provided an unlimited scope for crop improvement by wide hybridization in crop Brassica.

In India the mustard, Brassica juncea(L)Czern & Coss, is the ruling cultivated oil yielding species, accounting for 70% of the total area under cultivation of rapeseed and mustard crops. The larger area under this species is attributed to comparatively better sustainability. This work presents a report on the exploitation of recombination/introgression for the development of new genotypes of applied value,by interspecific hybridization between B. juncea and B. carinata(Braun) and B. juncea and B. campestris(L) var. yellow sarson(CV. YID-1). Shortcomings of present B. juncea varieties, the useful traits targetted in the new genotype(recombinant) and methods of their development are briefly described under the respective genotypes:

(1) High-yielding, dwarf, compact plant, short duration,large-seeded, extended pod bearing portion and shattering resistant genotype:

In the major Brassica growing regions of India(North and North-West part), the present day cultivated varieties of B. juncea grows over 200 cm tall with only top 30% bearing pods leaving the rest 70% unproductive mass. They are not only prone to lodging and bending but also shade the companion plant in pure crop stands and companion crop in intercroppings thus affecting their photosynthetic activity. The maturity period ranges 135-150 days and average yield is 18-20 quintals per hectare. To rectify the abovesaid undesirable traits of the B. juncea(AABB),it was crossed to B. campestris var. yellow sarson(CV YID-1)(AA). The F_1 was backcrossed to both the parents once to allow more homologous and non-homologous recombinations(Prakash, 1973). The recurrent selection finally resulted a stable genotype with 110 cm height, 110 days maturity, bold grain, 40% fruiting part, 30% harvest index

(24-26 % in cultivated var.) and resistance to lodging and pod shattering. Morphologically, the new plant type closely resembled with *B. juncea*. It was named as selection 165-93, and when tested for yield under normal plant density(20 plants per M^2), yielded 20 quintals as compared to best *B. juncea* var.,19q/Ha. Its performance in trials with increasedplant densities(40 plants/ M^2 or more) in pure and intercropping with matching height companion crops, is being tested. The new plant type is of great importance as in addition to the abovesaid desirable traits, it escapes the critical period of insects and diseases attack, requires less inputs and offer higher yield per unit area and time, fits under the specific situations where moisture is available for shorter period or where crop season is shorter.

(2) Disease resistant genotype:

Alternaria black spot(*A. brassicae*), downy mildew (*Peronospora parasitica*) and white rust(*Albugo candida*) are 3 major diseases in India, inflicting heavy losses(up to 30%) to seed and oil yield and quality. Almost all present var. of *B. juncea* are succeptible to abovesaid diseases and natural inoculum present in the field is enough to damage the crop during pathogen favouring season. Therefore, a *B. carinata* (Braun) line, showing good degree resistance to abovesaid diseases, was crossed to *B. juncea* to combine the resistance in *B. juncea*.Segregating generations were screened for the resistance in the field during the years of natural disease spread, and carried forward as mass during the years of low or no incidence. In F_7 generation a selection showing stable and good degreeof resistance to Alternaria and white rust was isolated. This genotype is tall(195 cm), late maturing(150 d), possesses thicker-dark green and shining leaves. Very small lesions develop on leaves nearing completion of fruiting in this genotype as compared to large lesions with higher frequencies at flowering time in existing *B. juncea* var.Morphologically, the new genotype has greater resemblance with *B. juncea* but change in thickness, colour and serration of leaves and seed colour indicates its recombinant nature.

Besides its late maturity, the seed size is smaller and yield is lower than the existing *B. juncea* varieties. These defects are being rectified by crossing the resistant genotype with high-yielding and early maturing varieties.

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RFLP Mapping in Indian Mustard (*Brassica juncea*)

T. Mohapatra, A. Sharma, A. Upadhyay, V.L. Chopra and R.P. Sharma
NRC on Plant Biotechnology, I.A.R.I., New Delhi-110 012, India

RFLP linkage maps are useful for studying genome organization and tagging genes of agronomic importance. In the genus *Brassica*, linkage maps have been constructed in *B. oleracea* (1, 2), *B. campestris* (3, 4) and in *B. napus* (5). We have initiated molecular mapping in Indian mustard (*B. juncea*), considering its importance as an edible oil source in India. Construction of a partial linkage map of this crop is presently reported.

Materials and Methods

An intervarietal cross of *B. juncea* was generated using cv. Varuna as female and exotic collection BEC 144 as male. By selfing a single F_1 plant the F_2 population was obtained. A total of 48 random F_2 plants were genotyped. DNA was extracted as described by Mohapatra *et al* (6) and restricted with either *Eco* RI, *Hin* dIII or *Eco* RV. Southern blotting, hybridization and washing were carried out as described by Sharma *et al* (7). Thirty four random genomic DNA clones from *Pst* I subgenomic library of mustard cv. Varuna (6) were used as probes. Besides, *cab* 3C cDNA of tomato (8) was also used. Linkage relationship among markers was established at recombination frequency $\leq$ 50% and log likelyhood of odds (LOD) of 3.0 using the computer package, MAPMAKER/EXP. 3.0 (9).

Results and Discussion

All the thirty five probes used to genotype F_2 plants, hybridized to multiple restriction fragments indicating high degree of sequence duplication in the *B. juncea* genome. Due to occurrence of duplicate loci, these probes generated a total of 65 markers. The probe BJG 357 was hyper-polymorphic and revealed eight polymorphic bands in combination with *Hin* dIII. Similarly, *cab* 3C hybridized to more than twenty *Hin* dIII fragments and yielded six polymorphic loci. Thirty six (55-3%) markers were characterized by presence-absence polymorphism and the rest by band to band polymorphism. Predominance of presence-absence polymorphism suggested that differential chromosomal rearrangements, particularly, insertion/deletion events had contributed to genetic differentiation of the parents.

Segregation analysis revealed significant deviation from the expected 1:2:1 or 3:1 ratio for 21% of the markers. This is comparable to that observed in *B. napus* (5). Based on linkage analysis 45 RFLP markers and one seed coat colour marker (designated by the symbol r_1) could be arranged on 14 linkage groups, covering 407.9 cM of the genome (Fig. 1). Cosegregation of markers BJG 472a and 472b suggested tandem duplication of the sequence. There were another five linked pairs of duplicate loci on linkage groups 1, 2, 4, 6 and 7. Presence of linked duplicate loci is known in other *Brassica* spp. Addition of more markers to the map is in progress. A total of 500 genomic DNA clones are available with us in *Pst* I subgenomic library of mustard. Use of these probes will generate a fairly saturated RFLP linkage map that will facilitate characterization of important traits in mustard.

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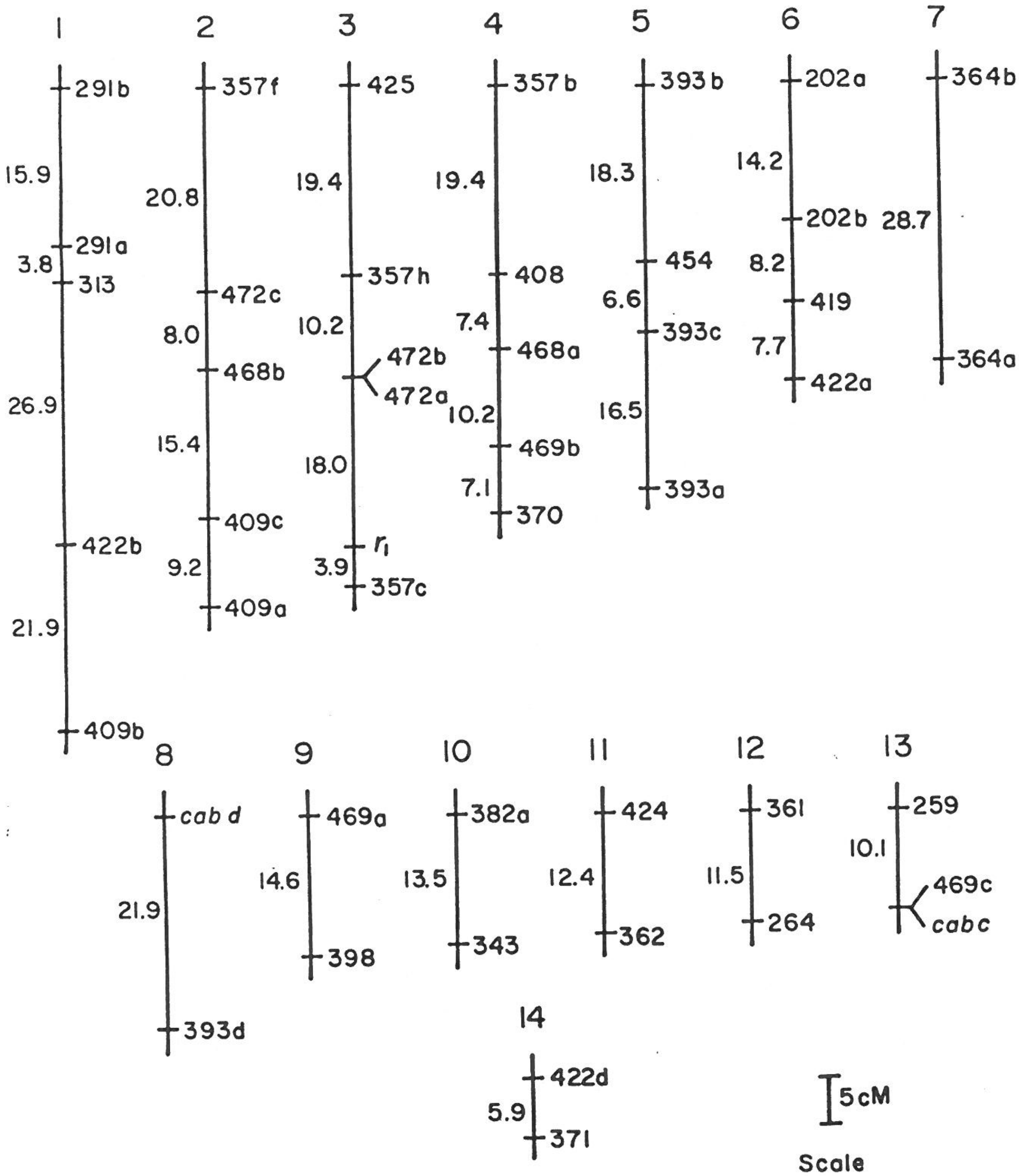

Fig. 1 RFLP linkage map of Indian mustard (*Brassica juncea*)

PLANT REGENERATION FROM MESOPHYLL PROTOPLASTS OF 'RAPID CYCLING' *BRASSICA OLERACEA* WITH 'CHIANG' CYTOPLASM*

T. Cardi[1] & E. D. Earle
Dept. of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853-1902, USA ([1]on leave from Res. Ctr. for Vegetable Breeding, National Research Council (CNR), Via Universita' 133, 80055 Portici, Italy)

Protoplasts can be used to manipulate cytoplasms either by inducing (somaclonal) variation in organelles or by transferring them even between incongruent species. In this note we report the results relative to protoplast culture of 'rapid cycling' (RC) 'Chiang' *B. oleracea* (CrGC # 1-10) and preliminary characterization of regenerated plants.

Protoplasts were isolated and cultured according to Walters and Earle (1) and Hansen and Earle (2), with minor modifications. Regenerated plants and controls were grown under continuous light, 180-220 $\mu E\ m^{-2}\ sec^{-1}$, 24 ^oC and analyzed for plant and floral phenotype, days from planting to flowering, and male fertility. Their nuclear DNA content was estimated following Arumuganathan and Earle (3).

On average, leaves from *in vitro* plantlets yielded 2.6 x 10^7 protoplasts per gram of fresh tissue (Table 1). About 0.15% of protoplasts plated produced calli that could be transferred on regeneration medium. Shoot regeneration was observed in all experiments except one in which, however, a small number of calli was analyzed, and it ranged from 3.0 to 41.5% (9.3% of total calli). The latter value is somewhat lower than that shown by fertile RC *B. oleracea* (2, 4). Previously, a negative effect of 'Ogura' cytoplasm on *in vitro* performance was reported (5).

Table 1 - Data from protoplast culture of 'rapid cycling' *Brassica oleracea* with 'Chiang' cytoplasm

Experiment	Protoplast yield ($x10^7$/g tissue)	Plating efficiency[a] (%)	Shoot regeneration: calli analyzed	Shoot regeneration: calli w/shoots (%)
1	2.2	0.10	99	3 (3.0)
2	2.9	0.06	28	0 (0.0)
3	4.2	0.28	147	6 (4.1)
4	2.3	0.20	122	10 (8.2)
5	1.5	0.19	111	11 (9.9)
6	-	0.09	53	22 (41.5)
Total	2.6 ± 1.0[b]	0.15 ± 0.08[b]	560	52 (9.3)

[a] (no. of calli recovered / no. of protoplasts plated) x 100
[b] mean ± SD

Sixty percent of regenerated plants were diploid, the remaining showing DNA contents equivalent to 4*x* ploidy level (Table 2). Some tetraploids showed a reduced size, but most of them could not be distinguished from diploids. Regenerated plants flowered as those coming from seeds. Some variation for flower morphology was observed in regenerated plants, and one of them showed partial restoration to fertility. *In vitro* culture induced restoration to fertility could be an interesting feature for elucidating the molecular basis of 'Chiang' CMS, as reported in T-CMS maize (6).

Protoplasts from CMS 'Chiang' *oleracea* were also used in donor - recipient fusion experiments with CMS 'Anand' *B. rapa*, and some plants were regenerated. One putative cybrid showed partial restoration of male fertility (4).

* Contribution no. 122 from Res. Ctr. for Vegetable Breeding

Table 2 - DNA content, ploidy level, days from planting to flowering, and male fertility of seed-derived and regenerated 'rapid cycling' *Brassica oleracea* with 'Chiang' cytoplasm

Genotypes	Ploidy level plants analyzed	2x	4x	Flowering plants analyzed	(days ± SD)	Male fertility plants analyzed	sterile	fertile
Seedlings	4	4 (1.51±0.12)[a]	0	3	31.3±0.6	15	15	0
Regenerants	30	18 (1.47±0.12)	12 (3.22±0.26)	6	30.8±1.2	31	30	1[b]

[a] mean DNA content (pg/nucleus ± SD)

[b] partially fertile: some flowers with stainable pollen; very fragile pollen grains

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Shoot Regeneration in the Genotypes of Cauliflower

P.K. Dash, R.P. Sharma and P.A. Kumar

Biotechnology Centre, Indian Agricultural Research Institute, New Delhi - 110 012, India.

Introduction

Cauliflower (*Brassica oleracea* var. Botrytis) is susceptible to two lepidopteran insect pests, *Plutella xylostella* and *Helicoverpa armigera*. Our programme envisages development of transgenic cauliflower expressing truncated and modified *cryIA(b)* gene of *Bacillus thuringiensis* for insect resistance. Towards this objective, we screened ten genotypes (five elite cultivars and five promising plant breeder's lines) of cauliflower for *in vitro* shoot regeneration response. We describe here the regeneration frequency from two different explants viz., hypocotyl and cotyledonary petiole, considered suitable for *Agrobacterium* mediated transformation.

Materials and Methods

Seed was surface sterilized in 0.1% mercuric chloride, rinsed three times with sterile distilled water and germinated on half strength MS medium with 0.8% agar. Six days old seedlings were used to cut hypocotyl (Narasimhulu and Chopra, 1988) and cotyledonary petiole (Moloney *et al.*, 1989) explants. The explants were placed on shoot regeneration medium containing benzyladenine (2 mg/L). Petridishes were incubated at 26°C 16h photoperiod for 6 weeks and scored for number of explants with shoots and average number of shoots per explant.

Results and Discussion

In our preliminary experiments we found that the shoot regeneration response was optimum from the explants prepared from 6d old seedlings. A comparison of the shoot regeneration responses of ten genotypes showed that three of them viz., Pusa Katki, 23000-7 and 41-5 were superior (Table 1). In all the genotypes tested the regeneration response was better from the hypocotyls than from the cotyledonary petioles. Similarly, the number of shoots per explant was more in the case of hypocotyls than cotyledonary petioles (data not shown). For instance, hypocotyls of the cultivar Pusa Katki produce 6-8 shoots per explant while 3-4 shoots arise from a single petiole. Both these systems were shown to be conducive for genetic transformaton mediated by *A. tumefaciens* in *Brassica* species (De Block *et al.*, 1989; Moloney *et al.*, 1989). Currently, efforts are underway in our laboratory to transform cv. Pusa Katki by *A. tumefaciens* GV 3850 (carrying a binary vector with modified *cryIA(b)* gene).

Table 1. *In vitro* shoot regeneration from hypocotyl and cotyledonary petiole explants of cauliflower genotypes

Shoot Regeneration %						
Cultivar	Hypocotyl	Petiole		Breeder's line	Hypocotyl	Petiole
1. Pusa Deepali	60	45	1.	23000-7	80	70
2. Pusa Katki	70	65	2.	98-4	50	30
3. Pusa Snowball	30	25	3.	41-5	50	20
4. Pusa Subhra	20	20	4.	KT-5	40	20
5. Early Kuari	25	22	5.	3-5-1-1	20	15

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General medium supporting shoot regeneration in Brassica species

Pushpa Kharb and J.B. Chowdhury
Department of Genetics, Haryana Agricultural University, Hisar, INDIA.

Introduction

Development of suitable plant regeneration protocols is pre-requisite for the success of in vitro manipulations for crop improvement. The success depends on critical factors such as genotype, hormonal constitution of the medium, source of explant etc. In the present study, the morphogenetic response of three diploid Brassica species (B. campestris, B. nigra; and B. oleracea) and three tetraploid species (B. juncea, B. napus and B. carinata) was observed under similar culture conditions.

Materials and Methods

Seed of all the above mentioned six Brassica species was obtained from the oilseed section, Department of Plant Breeding, Haryana Agricultural University, Hisar. The seeds were germinated aseptically and cotyledonary explants were excised from 6-days old seedlings. The explants were put on modified MS basal medium supplemented with 0.2 mg/l NAA and 2.0 mg/l BAP. About 60 explants of each species were used. The cultures were maintained at 25±1°C and provided 16 hr photoperiod of about 5000 lux intensity.

Results

Data on percentage of explants forming callus, roots and shoots are presented in the Table 1 below. Shoots alongwith roots were regenerated at the cut ends of explants after two weeks of culturing in all the six species. The morphogenetic response of different Brassica species varied considerably, maximum being that of B. oleracea (50 o/o) while B. nigra gave the least response (5 o/o). Fairly good shoot regeneration frequency could be achieved in B. napus (28.3 o/o) and B. juncea (25 o/o). B. carinata and B. campestris showed moderate shoot morphogenesis response (14.7 o/o and 8.7 o/o, respectively).

Discussion

The morphogenetic response of all the six Brassica species using cotyledonary explants has been studied by Narasimhulu and Chopra (1988) using MS basal and B5 media supplemented with various concentrations and combinations of growth regulators. On the basis of their study they established that regeneration in Brassica is governed by two sets of determinants: genetic and environmental. Genotype specific response of Brassica has been observed by other workers also by using some of the Brassica species or even cultivars of the same species (Murata and Orton, 1987; Jain **et al.**, 1988; Yadav **et al.**, 1991; Khehra and Mathias, 1992; Julliard **et al.**, 1992). In the present study, species specific response on the tested medium has been observed. This medium could support shoot regeneration in all the six Brassica species with shoot regeneration frequency not observed earlier on a single medium. This makes the study interesting as only a single medium is required for all the six Brassica species using cotyledons as explants. Cotyledonary explants have been successfully used to get salt tolerant plants (Jain et al., 1986) or Agrobacterium mediated transformations in Brassica (Mukhopadhyay et al., 1992). As shoot regeneration has been achieved after just two weeks, the present medium can be efficiently used for such in vitro techniques.

Table 1. Morphogenetic response of six *Brassica* species on modified MS basal medium supplemented with 0.2 mg/l NAA and 2.0 mg/l BAP

Species	o/o Explants forming			
	Calli	Roots	Shoots	# of shoots/ responding explant
B. campestris	48.9	42.5	8.7	1-4
B. nigra	30.0	0.0	5.0	1-2
B. oleracea	25.0	20.0	50.0	4-6
B. juncea	0.0	75.0	25.0	2-5
B. napus	34.3	19.4	28.3	1-6
B. carinata	17.6	0.0	14.7	1-2

Modified MS basal was obtained by omitting pyridoxine HCl and nicotinic acid from MS basal and adding 0.4 mg/l Thiamine-HCl.

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Induction, growth and development of somatic embryos from hypocotyl explant in mustard (Brassica juncea L. Czern & Coss).

Anita Kumari[1], G.S. Cheema[2], S.K. Munshi[1] and H.S. Dhaliwal[2]

Morphogenesis through somatic embryogenic pattern that results in production of discrete bipolar entities capable of growth and development like zygotic embryos is of immense practical importance for its efficiency in cloning for developmental studies, genetic manipulations and synthetic seeds. Efficient plantlet regeneration through somatic embryogenesis from hypocotyl explants has been reported in B. napus (Glimelius, 1984), B. nigra (Dietert et al., 1982) and B. juncea (Kirti and Chopra, 1989). However, we have induced somatic embryogenesis from hypocotyl explants of commercial B. juncea cv RLM 198 with much simpler system, which is effective and maintained for more than a year by regular subculturing.

One centimeter hypocotyl explant from 7 days old seedlings raised on modified Murashige and Skoog (1962) medium (HMS) were planted on modified MS (BMS) supplemented with 0.25 to 1.0 mg/l,2,4-diphenoxy acetic acid (2,4-D), 0.1 to 1.0 mg/l α-naphthalene acetic acid (NA), 0.25 to 2.0 mg/l benzyl aminopurine (BAP) singly and in combinations. The cultures were incubated at $25 \pm 3°C$ with 16/8 h light/dark regime.

Morphogenic expression revealed four different patterns emerging in cultures with time: callus, roots, shoots and embryos. In a particular combination of 2,4-D and BAP, 60-75% of the explants could be repeatedly induced to form somatic embryos. The embryogenic patterns were visibly evident in about 4 weeks of culture (Fig.1). The explant in control only showed scanty, whitish green callus restricted to the basal end. On the other hand, the embryogenic patterns were associated with none to scanty yellowish tissue mass very distinct from general callus. A definite polarity was again evident in such an organization.Some of the globular to cotyledonary stage embryos show greening and reared to complete plant development. The SEM studies of discretely organized embryogenic mass have shown many distinctly bipolar somatic embryoids in clustered orientation in different stages of growth. These features were further confirmed by TEM studies.

Our procedure relies, indeed,on developmental programming of cultured explants rather than using specific chemicals like zeatin (Jain et al., 1988) or BAP-ribozide (Kirti and Chopra, 1989). In primary culture, the developing somatic embryos show growth and greening upto 8 weeks after which they tend to undergo senescence. However, once isolated from the primary culture these could be maintained as repetitive somatic embryogenic cultures by regular subculture every 3-4 weeks for more than a year.

The biochemical analysis of somatic embryos raised on BMS medium showed that these contain wax and storage lipids as triacylglycerol (Table 1), sterols and partial glycerides, as visualized by TLC little less than zygotic embryo but significantly higher in comparison to general callus.

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[1] Department of Biochemistry [2] Biotechnology Centre, Punjab Agric.Univ. Ludhiana, India

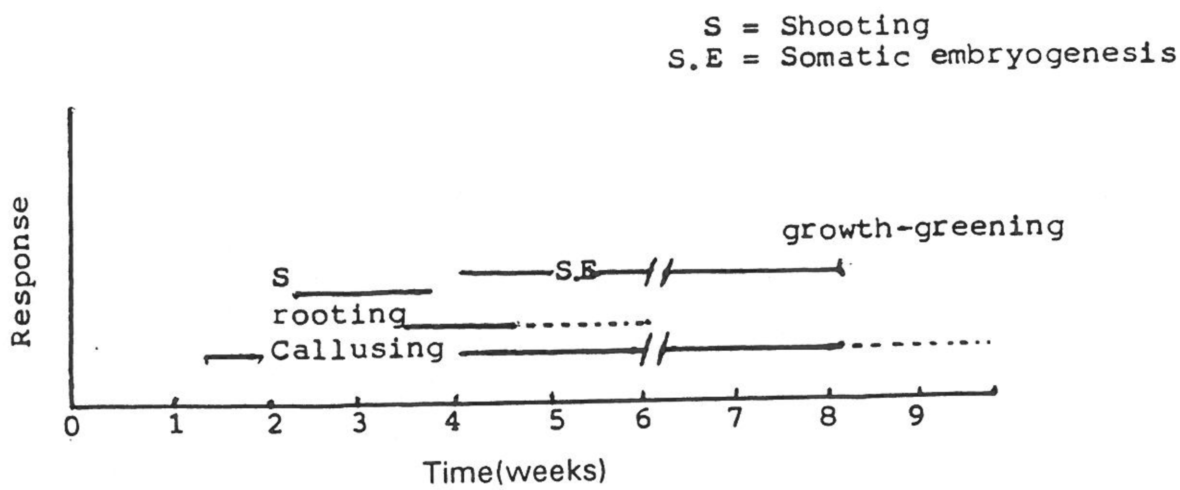

Fig.1. Time sequence of morphogenic expression in Brassica juncea cv RLM 198, excised hypocotyls cultured in vitro

Table 1. Biochemical parameters of somatic embryos on BMS in comparison to those of zygotic embryos in seed and general tissue mass

Parameters	General callus	Somatic embryo	Zygotic embryo
Dry matter(g/100g FW)	5.39	12.37	14.30
Lipid content			
(g/100g FW)	0.39	1.06	1.26
(g/100g DW)	4.64	8.57	18.00
Triglycerides by t.l.c.	+	+ + +	+ + + +

This system of repetitive somatic embryogenesis is being utilized for studies on lipid accumulation as influenced by certain physical (temperature, light/dark regime and polyethylene glycol-6000), metabolic (sucrose) and chemical (abscisic acid) factors.

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The interaction of genotype and growth conditions effects anther culture response of inbred swede lines

J.E.Middlefell-Williams
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Introduction:
Anther culture is a powerful tool for the genetic improvement of a number of brassicas (Keller, 1984). For example, the time taken to produce homozygous lines from F1 hybrids can be greatly reduced and would prove invaluable in the study of the fundamental genetical basis of heterosis. There have been no previous reports on the successful anther culture of *Brassica napus* L. subspecies *rapifera*.
It is well established that many factors influence embryogenesis . Preliminary experiments using seven inbred swede lines had indicated that a number of these factors were critical (results in preparation). Consequently, a series of replicated experiments were set up to test the effect of genotype, growth conditions of the donor plants, culture conditions, and media on two heterotic swede F1 hybrids between Bangholm Magres x Criffel (BMCR) and cv. Marian x Criffel (MC). A recognised highly responsive line of oilseed rape, *B.napus* L. subspecies *oleifera*, cv. Ariana, was used as a control.

Methods and Materials:
Donor plants were grown under two different environmental regimes, either 18 °c with continuous daylight at 240 µmol m^{-2} s^{-1}, or 15°c with a 16 hour photoperiod and a night temperature of 10 °c. The plants were fed twice weekly with Hoaglands solution. The donor plants were maintained in optimum condition and bud sampling was initiated at the onset of flowering. Buds from both apical and lateral inflorescences were collected, approximately 3 - 4·9 mm. long. Microspore viability and stage of maturity was assessed daily . Analysis of the results showed a range of pollen fertility from 11-86% (mean 50·2%) and maturity from uninucleate to the onset of the 2nd nuclear division. Analysis of the results indicated that pollen fertility had no significant effect on androgenesis. However stage of development was found to be vital, with mid to late uninucleate stage being most responsive. A total of 84 buds per treatment were sterilized using 10% Chloros for 5 mins followed by three washes in sterile water. Three buds per 55mm petri-dish were plated on Keller's (1984) modified B5 medium using two different physical support systems, either solid or solid/liquid bilayer. There were 28 plates per replicate, set up in four randomised blocks. All cultures were maintained at 35°c for 24 hours, then 30°c for either 14 or 6 days in the dark, prior to culture at 25°c in a 16 hour photoperiod. Embryos began to appear after the 15th day. The number of embryoids per bud and anther was assessed and the data was analysed using GENSTAT 5 analysis of variance, with the residuals plotted against fitted values to check the assumptions.

Results

Following data analysis it was found that there were no significant differences attributable to the growth conditions of the donor plants from any line, but significant differences between media type.

It was found that embryos and plants were readily regenerated from *B.napus* cv. Ariana and regenerated at acceptable levels from the inbred lines. BMCR was found to be more responsive than MC, and in all cases the bilayer medium producing a significantly greater number of embryos than the solid (P=<0.1%). No statistical differences found either between the two genotypes or from any other conditions. It was found that embryos derived from the bilayer medium were easily regenerated into phenotypically normal plants. For the inbred lines 60% of the resultant plants were diploid, 38% haploid, with isolated occurrences of plants with other ploidy levels.

This is the first report of embryogenesis using F1 hybrids of *B.napus* L *ssp. oleifera*. This resultant material will not only be invaluable in the study of the fundamental genetical basis of heterosis, but will contribute to progress in improving yields, quality and pest resistance in swede.

Table 1. Total number of embryos produced from each individual treatments (84 buds per treatment)

	continuous light		16 hour daylength		
	solid	bilayer	solid	bilayer	total
Ariana	662	2026	1066	1895	5649
BMCR	13	292	14	264	583
MC	1	107	0	150	258

Table 2. Mean number of embryos per bud and anther, data compiled from all treatments and adjusted to account for contamination losses (approx 15% overall)

	Ariana	BMCR	MC
no.embryos/bud	11·62	1·21	0·54
no.embryos/anther	1·94	0·20	0.09

Table 3. Compiled mean embryo yield from solid and bilayer media

	Ariana	BMCR	MC
solid	864	13·5	0·5
bilayer	1960	228·0	128·0

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EFFICIENT *IN VITRO* REGENERATION SYSTEMS FOR HIGH ERUCIC ACID RAPESEED CULTIVARS

Steve Millam, Ashley L. Craig and Susan M. Mitchell
Crop Genetics Dept., Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Erucic acid (C22:1) is synthesised by elongation of oleoyl-CoA via eicosenoate and is very stable at high temperatures with high fire and smoke points and high lubricity. Products of erucic acid have a number of current or proposed applications including, as a slip agent, lubricants and a range of useful derivatives. There is great interest in improving the currently available HEAR germplasm, including the development of lines with useful 'added-value' by-products.

Methods based on plant tissue culture techniques have been used successfully for the improvement of a range of Brassicas previously, at SCRI and elsewhere, but not HEAR lines to date. Such approaches offer means to genetically modify fatty acid profiles. We report techniques for tissue explant regeneration and for the isolation, culture and regeneration of protoplasts of three high erucic acid lines of oilseed rape. All HEAR lines investigated regenerated plants from tissue explants and from mesophyll protoplasts, with the cultivar 'Arthur' proving particularly responsive.

Tissue explant regeneration

The high-efficiency stem internode regeneration system developed at SCRI for the transformation of rapid-cycling *Brassica oleracea* (Millam *et al.*, 1994) was successfully adapted for the regeneration of shoots of all three HEAR lines. This method used an 8:1 ratio of cytokinin (BAP) to auxin (NAA) supplement to Murashige and Skoog medium, and produced shoots from internodes at a mean rate of over 4 per explant. It was found, in a separate experiment that, using internodes of the cultivar 'Arthur', shoot regeneration could be obtained using low levels of either a single auxin, a single cytokinin and a combination of both (Table 1). This may have significance in the range of subsequent induced fatty acid variation and suggest that the inherent responsiveness of this line would promote its use as a model for further studies.

Table 1. Shoot regeneration studies in HEAR cultivar 'Arthur'

Medium	Auxin NAA	Cytokinin BAP	Mean no. shoots per internode (n=40)
SM1	0.0	0.0	0
SM2	5.0 μm	0.0	2.60 ± 0.50
SM3	0.0	5.0 μm	2.40 ± 0.36
SM4	5.0 μm	5.0 μm	3.67 ± 0.47

Protoplast isolation, purification and regeneration

Protoplasts were isolated from 12 day old, dark grown, *in vitro* hypocotyl tissue using the methods of Glimelius (1984) and *in vitro* mesophyll tissue by the methods of Loudon, Nelson and Ingram (1989) modified by replacing the percoll purification step with the use of a 21% sucrose solution. The protoplasts were cultured in medium B (Pelletier *et al.*, 1983) at a density of 5000/ml in 50 mm dishes at 25°C in the dark for three days before exposing to light. After 30 days the cells were overlayed onto media TB1, and at the stage of calli development of diameter 1–2 mm onto TB2 for shoot regeneration (Barsby, Yarrow and Shepard, 1986).

Table 2. Summary of protoplast isolation and regeneration of HEAR lines

Cultivar	Source material	Mean no. protoplasts per gram fresh weight	Number of plants regenerated to date
Martina	leaf	$1.78 \pm 0.55 \times 10^6$	21
	hypocotyl	$3.30 \pm 0.89 \times 10^5$	15
Askari	leaf	$1.70 \pm 0.40 \times 10^7$	45
	hypocotyl	$5.80 \pm 2.10 \times 10^5$	12
Arthur	leaf	$4.10 \pm 0.60 \times 10^6$	>120
	hypocotyl	$4.20 \pm 1.10 \times 10^5$	n.a.

Populations of HEAR have been generated from both tissue explants and protoplasts. Preliminary analysis of glasshouse–grown samples have demonstrated interesting changes in fatty acid profiles and they now provide a most useful germplasm source for studies on the mechanisms of somaclonal variation. Prospects for the more widespread cultivation of the HEAR crop remain favourable and our investigations show that the plant is highly amenable to plant tissue culture manipulation and improvement.

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PROTOPLAST ISOLATION AND PRELIMINARY FUSION STUDIES USING *LUNARIA ANNUA*

Steve Millam, Ashley Craig & Irene Tierney[1]
Crop Genetics Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

[1]*Department of Molecular and Life Sciences, University of Abertay, Dundee DD1 1HG, UK*

Lunaria annua is a member of the Cruciferae family and a native of south-east Europe and western Asia. *L. annua* is usually grown as a biennial, but annual lines have been reported (Wellensiek, 1973). There has been recent interest in the seed oil content of this species as a source of industrially useful fatty acids (Millam *et al.*, 1993). The seeds of this species have been shown to contain a unique composition of 90% (n−9)-cis-monounsaturated fatty acids, of which 42% of the total comprises C22:1 erucic acid and 21% the C24:1 nervonic acid, both of which have a number of potentially valuable applications. Furthermore, the species has also been used in studies on the isolation and characterisation of key enzymes catalysing the synthesis of very long chain fatty acids (Fehling and Mukherjee, 1990).

Protoplast isolation and purification from Lunaria annua

Plants were maintained in an environmental growth cabinet under conditions of 24°C/20°C, 16 h light 8 hr dark at 75% relative humidity. Leaves were taken at two-thirds full expansion stage and surface-sterilised. Mesophyll protoplasts of *L. annua* were isolated and purified according to the methods of Craig and Millam (1994). From over twenty separate isolations, a mean yield of protoplasts was $2.86 \pm 1.51 \times 10^6$ per gram fresh weight was calculated. Those protoplasts not used in fusion studies were cultured in media c of Pelletier *et al.* (1983) where low levels of cell division were observed after ten days culture. Protoplasts were also successfully isolated from hypocotyl tissue. *Lunaria* seeds were germinated in compost at 20°C in darkness for the first ten days then in a 16 hr light, 8 hr dark regime for a further week. The seedlings were excised and surface-sterilised. The hypocotyls were cut into 2–3 mm sections and plasmolysed in media A of Glimelius (1984) for 40 mins, after which medium A was replaced by the enzyme solution medium b cited in the same paper. The material was incubated overnight at 20°C on a shaker (60 r.p.m.) in the dark. The protoplast suspension was then filtered through a 50 μm mesh, and 6 mls of CPW16 added. One ml of suspension was removed for counting. The protoplasts were then spun at 900 rpm for 8 mins. The floating band was then removed, diluted with 5 mls of W5 and spun at 800 rpm for 7 mins. The supernatant was then removed and the pellet resuspended in 1 ml of W5. Another count was made at this stage to ascertain the recovery of protoplasts. Mean yields of protoplasts were 3.07×10^6, with a final recovery of 37.5% of the original sample resulted.

Protoplast fusion and regeneration of fusion products

Mesophyll protoplasts of *L. annua* (mean diameter 42.2 μm + 5.2) were found to be significantly larger than those from *B. napus* (mean diameter 28.5 μm + 6.2), and were morphologically distinguishable from *B. napus* protoplasts due to colour and chloroplast configurations. Fusions were set up using a 1:1 ratio of either hypocotyl or mesophyll protoplasts of HEAR lines (see Millam *et al.*, this volume), and mesophyll protoplasts of *Lunaria*. Freshly isolated protoplasts were resuspended in a 0.5 M mannitol:1.5 mM calcium chloride solution and fused using a Braun Bioject 50 apparatus, with conditions of a fusion voltage of 90 V and one 30 microsecond pulse. The fusion mix were cultured in medium B as before, interestingly there was a high proportion of 1:1 fusions (over 80% of the totals) with a correspondingly low ratio of multiple fusions. The fusion material was taken through the protocol as far as a late callus stage but, to date, no shoot regeneration has been observed.

High rates of fusion were also obtained by using PEG (Polyethylene glycol 6000)–calcium chloride mediated fusion, but no cell divisions were observed following these procedures.

Development of randomly amplified polymorphic DNA–based markers (RAPDs) for identification of somatic hybrids

DNA was isolated from all three HEAR cultivars and from *L. annua* using standard miniprep procedures, with an additional phenol:chloroform extraction stage. A range of primers created at SCRI (SC10,13,35,53,64 and 74) were investigated for their suitability as a means of distinguishing between all three HEAR lines, and also *Lunaria*. A portfolio of such markers was assembled (results in preparation), and it is anticipated that these will be used for the early verification of putative somatic hybrid material. We hope to present further findings in due course.

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IN VITRO ANDROGENESIS OF BRASSICA OLERACEA VAR. CAPITATA L.

Svetla Petrova, Galina Antonova
The Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

Anther culture technique provides an efficient method of creating homozygous lines in a very short period in comparison to conventional breeding methods. For practical breeding, it is important that, in anther culture, haploids can be recovered in high frequency from every donor plant.

The tissue culture program to produce haploids in cabbage was started at the Institute in 1991. Aim of our experiments was to examine factors that might influence embryo production from anther culture of cabbage. Many factors may affect the yield of embryos, but the most important are the genotype, the conditions under wich the donor plants are raised, the size of the buds, the temperature and duration of the thermal shock, the medium composition.

The initial phase of cabbage androgenesis research consisted in the screening of numerous genotypes for responsiveness in anther culture. Many cultivars and experimental inbreds were tested. Anther were collected from plants grown in the greenhouse and in the field. Buds of about 2,0-4,0 mm length were collected before the first flower opened. The basic medium used was B_5 modified by Keller and Armstrong with different concentration of 2,4-D, NAA and silver nitrate. We have examined the impact of high temperature treatment in the first 1-2 days of culture.

We discovered several embryoids, sometimes they came out of the same anther. White embryoids appeared directly from the inside of the anther without callus formation. The rate of embryoid induction was very low and occured only in same genotypes. The highest embryo yields were obtained when anthers were isolated from buds with petals about 3/4 length of anthers, concentration of 2,4-D and NAA in the initial medium was 0,1 mg/l and cultures were incubated for 1 day at 35° C and then transfered to 25° C.

The results indicate a great variability in embryogenic response among genotypes. From the results, it could be concluded that genotype and composition of media play important role in androgenesis in cabbage. The main object of experiments in producing haploids via anther culture in cabbage is to increase the frequency of embryoid induction and the development of an efficient embryo to plant system.

Acknowledgment
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APPLICATION OF MICROSPORE CULTURE IN *BRASSICA NAPUS* CROSSES INVOLVING RESYNTHESIZED RAPESEED

S. WEBER, W. LÜHS and W. FRIEDT, Institute of Crop Science and Plant Breeding, Ludwigstr. 23, Justus-Liebig-University, D-35390 Giessen, Germany

INTRODUCTION

The resynthesis of amphidiploid rapeseed (*Brassica napus* L.) is a tentative way to increase the naturally limited genetic variation regarding fatty acid composition. With the aim of increasing the content of erucic acid in rapeseed oil novel genotypes have been created via interspecific hybridization (LÜHS and FRIEDT, 1994a, 1994b). Actually, microspore culture in rapeseed is very well documented and applied routinely in breeding programmes. This paper presents results on genotypic performance *in vitro* of F_1 donor plants originating from *B. napus* crosses between Canadian double low spring cultivars (*'Excel', 'Profit'* and *'Tribute'*) and selected resynthesized high erucic rapeseed lines.

MATERIALS AND METHODS

The F_1 progeny of 10 crosses (five parental combinations and their reciprocals, Tab. 1) were planted in groups of three plants in MITSCHERLICH pots (6.3 liters). Plants were grown under controlled conditions in a growth chamber with 13/7°C (day/night) and 16h light. Isolation and plating of microspores were carried out as described by COVENTRY *et al.* (1988). After four weeks the liquid medium was replaced by NLN-Medium with a decreased sucrose content (6.5%) which allowed storage of embryoids. These were transferred to solid medium (MURASHIGE and SKOOG, 1962), with 3% sucrose and 4g/l Gelrite five to seven weeks after initiation. At this time the rate of embryogenesis was scored. Differentiated shoots were cut, treated with rooting powder and put into soil. Plantlets were sprayed with chinosol-solution to prevent fungal infections. A second shoot was subcultured on MS-Medium (for storage *in vitro*) when embryoids gave rise to more than one shoot. The recovery of plants was scored when the plantlets were transfered to larger pots or subcultured *in vitro*.

RESULTS AND DISCUSSION

The rate of embryogenesis reached an average value of 10.6 embryoids/ml microspore suspension which significantly depended on genotype. The rate ranged from 0 to 26.0 embryoids/ml microspore suspension. Reciprocal effects were detected in three crosses (*'Profit'* x 91a, *'Profit'* x 232a; *Tribute'* x 223a) according to the statement of KELLER *et al.* (1987) that embryogenesis is controlled by genes located in the nucleus as well as by cytoplasmic genes. Additionally we detected a significant influence of the single plant within genotypes (K64, K56, K73, K43, K20), which is probably caused by an asynchrony of flowering as CHUONG *et al.* (1988) concluded. In the present study this effect might also be related to the residual heterozygosity of the parental cultivars, which were used for the production of the F_1.

Table 1: Genotypic performance of different hybrids cultured *in vitro*

Genotype	Code	Embryoid yield (embryoids/ml)	Regeneration (%)	Plants recovered (%) *in vitro*	*in vivo*
'Excel' x 125a	K64	12.5	20.9	-	62.8
125a x *'Excel'* [1]	K90	-	-	-	-
'Excel' x 232a	K56	8.6	10.4	82.5	71.0
232a x *'Excel'*	K31	6.3	18.5	44.0	73.8
'Profit' x 91a	K73	3.5	23.8	84.9	73.6
91a x *'Profit'*	K43	10.4	16.6	73.5	55.7
'Profit' x 232a [2]	K74	5.3		-	-
232a x *'Profit'* [2]	K30	7.8		-	-
'Tribute' x 223a	K20	11.7	15.9	59.0	51.9
223a x *'Tribute'*	K88	6.3	19.1	70.4	49.0
Total		10.3	18.2	71.5	64.5

[1] failed to produce embryoids because of infection
[2] no regeneration was carried out

In comparison to embryogenesis the regeneration of plantlets from embryoids was not influenced by the genotype of the donor-plant. Out of a total of 3,910 cultured embryoids 692 (18.2%) produced shoots. We assume that this low rate is caused by the relatively high density of embryoids in the culture dishes (7 embryoids/50ml MS medium per dish), accompanied by an increased competition for nutrients (data not shown). Hence the regeneration rate was counterbalanced by the remaining embryoids in culture dishes

where some embryoids failed to develop. Further, the high erucic acid content seems not to be a reason for reduced regeneration as described by HACHEY *et al.* (1991), since the resynthesized rapeseed material used in the present study showed a comparatively good regeneration capacity in other experiments (data not shown).
The correlation between the recovering of plants *in vitro* and *in vivo* was weak but it was closer when the extreme genotype K31 aws excluded. Therefore, we assume that subculture *in vitro* (carried out as *in vitro*-storage) underlies similar factors as a recovery *in vivo*. Furthermore, the rates of recovery are not correlated with the embryoid yield and the regeneration of shoots. We conclude that the *in vitro* reaction of undifferentiated tissue and the reaction of plants originating from such tissue is caused by different conditions.
There was no correlation between the rate of embryogenesis and the regeneration of shoots. This result supports the assumption of SIEBEL and PAULS (1988) that embryogenesis and regeneration are controlled by different genetic systems. The genotypes processed in the present study can therefore be classified as shown in Figure 1.

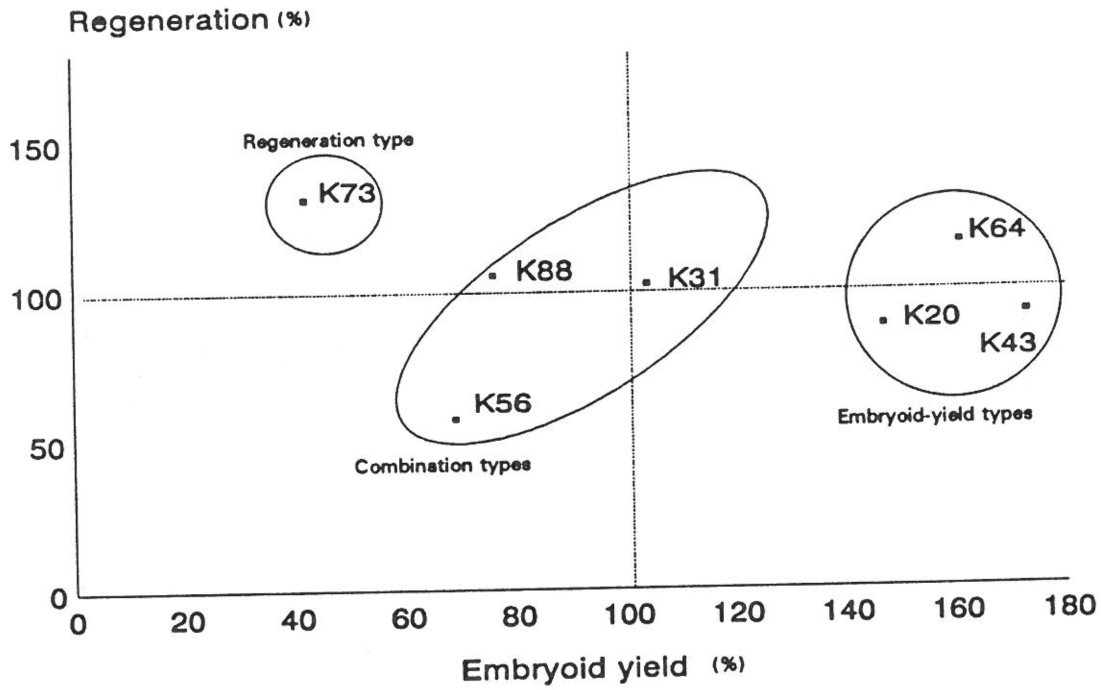

Fig. 1: Distinction of genotypes by embryoid yield (%) and regeneration (%) in relation to means (mean=100%)

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Plant regeneration from root explants of *Matthiola incana* (L.) R. Br. and transformation attempts.

Johannes Siemens, Carsten Köhn, and María Dolores Sacristán
Institute of Applied Genetics, FU Berlin, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany

Introduction

The annual cruciferous ornamental garden plant *Matthiola incana* was studied as an alternative crop due to high linoleic acid content in the seeds (Ecker et al. 1991) and its partial or complete resistance to *Fusarium oxysporum* (Bosland and Williams 1987), *Alternaria* spp. (Davis 1949), and *Plasmodiophora brassicae* (Torres Torres 1972). An obstacle for further studies was the lack of regeneration and transformation protocols for *M. incana*. Regeneration protocols from hypocotyl, leaf and cotyledon explants as well as a micropropagation protocol have been established, but no regeneration of a fertile plant was reported (Gautam et al. 1983, Mizozoe et al. 1992, Mensuali-Sodi et al. 1994). Recently the regeneration of fertile plants starting from protoplasts has been achieved (Siemens and Sacristán 1995). In this communication we report the adaptation of tissue culture methods established for *Arabidopsis thaliana* (Valvekens et al. 1988, Marton and Browse 1991, Czako et al. 1993) to *M. incana*. A protocol for regeneration of fertile plants from root explants of two lines of *M. incana* is described.

Materials and methods

Plant material: The wild type line MSC of *M. incana* and the mutant line MChl carrying a mutation for chlorophyll deficiency were used (Siemens and Sacristán 1995).
Culture conditions: Surface sterilized seeds were germinated on AM medium (Damm and Willmitzer 1991) at 22-26°C and 16 h light (60-80 µmol $m^{-2}s^{-1}$). After 3-8 weeks roots were harvested for root culture or callus induction. The remaining shoots were placed on G1-medium (Sacristán et al. 1989) and further cultivated as shoot culture.
Root culture in liquid media was performed according to Czako et al. (1993) with prolonged initial auxine incubation. Roots were cultivated in a bipartite cycle of ARC supplemented with 0.05 mg/l IAA for 7 d and subsequently in ARC for three weeks.
Callus induction and regeneration was performed according to Valvekens et al. (1988) with modifications. Roots were transferred to callus induction medium (CAIM) for 7 days and then further cultivated on shoot regeneration medium (SHIM) for three weeks. Afterwards the callus was placed on three different *Arabidopsis* shoot regeneration media: SHIM, AIIR (Marton and Browse 1991), and SRM (Damm and Willmitzer 1991). Subsequently the media were renewed every three weeks.
Agrobacterium-mediated transformation of root explants was performed according to Valvekens et al. (1988) with the *A. tumefaciens* strain p35S GUS INT (Vancanneyt et al. 1990). The disarmed Ti-Plasmid contains the neomycin-phosphotransferase gene and the ß-glucuronidase gene under the control of the CaMV 35S promoter. An intron is placed into the GUS-gene to prevent expression in *A. tumefaciens*.
Cultivation of regenerated shoots: Regenerated shoots (2-5 mm length) were cut off and placed on AM. After 10-30 days the shoots were transferred to G1 to promote root development. Rooted shoots were transferred to soil.
Chromosome analysis were made according to Sacristán and Gerdemann-Knoerck (1986).
GUS-assay was performed according to Jefferson (1987).

Results and discussion

More than 97 % of all root explants of young seedlings developed smooth and white-yellowish callus within two weeks after transfer into CAIM, which became green during the next 2-4

weeks of subculture. After 55-65 days on shoot regeneration media regenerating shoots were visible. The regeneration rate of the two lines on the different media is given in table 1. The number of shoots per initial root length could be increased by continuous subcultivation on shoot regeneration media and regular detaching the shoots from callus. Callus development also occurred from root explants of shoot culture plants and from roots growing for 4 months in root culture media, but regeneration of shoots was a rare event in these calli.
Shoots of 2-5 mm length were transferred to hormone-free AM-medium. About 40 % of the shoots developed dark green leaves and could be placed on G1-medium to promote rooting. About 10 % of these shoots developed roots within the next 30 days. The transfer to soil of rooted shoots was performed without loss if an adaptation time for about one week regarding the air humidity was realized. Twelve plants of each MSC and MChl were brought to the greenhouse, where they flowered. Root-tips of these plants were used for chromosomal analysis. All analyzed plants were diploid. No morphological differences with respect to the original lines could be detected. MSC and MChl regenerants were self-fertile and showed with more than 1000 seeds per plant the same seed-set as seed-grown plants.

Table 1: Number of regenerated shoots per root length of the initial transferred explants (cm^{-1}) of two lines of *Matthiola incana* after 80 d of cultivation on different shoot regeneration media. The data are mean values of three independent experiments.

line	Medium		
	SHIM	AIIR	SRM
MSC	0.06	0.75	1.5
MChl	0.11	0.27	0.33

Preliminary transformation experiments of root explants were performed by cocultivation with *Agrobacterium tumefaciens* after 7 days of cultivation in CAIM according to Valvekens et al. (1988). The GUS-gene construct was used for the early detection of transformation events. GUS-assays were performed with root explants 7 days after cocultivation. Transformation events became apparent as GUS-positive cells (blue spots) whose number per initial root length varied from 0 to 0.9 cm^{-1} (mean 0.29 cm^{-1}). Nearly all blue spots consisted on stained single cells, clusters of stained cells were rarely observed. A selection of kanamycin-resistant callus (50 mg/l) failed.

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TRANSFER OF CMS 'ANAND' CYTOPLASM FROM *BRASSICA RAPA* TO *B. OLERACEA* THROUGH PROTOPLAST FUSION*

T. Cardi[1] & E. D. Earle
Dept. of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853-1902, USA ([1]on leave from Res. Ctr. for Vegetable Breeding, National Research Council (CNR), Via Universita' 133, 80055 Portici, Italy)

The production of new CMS types in *Brassica oleracea* is a highly desirable objective in view of their utilization for F_1 hybrid seed production. The 'Anand' cytoplasm was originally discovered in *B. juncea* (1), but recently attributed to the wild species *B. tournefortii* (2). We attempted to transfer it from 'rapid cycling' (RC) *B. rapa* (CrGC # 1-31) to RC *B. oleracea* (CrGC # 3-1).

Isolation, culture, and fusion of protoplasts were carried out according to Hansen and Earle (3) and Walters *et al.* (4), with minor modifications. Donor and recipient protoplasts were treated prior to fusion with 30 krad γ-rays and 3 mM iodoacetate, respectively. Regenerated plants and controls were grown under continuous light, 180-220 $\mu E\ m^{-2}\ sec^{-1}$, 24 ^{o}C and analyzed for plant and floral phenotype, and male fertility. Their nuclear DNA content was estimated according to Arumuganathan and Earle (5).

Pre-treatments of protoplasts inhibited colony formation in parental protoplasts and in their unfused mixture. On the other hand, about 200 putative cy(hy)brids were regenerated after fusion, and a sample of 44 from 26 calli was analyzed further. On the basis of their DNA content, they could be divided in 4 groups with 2*x*, 2*x*-4*x*, 4*x*, and >4*x* ploidy level, respectively (Table 1). About 60% of plants were diploid.

Table 1 - DNA content and ploidy level of seed-derived and regenerated 'rapid cycling' *Brassica oleracea, B. rapa* with 'Anand' cytoplasm, and putative cybrids

Genotype	No. of plants	No. of plants w/ ploidy level 2*x*	2*x* - 4*x*	4*x*	> 4*x*
Seedlings					
B. oleracea	5	5 (1.43±0.14)[a]	0	0	0
B. rapa 'Anand'	5	5 (1.22±0.13)	0	0	0
Regenerants					
B. oleracea	26	21 (1.39±0.12)	0	5 (3.35±0.32)	0
B. o. (+) *B. r.* 'An.'	44	27 (1.51±0.09)	4 (2.06±0.26)	11 (3.31±0.20)	2 (6.77±1.43)

[a] mean DNA content (pg/nucleus±SD)

Plant morphology closely resembled that of the recipient parent. Sixty-four percent of putative cybrids were male sterile with empty anthers, and showed stamens and petals varying in size and shape. The remaining plants were fertile and had normal flowers (Table 2).

Preliminary data from crosses with fertile pollinators indicated maternal inheritance of male sterility and good female fertility in some of the lines, both after hand and insect pollinations in cages (Table 3). No cold temperature chlorosis was seen in male sterile plants, unlike 'Ogura' CMS.

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Table 2 - Male fertility of seed-derived and regenerated 'rapid cycling' *Brassica oleracea, B. rapa* with 'Anand' cytoplasm, and putative cybrids

Genotype	No. of plants analyzed	w/ dehiscent anthers	w/ indehisc. anthers	w/ empty anthers	w/ intermed. phenotype[a]
Seedlings					
B. oleracea	15	15 (100)[b]	0	0	0
B. rapa 'Anand'	15	2 (13)	7 (47)	4 (27)	2 (13)
Regenerants					
B. oleracea	34	32 (94)	0	2 (6)	0
B. o. (+) *B. r.* 'An.'	44	16 (36)	0	28 (64)	0

[a] Plants showing variable pollen production and pollen stainability
[b] Percentages in parentheses

Table 3 - Seed set of diploid CMS cybrids

Flower morphology	Plant number	Seed set: Hand pollination[a] Pod set	Seeds	Insect pollin.n[b]
Good	94-013	60/176	137	good
	94-016	21/33	not counted	good
	94-017	71/128	606[c]	good
Fair	94-014	26/61	not counted	not tested
	94-015	47/128	45	not tested
	94-019	24/73	90	good
	94-020	56/65	557	fair-good
	94-031	4/116	not counted	not tested
	94-041	27/75	not counted	not tested
	94-064	44/81	168	in progress
Poor	94-011	18/41	89	not tested
	94-012	13/32	50	not tested
	94-018	30/33	67	not tested
	94-021	71/111	795	fair-good
	94-030	25/61	125	fair
	94-032	13/162	not counted	very poor
	94-035	24/37	108	fair
	94-036	0/47	0	not tested
	94-052	126/251	746	not tested
	94-055	45/104	307	not tested

[a] With rapid cycling *B. oleracea*
[b] With broccoli (2403, SC). Plants were caged with flies
[c] Sixteen plants grown from these seeds were all CMS

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Studies and application of CHA and its hybrid of winter rapeseed *(B.napus)* in China

C. Y. Guan

Department of Agronomy, Hunan Agricultural University,

Changsha, Hunan 410128, China

CHA (chemical hybridizing agents) can selectively induce only pollen sterility without affecting female fertility. China is one of the earliest country in CHA and hybrid research of rapeseed. An initial research was started by Guan, C. Y. in 1977. The application of first CHA hybrid in China was in 1989. Up to now the area of CHA hybrids in China is about 60 thousant hactars, which account for about 1%the total rape area in China (600 thousant hactars).

I. SCREENING OF CHA

Since 1977 more than 30 kinds of CHA's have been studied by Guan, C. Y. et al.. The main CHA's of inducing male sterility plant more than 80% are listed at Table 1.

Table 1. The CHA of inducing male sterility plant more than 80% in rapeseed.

CHA	Concentration (ppm)	Stage treated	% of male sterility			Female fertility reduction
			CMSP⑥	PMSP⑦	Total	
ZMA①	300	uni.⑤	80.3	8.5	88.8	few
GA3②	4000	uni.	65.6	34.4	100.0	no
SMA③	300	uni.	81.0	6.3	87.3	high
DPX④	15000	uni.	81.1	3.9	85.0	very high

①ZMA— Zinc methyl arsenate; ②GA3— Gibberellic acid; ③SMA—Sodium methyl atsenata; ④DPX— 3-(p-chlorophenyl)-6-methoxy-3-triazine-2, 4(1H, 3H)dione; ⑤uni.— Uninucleate stage(initial bud stage) of rapeseed; ⑥CMSP —Complete male sterility; ⑦PMSP— Partial male sterility.

The mechanism of ZMA inducing male sterility in rapeseed are: the tapetum of CMSP anthers is disintegrated earlier, the anthers of CMSP have only 3 kinds of amino acid(Ala, Asp an Asn), the activity of ensyme in anther is reduced, the respiratory intensity in anthers is weak.

Ⅱ.PRUDUCTION OF CHA'S HYBRID SEED

1. Isolation spot. The production of CHA's hybrid seed must be arranged in islation spot. The isolation spot has not growed the *Brassica* species within the past 4 years. The isolation distance at the plain region must be more than 1000 M away from other *Brassica* species.

2. The row ratio of parents. The row ratio is always 3 or 4 rows (female):2 rows (male). The row width spacing of female parent is about 30 cm and the spacing in the row is about 20-30 cm. The row width spacing and the spacing in the row of pollen is all about 40 cm. The row width spacing between of female perent and pollen parent is about 50-60 cm.

3. Sowing of the parents. The sowing of the parents is usually at same time. In the case of earlier or later of the maturity of parent, the time for sowing must be adjusted. If the flowering date can not synchroniza at field, the branches and flowers can be trimed so as to synchronize flowering.

4. Application of CHA. The female parent is spraied by CHA when 50% bud appear by using the fog sprayer. If application 2 times the second time spraying CHA is after 7-10 days. The pollen parent plant must be covered by plastic sheet during spraying.

5. Artificial supplememtary pollination. The feather broom always is used as a tool of supplememtary pollination. Thefeather broom is first moved on infloresecence of pollen parent, then it is moved on infloresecence of female parent.

6. Harvest of hybrid seeds. During maturity the pollen parent is first harvested and moved out of the filed, then the female parent is harvested and the seeds are threshed. The hybrid seed yield is usually about 450-600 kg/ha.

Ⅲ.MAIN CHA'S HYBRIDS

There are more than 10 CHA's hybrids (*B. napus*) planted with larger area in China. They are Xingzayou 1, Xingyou11×85-110, Zhuza 2, Zhuza 3, 3529×Oro, Yuza 05 and so on. The increase of the seed yield is over 10% compared with recommended conventional rapeseed varitiey.

The studies on some new CHA, new crosses and F_2, F_3 use et al. are carring out now.

The main problem of CHA and hybrids in rapeseed are their stability. Sometimes the female fertility reduction, severe plant toxicit and death occured. It can be explained that genotype/chemical/environmental have not been evaluated clearly.

EFFECT OF PARTHENIUM EXTRACTS ON POLLEN TETRAD AND POLLEN STERILITY IN RADISH (RAPHANUS SATIUS. L)

U.C. Mehta, K.C. Mahato and J.N. Singh
Giridih College, Giridih, Bihar(INDIA)

Out of root crops commonly cultivated in India, radish, the "Cindrella" of the Indian vegetables, is by far one of the most important (Dayal 1985).

Although being one of the most important root crops, no report on the effect of parthenium extracts on pollen tetrad and pollen sterility is available so far. It is known that parthenium alkaloid is highly carcinogenic and causes cancer. We also know that radish is high resistant to physical and chemical mutagen (Gomez-campo and Delgado, 1964, Zoz and Rappoport 1971).

Only two varietal populations of radish namely Bombay Red(BR) and Japanese White(JW) were used in the present investigation, 100-200 air dried seeds were treated with different concentrations (10%, 20%, 30% and 40%) of parthenium alkaloids for 6 hrs, 12 hrs, 24 hrs and 48 hrs. The seeds were sown simultaneously in identical field condition and plants were raised. Methods of cytological analysis are as used earlier (Mehta and Dayal, 1991).

The frequency of abnormal sporoids i.e. monads, diads, triads and pentads was quite high in treated plants in comparison to the control. Some hexads were also found. The frequency of abnormal sporoids was directly proportional to the dose of treatment in both the varieties. Parthenium extracts had a marked effect on pollen sterility in both varieties. The pollen sterility gradually increased with increase in dose of treatment. The effect was more severe at 40% causing very high percentage of sterility (Table I). Our study on the tetrad formation and pollen sterility showed parallelism with those of Lesely and Lesely (1950), Gaul (1958), Ahmad (1978) and Hague and Godward (1984).

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TABLE-I : Tetrad analysis and pollen sterility in two varieties of radish at different concentrations and different hours of treatment.

Materials	Types of pollen sporoids						Total No. of Abnormal Sporoids	Abnormal Sporoids Mean ± SE	Pollen Sterility (%) Mean ± SE
	0	00	00 0	00 00	00 0 00	000 000			
JWK	-	-	-	500	-	-	-	00	5.0 ± 0.50
JW 10% 6 hrs.	4	8	11	467	10	-	33	6.6 ± 1.11	40.0 ± 1.78
JW 20% 12 hrs.	5	10	13	460	12	-	40	8.0 ± 1.21	44.0 ± 1.70
JW 30% 24 hrs.	12	18	25	445	10	-	55	13.0 ± 1.50	47.0 ± 1.62
JW 40% 48 hrs.	15	25	20	420	15	5	80	16.0 ± 1.63	87.0 ± 1.89
BRK	-	-	-	500	-	-	-	0.0	8.0 ± 85
BR 10% 6 hrs.	5	12	22	458	1	2	42	8.4 ± 1.24	44.5 ± 1.75
BR 20 % 12 hrs.	12	17	24	449	8	-	51	10.4 ± 1.36	50.0 ± 1.50
BR 30% 24 hrs.	10	25	15	440	10	-	60	12.0 ± 1.45	45.0 ± 1.58
BR 40% 48 hrs.	11	29	18	430	12	-	70	13.4 ± 1.50	57.5 ± 1.50

Characteristics of male sterility in alloplasmic *Brassica campestris* L.

S. MEKIYANON, Y. KANEKO and Y. MATSUZAWA

Lab. of Plant Breeding, Fac. of Agriculture, Utsunomiya Univ., Utsunomiya 321, Japan.

Introduction

In 1993 and 1994, 6 types of male sterility of petaloid, partial petaloid, antherless, partial antherless, petaloid-antherless and brown anther were derived from the substitution of *Brassica campestris* nuclear genome into *Eruca sativa* cytoplasm (Mekiyanon *et al.* 1994). In 1994, these male sterile plants were studied on morphology and anatomy in nectary development, number of pollen grain, pollen viability, female fertility, chlorophyll deficiency and cyto-histology.

Materials and Method

The scale of nactary development was scored by the method of Leung et al. (1983). Heidenhain's iron hemalum method was applied for cyto-histological study. A little pollen may be produced in some male sterile plants. In this case, number of pollen grain and pollen viability would be observed by the modification of Murakami's method (1992). Male sterile plants were cross with *B. campestris* and number of pollinated flowers, siliques and good seeds were recorded to calculate female fertility as followed: good seeds/pollinated flowers, good seeds/siliques. The leaves of male sterile plants were observed as to chlorophyll content by the method of Melo and Giordano (1994).

Results and Discussion

In nectary development, these male sterile plants showed low and medium index value of development scale when compared with fertile plant (Table 1). From the result of cyto-histological study, the stamens of petaloid type male sterility were completely converted into petal (called petaloid stamen) and normal stamens were not recognized. In antherless type, stamens showed lack of four-loculed anther, whereas brown anther type had anther like fertile one but the breakdown of microsporogenesis occurred during pollen development resulting in pollen abort. In this male sterility, only brown anther type male sterile plants produced some pollens at the end of flowering stage, the number of pollen grains and pollen fertility are shown in Table 2 and Table 3, respectively. Phenotypic expression in brown anther type male sterility was suggested to be more sensitive for thermal condition where high temperature might be effective in producing viable pollens. The pollen produced in brown anther type male sterility has relatively high fertility when compared with fertile plants. The female fertility of these male sterile plants was wide range variation in both of seeds/pollination (3.11~5.44) and good seeds/silique (3.47~6.00). These male sterile plants showed a little chlorophyll deficiency when compared with fertile plants.

Petaloid and antherless type male sterility newly bred in this study showed high potential to develop male sterile line in *Brassica* crop, because they could not produce any pollen throughout the flowering stage. To develop F_1 hybrid seed production in *Brassica* crops, based on these male sterility, selection for high female fertility, no chlorophyll deficiency and complete development of nectary glands are required.

Table 1 The development of nectary glands male sterile plants.

Male sterility	No. of plants observed	Average scale of nectary development *
Petaloid type	20	1.17
Partial petaloid type	5	6.20
Partial antherless type	10	0.70
Brown anther type	5	5.60
Fertile plants	5	9.00

* 0~1 = low development, 3~5 = medium development, 7~9 = high development

Table 2 Number of pollen in brown anther type male sterility when compares with fertile plant

Combination	No. of plants observed	Average number of pollen grains ($\times 10^4$)	Relative percentage
BC_2 of 8-9×Bomb	3	5.11	59.74
BC_2 of 15-5×Solo	3	3.11	36.33
BC_2 of 6-11×Kyomizuna	3	2.67	31.15
BC_2 of 7-12×Taibyo Shin-matsushima	3	2.44	28.54
BC_2 of 11-9×Yukishiro Taisai	3	2.45	28.58
Average	-	3.16	36.92
Fertile plant	3	8.56	100.00

Table 3 Pollen viability of brown anther type male sterility compares with fertile plant

Combination	No. of plants observed	Pollen fertility (%)
BC_2 of 8-9×Bomb	3	90.7
BC_2 of 15-5×Solo	3	25.9
BC_2 of 6-11×Kyomizuna	3	89.1
BC_2 of 7-12×Taibyo Shin-matsushima	3	91.9
BC_2 of 11-9×Yukishiro Taisai	3	92.0
Average	-	77.6
Fertile plant	3	96.8

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Petaloid type MS transferred from *Brassica campestris* into other *Brassica* crops

S. MEKIYANON, Y. KANEKO and Y. MATSUZAWA

Lab. of Plant Breeding, Fac. of Agriculture, Utsunomiya Univ., Utsunomiya 321, Japan.

Introduction

Nowadays, F_1 hybrid seed production in *Brassica* crop as cabbage based on self incompatibility (SI) that is controlled by many *S* alleles which are unstable to environmental factors especially under high temperature and high humidity (Johnson 1972). Therefore, the F_1 hybrid seeds would be the mixture of sib-crossed and successful F_1 seed, depending the altering of environmental condition. Moreover, the overcoming of self incompatibility through bud pollination, which is the less trouble, requires a great amount of work and time (Melo and Leonado 1994) and other techniques are still difficult to do in the simple process. Some types of male sterility were successful in many plant species for F_1 hybrid seed production such as zinnia, carrot, onion etc. (Pearson 1981, Cowen and Edward 1990), due to the high stability and advantage in possible combinations, in contrast to SI system.

In 1993, four types of male sterility of full-petaloid, partial-petaloid, antherless and brown anther type were derived from the substitution of *Brassica campestris* nuclear genome into *Eruca sativa* cytoplasm (Mekiyanon *et al.* 1994). According to the stability for environment, petaloid type male sterility was selected for the male sterile donor for inducing into *B. oleracea* and *B. juncea*.

Materials and Method

In 1993, interspecific hybrid between petaloid type male sterile *B. campestris* with *B. oleracea* and *B. juncea* were produced by ovary culture, when male sterile *B. campestris* was used as female and *B. oleracea* and *B. juncea* as pollen donor. Interspecific hybrid seedlings were transplanted into vermiculite and incubated in 15°C of phytotron for a month. Two weeks after transplant, seedlings were treated with 0.2% colchicine for chromosome doubling. At the flowering stage in 1994, interspecific hybrids were backcrossed with *B. oleracea* and *B. juncea*. In this step, embryo culture was applied to overcome incompatibility barrier between hybrid and male parent of *B. oleracea*. Backcross progenies of *B. juncea* were grown side by side with *B. juncea* to induce following backcross by open pollination. Fig. 1 shows the breeding diagram to transfer petaloid type male sterility of *B. campestris* into *B. oleracea* and *B. juncea*.

Results and Discussion

A large number of interspecific hybrids between petaloid type male sterile *B. campestris* and normal male fertile *B. oleracea* were obtained (data not shown), and moreover 4 interspecific hybrids between *B. juncea* were produced. At the flowering stage in 1994, some plants of interspecific hybrid, both of *B. oleracea* and *B. juncea* as pollen donor, showed complete petaloid type male sterility but some plants could produce some pollen at the edge of petaloid stamen. For this phenomenon, Fu *et al.* (1990) reported that cytoplasmic male sterility could produce a few fertile pollens because some fertile cytoplasm of male parents was transferred to sterile cytoplasm. Therefore, it may be expected that some male sterile progenies in this study which produced pollen at the edge of petaloid stamen may incorporate cytoplasmic factor(s) for fertility from pollen donor. The complete petaloid interspecific hybrids were backcrossed by *B. oleracea* resulting a large number of embryos for culture *in vitro* (data not shown). In this step, embryo culture was successfully applied to make backcross progenies between *B. oleracea*, because interspecific hybrid which carrying a half genome of *B. oleracea* and *B. campestris* (CCAA genome) cannot completely develop embryo, resulting in following embryo abortion (Yamamoto 1994). For *B. oleracea* as pollen donor, 2 backcross progenies were derived. Five open pollinated seeds were derived in backcross by *B. juncea* as pollen donor and 3 seeds were sown in October 1994. Table 1 shows the expected genome constitution and characteristics of both BC_2 progenies.

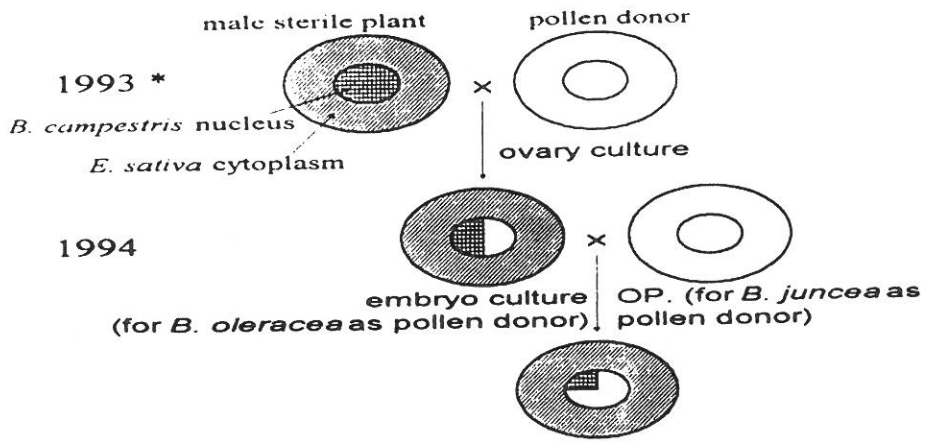

Fig. 1 Breeding diagram to transfer the petaloid type male sterility of alloplasmic *B. campestris* carrying *E. sativa* cytoplasm into *B. oleracea* and *B. juncea*, pollen donor was used in place of *B. oleracea* and *B. juncea*.
* year

Table 1 Expected genome constitution and characteristics of BC_2 progenies of MS *B. campestris* × *B. oleracea* and MS *B. campestris* × *B. juncea*

Combination (BC_2)	No. of derived plants	Plant no.	expected genome constitution	Characteristics
MS *B. campestris* × *B. oleracea*	2	1	ACC	hairy, waxy leaves
		2	ACC	hairless, waxy and dark green leaves
MS *B. campestris* × *B. juncea*	3	1	AAABB	hairy, light green leaves and look like *B. juncea*
		2	AAABB	hairless, light green leaves and look like *B. juncea*
		3	AAABB	hairless, light green leaves and look like *B. juncea*

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Induction of fertility by Chemical Mutagens in *Brassica rapa* L.

S.K. Yadav and N. Kumar

Fertility is a complex character and depends upon a number of factors such as quality of male and female repreductive organs, embryology, S-alleles, cytoplasmic male sterility, chromosomal aberrations and environmental factors (Tilney-Basset,1973). The fertility characteristics such as number of ovules/ovary, number of seeds/silique and FV(%) have well studies in radishes (Dayal, 1975) and *Brassicas* (Kumar *et.al*. 1992). It has been reported that the cause of low fertility in the inbred lines of radish is the abnormolities in micro and magasporogenesis as well as micro and megagametogenesis.

In the present investigation the effect of HZ and EMS on fertilization value in *Brassica rapa* L. has been studied in order to get mutants with enhanced fertility.

MATERIAL AND METHODS :

The material used in the present investigation comprised of cultivated turnip (*Brassica rapa* L.) and two chemical mutagens i.e. hydrazine (NH_2-NH_2) and ethyl methane sulphonate ($CH_3.CH_2.SO_3.CH_3$). The seeds were treated with HZ and EMS solutions of different concentrations for 12 hours. The treated seeds along with their control were sown in the experimental plots in rows at a distance of 12 inches.

In order to study fertility characteristics such as number of ovules, number of seeds and FV(%) 25 plants raised in the experimental plots were selected and analysed carefully. Fertilization value was culculated by dividing the mean number of seeds/silique with the mean number of ovules/ovary usually expressed in precent.

$$FV = \frac{\text{Mean number of seeds/silique}}{\text{Mean number of ovules/ovary}} \times 100$$

RESULTS AND DISCUSSION.

Our results showed that the lower concentrations of HZ and EMS significantly induced the mean number of seeds/silique, number of ovules/ovary and fertilization value (FV%) however higher concentrations of mutagens reduced the number of seeds, ovules and FV. (Table-I).

Earlier studies of fertility in *Raphanus sativus* L. revealed that FV(%) and seed setting are genetically controlled (Dayal *et.al*. 1975,1986).

In the present study, the results clearly indicated that Lower concentrations of hydrazine and ethyl methane sulphonate suitably induce fertility which is due to gene mutation. However higher concertrations of chemical mutagens show harmful effects and reduce fertility.

Department of Botany Magadh University, Bodh-Gaya-824234

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TABLE - 1

Mean number of seeds/silique, mean number of ovules/ovary and fertilization value (FV%) in HZ & EMS treated and control plants of Brassica rapa L.

Concentrations of Chemical mutagens.	Duration of treatment.	No.Of Seeds/ silique.	No. of Ovules/ ovary.	% Fertilization value.
	Hours.	M + SE	M + SE	M + SE.
Control	12	18,6 ± 1.30	22.20 ± 0.64	83.78 ± 0.24
HZ - 0.01	12	24.3 ± 0.33**	25.50 ± 0.76*	95.29 ± 0.37***
HZ - 0.05	12	23.7 ± 2.77**	25.20 ± 1.20*	94.05 ± 0.46***
HZ - 0.10	12	20.9 ± 0.23	24.14 ± 0.65	86.58 ± 0.37
HZ - 0.5	12	20.0 ± 0.58	23.50 ± 0.52	85.11 ± 0.50
HZ - 1.0	12	19.1 ± 0.23	23.10 ± 0.76	82.68 ± 0.54
EMS -0.001	12	24.3 ± 0.86**	26.50 ± 1.25***	91.70 ± 1.38***
EMS -0.005	12	22.1 ± 0.58**	25.00 ± 0.76***	88.40 ± 98***
EMS -0.01	12	20.0 ± 0.45	23.33 ± 0.76	85.73 ± 0.34

* Mean difference from the control at 0.05 P

** Mean difference from the control at 0.01 P

*** Mean difference from the control at 0.001 P

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Attempts to induce haploidy *in vivo* in *Brassica napus* L.

H.J. Yoell and G. Séguin-Swartz
Agriculture and Agri-Food Canada Research Centre, Saskatoon, SK S7N 0X2, Canada

Although the occurrence of spontaneous haploidy in *B. napus* has been reported by several workers (1, 2, 3, 4, 5), and two oilseed rape varieties have been derived from chromosome-doubled spontaneous haploid plants (6, 7), the frequency of spontaneous haploidy is too low for the phenomenon to be of routine use in breeding programmes. Anther and microspore culture are well-developed techniques in *B. napus* for generating haploid plants. However, given that there may be deleterious effects associated with anther culture in *B. napus* (8), it would be of interest to develop a method for inducing haploidy *in vivo*.

We hypothesized that pollination followed by failure of fertilization of the egg cell might induce haploid parthenogenesis, and that a subsequent pollination might produce a normal endosperm without affecting a developing parthenogenetic embryo. Six treatments were compared for their effectiveness in inducing haploid plants: 1) flower emasculation and bagging without pollination; 2) pollination of pistils of emasculated flowers with heat-killed pollen; 3) pollination of pistils of emasculated flowers with fresh pollen followed by ablation of the stigma and style at hourly intervals from 0-6 hours after pollination; 4) pollination of pistils of emasculated flowers with fresh pollen 1-7 days after emasculation (delayed pollination); 5) pollination of pistils of emasculated flowers with pollen irradiated with 100, 200, 300, 400, and 500 x 10^3 cGy γ-irradiation; and 6) pollination of pistils of emasculated flowers with pollen irradiated with 300 x 10^3 cGy γ-irradiation followed by pollination with fresh pollen, 1, 3 and 5 days after the first pollination. The control treatment was pollination of pistils of emasculated flowers with fresh pollen 1 day or less before anthesis.

Erucic acid content, a simply-inherited trait determined by the genotype of the embryo (9), was used as a marker to distinguish maternal from hybrid seeds. Cultivar Westar (<0.5% erucic acid) was used as the female parent and cv. Argentine (42-45% erucic acid) was used as the male parent. Erucic acid content was determined by the half-seed technique (10) modified by J.P. Raney (AAFC, Saskatoon Research Centre). Seedlings from seeds with a low erucic acid content were grown and the ploidy of the plants was assessed with guard cell length measurements (Yoell *et al.* unpublished).

No seed was produced by unpollinated flowers or by flowers pollinated with heat-killed pollen. The stigma and style ablation experiments revealed that pollen germination occurred as early as 30 minutes after pollination and that pollen tubes were present in the ovary 5 hours after pollination. No seed was obtained in treatments involving ablation 0, 1, 2 or 3 hours after pollination, indicating that parthenogenesis was not triggered by pollen germination and growth independent of fertilization. Treatments involving ablation 4, 5, and 6 hours after pollination did not differ significantly from the control in the numbers of haploid plants produced per total number of seeds. Delayed pollination was not effective in enhancing haploidy rates. γ-irradiation reduced the ability of the pollen to germinate and produce a tube *in vivo*, and γ-irradiation with 200 x 10^3 cGy and larger doses reduced the number of ovaries containing pollen tubes by 24 hours after pollination, although pollen tubes in the style and ovary were observed in irradiation treatments of up to 400 x 10^3 cGy γ-irradiation. Pollinating pistils with irradiated pollen had a marked effect on seed set with decreased seed size, increased number of collapsed seeds, and delayed embryo development. In the pollination treatments with pollen irradiated with 100 and 200 x 10^3 cGy γ-irradiation, the number of haploids per total number of seeds exceeded that of the control treatment (11 in 731 and 4 in 72 vs. 28 in 4544 for the control; significant at the 5% and 1% levels, respectively). However, the number of haploids produced per pollination in these treatments was significantly lower than that of the control treatment (11 in 348 and 4 in 358 vs. 28 in 297). Pollination with γ-irradiated pollen followed by delayed pollination did not produce more haploids than the control treatment.

Although only two of the treatments were effective in enriching the population for haploids, this does not entirely invalidate the hypothesis of parthenogenetic induction via independent stimulation of parthenogenesis and fertilization of the polar nuclei. The separation of the two processes in the present experiments was not successful; however, a treatment involving both processes simultaneously could be tested.

In *Solanum tuberosum* (4*x*), pollination with lines of *S. phureja* (2*x*) that produce high frequencies of pollen grains containing single, unreduced (2*x*) sperm nuclei results in the fertilization of the polar nuclei and the parthenogenetic development of the egg cell (11). Pollination of *S. tuberosum* with colchicine-treated pollen of other 2*x Solanum* species and lines which produce low frequencies of pollen grains containing single 2*n* sperm nuclei results in haploidy induction frequencies similar to those produced through pollination with *S. phureja* (12). This system may be difficult to apply directly to *B. napus*, since *Brassica* pollen grains are shed at the trinucleate stage, rather than at the binucleate stage as in *Solanum* species. Unreduced pollen grains are known to occur in the Brassicaceae (13), although they generally contain two sperm nuclei. It is possible that the increased haploidy frequency observed by Luo and Wu (14) after pollination of *B. napus* with *B. rapa* was due to the fertilization of the polar nuclei by unreduced sperm nuclei, combined with either the lack of a second sperm nucleus or the failure of an unreduced sperm nucleus to fertilize the egg cell. It would be of interest to test the haploidy inducing ability of 2*n Brassica* species with high frequencies of pollen grains containing one or two unreduced sperm nuclei. Alternatively, it might be possible to cause the production of pollen grains containing single restitution sperm nuclei by treating flower buds or anthers at the binucleate stage with colchicine.

The comparison of the field performance of doubled haploid lines obtained from anther or microspore culture-derived haploids, ovary culture-derived haploids, and haploids arising *in vivo* will allow breeders to determine the effects of the male versus female cytoplasm and of *in vitro* culture versus *in vivo* development, and to select the most appropriate way of producing homozygous lines.

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YELLOW SEEDED TROMBAY MUSTARD VARIETIES WITH INCREASED OIL AND PROTEIN CONTENT

V.Abraham and Shashi A.Kotwal, Nuclear Agriculture Division,

Bhabha Atomic Research Centre, Trombay, Bombay 4000085, India

Indian mustard with yellow seed coat colour was first developed at this Research Centre (1). Even though seed yield increase is the main objective of any crop improvement programme, characters like higher oil and protein content in the oil cake are equally important in oilseed crops. The main emphasis in our breeding programme was to develop varieties with earliness (2), higher yield, higher oil content (3) and better pest and disease resistance (4,5).

Among the 28 varieties of Trombay mustard (TM- series) developed at this centre, 17 are with yellow seed coat colour and the remaining are black seeded cultures. In general, the yellow seeded mustard has a number of advantages over the black seeded varieties such as early maturity, thinner seed coat resulting in higher oil content, lighter oil colour, more crude proteins, and less crude fibre (6,7). The advantageous attributes of yellow seeded mustard are being exploited in India now. Till the 1993-94 crop season, as on record, these yellow seeded TM-series were utilised in more than 500 crosses, by the major mustard breeding centres in the country mainly to incorporate earliness and yellow seeded characters (8).

The oil content of eight yellow seeded TM-cultures and two black seeded national checks were estimated in three replications, using soxhlet method. The total crude proteins were estimated in the remaining oil cake also in three replications, using nitrogen analyser model Technicon Autoanalyser (Industrial Method NO.334-74, W/B of Technicon).

The data are shown in Table-1. It is clear from the table that in the yellow seeded varieties, oil content was significantly higher, in seven out of the eight cultures and the crude protein in four of them were also higher. Three of these four cultures had both high oil and high protein content whereas the early variety TM-18-8 had the lowest oil content among the selections but with higher protein content. The seven selections, S2-1 to TM-18-8 were developed from the cross TM-4 x Lethbridge, a Canadian variety (3).

The oil cake with higher protein content has the potential to be utilised as a raw material to manufacture protein rich products, if low glucosinolate lines are developed in these yellow seeded cultures.

Table-1 **Oil and crude protein content of Trombay mustard cultures**

culture	Oil content in seeds (%)	crude protein content in oil cake (%)
Varuna	34.56	40.62
Pusa bold	34.20	38.54
TM-4	37.92*	41.40
Selection 2-1	39.75*	43.74*
" 3-3	39.38*	41.01
" 5-13	38.50*	42.96
" 22-1a	38.17*	43.49*
" 25-1	38.17*	42.44
TM-18	38.00*	44.01*
TM-18-8	35.68	43.95*
L.S.D.(at 5% level)	2.42	2.85

* significant at 5 % level

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INHERITANCE OF SEED COAT COLOUR AND ITS USE IN DETERMINING NATURAL CROSS- POLLINATION IN YELLOW SARSON

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

A carmel seed coat colour mutant in yellow sarson (*B. campestris* var. yellow sarson) was isolated in the strain YSIK 4 at 50 KR . It was high yielding, bold seeded with carmel seed coat colour (Chauhan and Kumar,1986).In order to study its inheritance, the mutant was crossed with yellow sarson strain NDYS 1 possessing yellow seed coat colour during 1986-87 and required generations were developed. The parents, F1,F2 ,BC1 and BC2 were grown during 1988-89. The F1 seeds were carmel in colour indicating its dominance over yellow seed colour. The F2 generation segregated into 188 carmel seeded and 45 yellow seeded plants indicating monogenic dominant inheritance for this trait(χ^2 = 4.01 at p = 0.05-0.02). Segregation pattern in BC2 generation also confirmed the mode of inheritance observed in F2 generation. Monogenic dominant gene action of brown seed colour over yellow seed colour has been reported by Singh (1958).

This mutant was used as dominant marker to study extent of natural cross-pollination in this crop. To allow natural cross-pollination, 28 small concentric plots each consisting of one plant of the strain NDYS 1 (yellow seed colour, recessive marker) in the centre and surrounded by carmel seed colour mutant. The percentage of natural cross-pollination was determined in the progenies of open-pollinated yellow seeded plants grown during 1990-91.

The range of natural cross-pollination in the present study varied from 0 % to 6.67 % with an average of 2.79% (Table 1) under eastern U.P. conditions. The natural cross-pollination in this crop has been reported earlier to the extent of 5 to 12 % (Singh,1958).

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Table 1. Percentage of natural cross-pollination in yellow sarson

Test progeny	Number of plants with		Total plants	Per cent cross-pollination
	yellow seeds	carmel seeds		
1	96	02	98	2.04
2	74	04	78	5.12
3	81	03	84	3.57
4	60	02	62	3.22
5	76	01	77	1.29
6	55	02	57	3.51
7	103	03	106	2.83
8	97	02	99	2.02
9	84	04	88	4.54
10	80	05	85	5.88
11	83	02	85	2.35
12	49	02	51	3.92
13	76	03	79	3.79
14	61	00	61	0.00
15	55	01	56	1.78
16	57	03	60	5.00
17	75	01	76	1.31
18	59	02	61	3.27
19	64	00	64	0.00
20	88	01	89	1.12
21	78	01	79	1.26
22	51	02	53	3.77
23	51	00	51	0.00
24	25	01	26	3.84
25	30	00	30	0.00
26	30	02	32	6.25
27	29	00	29	0.00
28	28	02	30	6.67
Total/Average	1795	51	1846	2.76

INVESTIGATIONS ON THE EFFECT OF PARTIAL DEFOLIATION ON ^{14}C-TRANSPORT IN INDIAN MUSTARD

M.L.Chhabra[1], H.N. Krishnamoorthy[2]
Oilseeds Section, CCS HAU Hisar-125 004(INDIA)

While attempting to enchance the seed yield through selection for particular physiological attribute, it would be more helpful if the critical requirement of the source and sink size, translocation efficiency of photoassimilates and compensatory mechanism operating subsequent to defoliation are known prehand in that particular crop. Findings from this laboratory (Chhabra and Dhawan, 1982) revealed, that defoliation to the extent of fity per cent causes no reduction in seed yield in Indian mustard. This speculates high compensatory mechanism in Indian mustard. The present experiment is an attempt to examine the compensatory mechnism operating in translocation of assimilates subsequent to partial defoliation.

The experiment was conducted on 68 day old plants of B. juncea var. RH-30. Six representative plants were selected. Three of these were 75% defoliated while remaining three were kept undefoliated controls.

$^{14}CO_2$ was fed to the middle leaf on main shoot and the incorporation of radioactivity was examined in various components listed in Table-1. It was observed that the assimilates translocated to the inflorescence were about 3-folds higher in 75% defoliated plants compared to undefoliated control plants.

When the middle leaf was fed with $^{14}CO_2$ about 2 folds higher labelled assimilates were translocated to the lower than to the leaf immediately above the fed leaf. This indicates that the lower leaves are serving as "sink leaves". In undefoliated plants, no counts were retained in the leaf immediately above the fed leaf or in the top-3-leaves. The possible reason is that the two very strong sinks namely, the inflorescence and lower leaves, do not allow the assimilates to be retained in these leaves. But, in 75% defoliated plants, when some of the "sink leaves" are plucked, the assimilates otherwise to be translocated to these leaves are diverted towards the upper leaves and the inflorescence. The data further revealed that the per cent of total incorporated assimilates retained by the fed leaf in the undefoliated plants were higher retained by fed leaf of defoliated plants. This seems because of relatively less translocation of assimilates to the leaves above the fed leaf in the undefoliated plant than in the defoliated plant.

Therefore, these observations clearly support the hypothesis that when the foliage is surplus, these surplus leaves block the path of translocation of assimilates to the reproductive sinks.

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2. Prof. & Head, Deptt. of Botany, CCS HAU Hisar (INDIA)

Table 1 : Translocation pattern of ^{14}C-assimilates in partially defoliated and undefoliated plants of *B. juncea* Var. RH-30.

Plant Part	Undefoliated control				75% defoliated			
	cpm/g dry wt.			Incor- poration: % of total	cpm/g dry wt.			Incor- poration: %of total
Tagged Leaf	68251	±	18186	97.5	51128	±	15138	91.9
Leaf below tagged leaf	1435	±	234	2.0	993	±	97	1.8
Leaf above tagged leaf	0	±	-	0.0	546	±	46	1.0
Top-3-leaves	0	±	-	0.0	195	±	38	0.4
Flower-buds and flowers	168	±	32	0.2	2012	±	236	3.6
Total	70061				55590			

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^{14}C-ASSIMILATE TRANSLOCATION IN VARIOUS COMPONENTS OF INDIAN MUSTARD SILIQUA.

M.L. Chhabra[1], H.N. Krishnamoorthy[2]
Oilseeds Section,CCS HAU,Hisar-125 004(India)

Under optimal set of conditions, the source size is never a factor limiting productivity in Indian mustard (B. juncea (L.) Czern & Coss.) whereas translocation efficiency is a factor limiting productivity (Chhabra, 1986). One possible reason of poor translocation efficiency is poor sink strength in Brassica. The siliquae are main reproductive sinks but heitherto the sink strength of various components of siliqua e.g. seed coat, cotyledons, embryo, placenta, replum, pedicel, beak and siliqua wall etc. is unreported. As the sink strength of siliqua in absolute terms is collective contribution of its constitutive components; needs to be examined. The present paper is an attempt in that regard.

The experiment was conducted on B. juncea (L.) Czern & Coss. The plants were simplified by removing all the branches on the main shoot 120 dys after its sowing. $^{14}CO_2$ was fed by inserting middle leaf in assimilation chamber and to it 1 ml. of $NaH^{14}CO_3$ was attached below, in a vial. The chamber was sealed and $^{14}CO_2$ was generated by injecting 2 ml 2N-HCl for 1 hr. After allowing for 20 hours for translocation, various components of siliqua (listed in Table 1) were separated and analysed for ^{14}C soluble sugars. The incorporation of ^{14}C- in various components was as follows :

1) Tagged leaf : The tagged leaf incorporated relatively less counts as the crop was nearing maturity and leaves were senescing (Table 1).

ii) Siliquae and seeds: The siliquae incorporated relatively lower counts than the seeds alone.

iii) Pedicels, beak and siliqua wall : Among these three components of siliqua (which forms its outer morphology), the cpm incorporation was about 5 to 6 folds lower in the siliqua wall than in the pedicel and the beak.

iv) Seed coat, cotyledons and embryo : In these three components of the seed, the assimilates were incorporated in the decreasing order in embryo, cotyledons, seed coat.

The counts incorporated by the embryo were about 5 and 11 times more than the cotyledons and seed coat respectively.

v) Replum and placenta: The counts incorporated by the placenta were about 4 times more than by the replum.

Thus, based upon cpm incorporation in different components of siliqua, it is evident that the embryo and the

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2. Prof. & Head, Deptt. of Botany, CCS HAU Hisar (India)

placenta are two very strong sinks, which favour the movement of assimilates toward the seeds.

One striking observation was that the siliqua wall and seed coat retained the minimum counts. This is because, close to the siliqua wall and seed coat are very strong sinks namely seeds and the embryo respectively, which draw the assimilates very quickly and hence as a result siliqua wall and seed coat retain the minimum counts. In other words, the retaining capacity of assimilates by any component depends primarily upon the strength of the sinks present in the vicinity of the component in question.

Table 1: Translocation pattern of assimilates in various components of siliqua in *B. juncea* Var. RH-30.

Plant part	cpm/g fresh wt.
Pedicel	269 ± 34
Beak	230 ± 12
Siliqua wall	38 ± 4
Seed Coat	64 ± 12
Cotyledons	150 ± 19
Embryo	715 ± 62
Replum	239 ± 13
Placentae	867 ± 48
Siliqua	146 ± 11
Seeds	198 ± 26
Tagged leaf	4517 ± 732

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INHERITANCE OF FLOWER COLOUR AND LEAF WAXINESS IN *BRASSICA CARINATA* A.Br.

S.J.JAMBHULKAR* AND R.N.RAUT**

*Nuclear Agriculture Division, Bhabha Atomic Research Centre, Bombay - 400085
**Division of Genetics, Indian Agricultural Research Institute, New Delhi-110012.

Efforts are being made to introduce *Brassica carinata* popularly known as Ethiopian mustard for cultivation in India as an oilseed crop in view of its resistance to biotic and abiotic stresses. But due to the non adaptability of direct introductions, it was synthesized in India (Sarla and Raut,1988). White flower and waxy leaves were observed in synthesized *B.carinata* . Possible association of such morphological traits with aphid tolerance/resistance could be of great use in resistance breeding. Therefore an attempt was made to study the inheritance pattern of these two characters.

True breeding lines for the contrasting characters viz. yellow vs. white flowers, and waxy vs. glossy leaves were crossed reciprocally . F_1 were planted in single row plot of 5 meter length and large F_2 population were grown. Chi-square test was applied to the observed ratio.

Both the traits in F_1 showed intermediate expression between two parents (Table 1.). This indicated partial dominance of these two traits. The F_2 segregation showed a good fit to 1:2:1. This ratio confirmed monogenic inheritance with incomplete dominance. This is first report on the inheritance of these characters in synthesized *B.carinata*. Whereas flower colour was observed under the control of single dominant gene in *B.hirta* (Mitchell,1988) and *B.juncea* (Angadi *et.al*. 1987), two dominant genes interacting epistatically in *B.juncea* was reported by Rawat and Anand (1986) and Brar *et. al*.(1991).

We observed that white flower colour and leaf waxiness confer tolerance against aphids or are closely associated with resistance to aphids, white rust and *Alternaria blight*.Their simple monogenic inheritance assumes importance in transfering these traits by simple breeding methods. Chattergi and Sengupta (1987) observed that white colour of petals does not attract the alate, while alite have preference to yellow flower. Tewari and Skoropad (1976) concluded that epicuticular wax in rapeseed confers resistance to *Alternaria brassicae* (Berk.) Sacc.Therefore white petals and leaf waxiness could be exploited in breeding programme as a tool to develop resistance against aphids and *Alternaria* respectively.

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Table 1 : F_2 segregation in reciprocal crosses for flower colour and leaf waxiness.

Character	cross	F_1	F_2 segregation			X^2 (1:2:1)	P value
			Yellow	cream	White		
Flower colour	Yellow x White	Cream	135	270	128	0.275	0.90-0.80
	White x Yellow	-do-	31	53	24	0.943	0.70-0.50
			166	323	152	0.649	0.80-0.70
			Waxy	Inter-mediate	Non-waxy		
Leaf waxiness	Waxy x Glossy	Inter-mediate	31	72	38	0.757	0.30-0.50
	Glossy x Waxy	-do-	41	83	38	0.209	0.90-0.80
			72	155	76	0.265	0.90-0.80

Association Analysis for Seedling Characters in *Brassica carinata* A. Braun

N.K. Thakral and Hari Singh
Department of Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, INDIA

Rapeseed and mustard are generally grown in semi-arid and arid regions which receive scant and erratic rainfall and characterised mainly by inherent salinity problems. The ground water of these areas is also of poor quality, and its prolonged use results in salinity problem. But *Brassica carinata* A. Braun, newly introduced species has been reported to perform better than other species under problematic conditions like late sowing, rainfed and saline conditions (Malik, 1990). Salt concentration in the root zone is deterimental for plants, imposing effects on growth stages and ultimately adverse effects on yield (Mass & Hoffman, 1977; Kumar **et al.**, 1981). Therefore, a knowledge with respect to the association of various traits with seed yield would be of immense help in formulating effective and efficient screening programme. Keeping in view the above, the present experiment was devised to investigate the inter-relationship of various seedling parameters in Ethiopian mustard.

The seeds of six parents and their 15 F_1 s were placed on the top of filter paper in petriplates containing 0, 125 and 175 meq/1 chloride dominated salt solutions in three replications. The data on germination was recorded daily to calculate speed of germination as per Maguire (1962). Seedling vigour index was calculated as (Root length + shoot length) x seedlings dry weight.

Seed yield failed to show any kind of (positive or negative) significant correlation with seedling parameters viz; germination per cent, speed of germination, root length, shoot length, seedlings fresh weight, seedlings dry weight and seedling vigour except seedlings fresh weight. On the other hand all these characters had significant positive association with seedling vigour in both the environment except shoot length on normal sown condition. This indicate the importance of these characters from ecological point of view which provides the plants an ability to survive under stress conditions, results in better plant stand and untimately increases the seed yield. Therefore, these characters can be used as selection criteria at early seedling stage as already advocated by Singh and Rana (1989) and Kuhad **et al.** (1989).

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Table 1. Genotypic (G), phenotypic (P) correlation coefficients between seed yield, germination per cent and seedling characters under normal (above diagonal) and saline (below diagonal) environments in Ethiopian mustard

Character		Seed yield	Germination per cent	Speed of germination	Root length	Shoot length	Seedlings fresh weight	Seedlings dry weight	Seedling vigour
Seed yield	G		0.210	0.312	-0.012	0.171	0.518	0.253	0.260
	P		0.157	0.307	-0.014	0.164	0.514*	0.249	0.255
Germination per cent	G	0.128		0.625	0.733	-0.441	0.720	0.746	0.704
	P	0.126		0.512*	0.602*	-0.348	0.582*	0.598*	0.581*
Speed of germination	G	0.203	0.942		0.350	0.346	0.784	0.617	0.745
	P	0.202	0.853*		0.342	0.336	0.777*	0.600*	0.732*
Root length	G	0.457	0.712	0.698		-0.671	0.390	0.515	0.465
	P	0.449*	0.644*	0.691*		-0.654*	0.389	0.507*	0.462*
Shoot length	G	0.380	0.458	0.536	0.620		0.252	0.062	0.209
	P	0.367	0.412	0.525*	0.607*		0.246	0.062	0.213
Seedlings fresh weight	G	0.187	0.606	0.749	0.713	0.558		0.871	0.908
	P	0.185	0.548*	0.745*	0.708*	0.545*		0.862*	0.901*
Seedlings dry weight	G	0.290	0.545	0.688	0.733	0.572	0.964		0.971
	P	0.283	0.482*	0.681*	0.727*	0.553*	0.956*		0.962*
Seedling vigour	G	0.362	0.604	0.719	0.840	0.759	0.925	0.957	
	P	0.358	0.541*	0.714*	0.836*	0.748*	0.921*	0.952*	

* Significant at P = 0.05

Genetic Components of Seed Yield and Oil Content Under Normal and Saline Environments in Indian Mustard

N.K. Thakral and Hari Singh
Department of Plant Breeding, CCS Haryana Agricultural University, Hisar-125004,INDIA

Indian mustard (Brassica juncea (L.) Czern & Coss.) is high yielding and widely grown species in India. A large area of this species is under dryland conditions which have the problem of salinity and alkalinity. Only a little success has been made so far for breeding and selection of varieties especially suitable for soil stresses like salinity and alkalinity (Epstein and Rains, 1987). Information on precise genetic control systems with respect to plant responses to edaphic environment is still limited particularly in rapeseed and mustard and whatsoever is available need confirmation. So, three relatively salt tolerant viz; RH 7859, RH 7846, RH 781 and three susceptible namely RH 8315, RWH 1 and RH 8113 genotypes were selected from the germplasm. These were mated in diallel fashion to derive genetic informations.

The seeds of six parents and their 15 F_1 progenies were grown to maturity in polyethylene lined earthen pots. The pots were saturated to field capacity with salt solution, prepared by dissolving chloride and sulphate salts in the ratio of 7:3. Ten seeds per pot were sown and two uniform plants/pot were retained for further studies. The component analysis was done as per the method of Hayman, 1954.

The additive (D) and dominant (H_1 & H_2) components were significant for seed yield in both the environments. The values of h^2 and F were also significant under stress condition. The estimates of degree of dominance $(H_1/D)^{1/2}$ revealed over dominance consistantly over the environments. The ratio h^2/H_2 was less than unity, indicating possibility of one major gene group for the control of this character. Heritability in narrow sense was low under normal environments and moderate under stress environment.

The genetic parameter D was non-significant, whereas H_1 and H_2 were significant under both the environments for oil content. The estimates of $(H_1/D)^{1/2}$ showed over dominance under both the environments. The positive F values in both the environments indicated preponderance of dominant alleles in the parents. The ratio h^2/H_2 showed one gene group to control this character. Heritability in narrow sense was low in normal and moderate in saline environment.

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Table 1. Estimates of genetic components for seed yield and oil content under two environments in Indian mustard

Components of variation	Seed yield per plant		Oil content	
	E_1	E_2	E_1	E_2
D	10.946* ±4.776	8.743* ±0.513	1.874 ±2.295	2.439 ±1.187
H_1	41.173* ±12.125	10.600* ±1.320	17.694* ±5.806	7.998* ±3.012
H_2	30.199* ±10.831	6.556* ±1.163	16.323* ±5.187	6.102* ±2.691
h^2	0.011 ±7.297	5.281* ±0.783	5.046 ±3.491	6.149* ±1.811
F	18.866 ±11.668	11.312* ±1.253	1.138 ±5.587	2.421 ±2.899
E	0.002 ±1.806	0.001 ±0.194	0.001 ±0.864	0.001 ±0.448
Degree of dominance	1.939	1.101	3.073	1.811
Symmetry of genes	0.183	0.155	0.231	0.191
Proportion of dominance and recessive genes	2.598	3.849	1.220	1.755
Groups of genes exhibiting dominance effects	0.0004	0.806	0.309	1.008
h^2 (n.s.)	0.168	0.310	0.205	0.386
t^2	0.020	0.015	7.182	0.721

* Significant at P = 0.05

E_1 = Normal condition

E_2 = Saline condition

RESPONSE OF CULTIVARS AND HYBRIDS OF WHITE HEAD CABBAGE, BRASSICA OLERACEA VAR. CAPITATA TO DOWNY MILDEW, PERONOSPORA PARASITICA

Galina Antonova, Stoika Masheva

The Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

The response of the pathogen was determined under controlled conditions at cotyledon stage during the last decade of November 1994 with two isolates: PPI-B (from broccoli) and PPI-C (from head cabbage).

The rearing of the pathogens and the conditions of the inoculation were performed after the Williams· method (1985). The cotyledons were spilt with spore suspension with concentration of 10 spores/ml (a drop for each cotyledon). After that the plants were placed into moist cabinet at a temperature of 20° C.

The reading of the symtoms was carried out after 10 days according to the 5-grade scale of Kluszewski (1980) for each plant (0 = symptomless; 4 = abundant sporulation and widespread necroses).

The hybrid Impala F_1 was used as a susceptible control.

The results obtained (Table 1) do not give ground to be determined resistant cultivars and hybrids out of the tested ones. In some of them were selected individual plants as a sourse of resistance to downy mildew.

By type of sporulation the two isolates almost do not distin- guish.

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Table 1. **Response of Brassica oleracea var capitata to downy midew. Perospora parasitica**

Cultivar, hybrid	PPI-C Total number of plants	PPI-C % of plants R	PPI-C % of plants T	PPI-C % of plants S	PPI-C Sporulation	PPI-B Total number of plants	PPI-B % of plants R	PPI-B % of plants T	PPI-B % of plants S	PPI-B Sporulation
Masada F_1	17			100	2	18			100	1
Parel F_1	19			100	3	16			100	3
Atleta F_1	15	7	13	80	3	13		15	85	3
Plana F_1	19		5	95	2	18		6	94	2
Erma F_1	19		5	95	3	14	21	50	29	1
Rinda F_1	15		7	93	3	20		10	90	4
Gideon F_1	17		6	94	0	18			100	0
Fieldsport F_1	18			100	4	20			100	1
Provita F_1	20		5	95	4	20	5	15	80	4
Duncan F_1	17		6	94	3	18			100	4
Palorama F_1	17		6	94	1	19			100	4
Farao F_1	18			100	2	18	6	6	88	2
Fresco F_1	15		13	87	3	16			100	0
Trinity F_1	17			100	1	15			100	0
Cortina F_1	14			100	3	20	5	10	85	0
Impala F_1	19			100	4	18	17		83	0

R - resistant, class = 0
T - tolerant, class = 1-2
S - susceptible, class = 3-4

PATHOGENICITY OF *LEPTOSPHAERIA MACULANS* ISOLATES ON ONE ECOTYPE OF *ARABIDOPSIS THALIANA*.

Hortense BRUN and Michel TRIBODET

INRA - Station de Pathologie Végétale
Domaine de la Motte BP 29 35650 - LE RHEU (FRANCE)

The blackleg fungus [*Leptosphaeria maculans (Desm.)* Ces. et de Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] is the cause of the devastating blackleg disease of oilseed crops (*Brassica napus* and *Brassica campestris*), and infects also other Crucifers. In France, natural infection of *A. thaliana* by *Peronospora parasitica* and *Albugo candida* are frequently observed. Different authors reported on the susceptibility of *A. thaliana* to *Plasmodiophora brassicae* (Koch et *al*, 1991), *Peronospora parasitica* (Koch and Slusarenko, 1990) and *Sclerotinia sclerotiorum* (Dickman and Mitra, 1992), but symptoms of *L. maculans* have never been observed on *A. thaliana*, even in fields showing severe blackleg damage. We report here on inoculation experiments performed under controlled conditions, in order to assess the pathogenicity of different isolates of *L. maculans* on *A. thaliana*.

Thirty-six isolates of the fungus were compared. Fungal strains were isolated from different kinds of symptoms (stem canker, blackspot on leaves, taperoot darkening from prematurely ripening plants) on oilseed rape cultivars Darmor, Bienvenu, Westar, Ceres, and Primor, originating from different parts of France. One isolate was from Canada (Saskatchewan). Twenty-five isolates were obtained from single ascopores. Two isolates have pigmentation and grow very fast on agar medium. All of them were aggressive on cultivar Westar. Seeds of *A. thaliana* (ecotype "WS"; provided by G. Pelletier, INRA Versailles) were grown in sterile soil at 22°C with 16h light in a growth chamber. After developpement of true leaves, three leaves per plant were wounded with a needle, and a 10µl drop of a pycniospore suspension (10^7 spores/ml) was deposited on the wounds. A 10µl drop of water was used in controls. Inoculated plants were kept for 24h at about 100% RH, and later above 90% RH, at 20°C with 16h light. Three plants (randomized replicates) were inoculated with each isolate. Symptoms were recorded at several times, until 21 days after inoculation.

Three days after inoculation, a restricted dark necrotic lesion became visible around the wounds on all plants infected with any *L. maculans* isolate, but not on the controls. After this date, symptoms did not increase in size except with 2 isolates (symptoms reaching up to 2 mm). Five to 6 days after inoculation, chlorosis was observed at the extremity of contaminated leaves, and became generalized to the entire leaf surface within a few days.

In conclusion, none of the 36 *L. maculans* isolates was aggressive on *A. thaliana* ecotype "WS" under our experimental conditions. This suggests that some defense mechanisms against this fungus are involved. It will be interesting to test these isolates against different ecotypes to look for possible differential interactions.

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Screening for Resistance to *Alternaria brassicae* (Berk.) Sacc. in *Sinapis alba* L.

Lise N. Hansen, Stephen R. King and Elizabeth D. Earle
Department of Plant Breeding, Cornell University, Ithaca, NY 14853-1902

Introduction

Alternaria brassicae causes blackspot disease in crucifers, one of the most important diseases of the *Brassica* crops. Sufficient resistance is not available within the genus. High resistance has previously been identified in *Sinapis alba* (Brun et al., 1988; Zhu and Spanier, 1991). In addition to resistance to *A. brassicae*, *S. alba* bears resistance to flea beetles (Putnam, 1977; Lamb 1984; Palaniswamy and Lamb, 1992), to the beet cyst nematode (Lelivelt and Hoogendoorn, 1993), to drought (Primard et al., 1988), and possibly to the cabbage maggot (Finch and Ackley, 1977). *S. alba* is therefore a very valuable source for introgression of desirable traits into the *Brassicas*. In this study, we evaluated the resistance to *A. brassicae* in several lines of *S. alba*.

Materials and Methods

Thirty seven *S. alba* lines were provided by the Israel Gene Bank for Agricultural Crops, Bet Dagan, Israel. Isolates of *A. brassicae* were obtained from Dr. J. P. Tewari, Canada (canola isolate), and Dr. T. A. Zitter, Ithaca, NY (turnip isolate). Sporulation was achieved on 20% V-8 juice agar, when plates were inversely stacked at room temperature with a 14 h photoperiod of 30-60 $\mu E\ m^{-2}\ sec^{-1}$. Spores were harvested 3-4 weeks after subculturing.

Plants were inoculated when 3-4 weeks old. Six plants of each accession were sprayed to run-off with a 50,000 spores/ml suspension and incubated at 20°C for 5-7 days. The *S. alba* lines were screened three times in separate trials, once under mist and twice in plastic bags. Disease severity ratings ranged from 1 to 10, where 1 = no disease, 2 = few small flecks, 3 = small flecks, but no large lesions, 4 = small flecks and one or two lesions, 5 to 9 = increasing numbers and size of lesions, and 10 = dead. Plants with scores of 3 or less would be classified as having resistance.

Results

The mean disease severity rating in the 37 *S. alba* lines varied from 1.3 to 5.0 in the three trials (Table 1). There was a high variability within genotypes, and genotypes also responded differently in the three screens. In general, the bag inoculations were more severe than inoculation under mist. However, most lines appeared to have potential for contributing resistance to the *Brassicas*, which usually would receive a disease severity rating of 5 or higher.

From ten different lines, individual plants showing high resistance were selected, and selfed seeds were harvested. Plants from these seeds were screened for resistance to *A. brassicae* and compared with the parental lines to see if the genetic resistance could be improved. No differences between offspring and parental lines were found.

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Table 1. Screening for resistance to *A. brassicae* in *Sinapis alba*.

Sinapis alba line	Trial 1[a] (Mist)	Trial 2[a] (Bags)	Trial 3[a] (Bags)
ALT-378	NT[b]	3.6	2.8
ALT-533	2.5	2.8	4.0
ALT-534	3.7	3.4	4.0
ALT-535	3.0	4.0	3.7
ALT-536	2.8	4.3	3.2
ALT-537	2.3	3.2	2.5
ALT-538	3.3	5.0	2.2
ALT-539	3.0	3.9	2.7
ALT-540	3.0	4.5	2.3
ALT-541	1.8	4.5	2.0
ALT-542	1.3	4.5	3.0
ALT-543	2.7	4.3	2.3
ALT-544	2.3	4.5	3.2
ALT-545	1.7	4.5	4.8
ALT-546	2.7	4.9	4.5
ALT-547	3.0	4.5	4.0
ALT-548	2.7	4.5	4.5
ALT-549	2.8	4.5	4.7
ALT-550	2.0	2.7	4.3
ALT-551	3.0	4.3	3.8
ALT-552	2.0	4.0	2.7
ALT-553	2.8	4.9	3.0
ALT-554	2.3	3.7	3.0
ALT-555	2.6	4.8	3.8
ALT-556	2.0	4.2	2.8
ALT-557	2.3	3.7	4.5
ALT-558	2.2	3.0	4.7
ALT-559	2.8	3.0	2.8
ALT-560	1.8	3.5	4.2
ALT-561	1.8	4.0	4.3
ALT-562	2.3	4.4	3.2
ALT-563	2.3	4.4	4.2
ALT-564	3.7	3.3	4.7
ALT-565	3.6	3.8	3.5
ALT-566	3.5	3.6	4.7
ALT-567	3.7	4.8	4.7
ALT-568	5.0	4.9	4.2
Mean	2.6	4.1	3.6
Std[c]	0.9	0.9	1.2

[a] Disease severity ratings: 1 - disease free, 2 - highly resistant, 3 and 4 - moderately resistant, 5 to 10 - increasing degrees of susceptibility.
[b] Not tested
[c] Standard deviation.

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SPECIFITY OF PERONOSPORA PARASITICA ISOLATES TO SOME BRASSICA OLERACEA CULTURES

Stoika Masheva, Galina Antonova

The Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria

The response of two isolates of P. parasitica was studied on assortment of White Head Cabbage (B. oleracea var.capitata), Cauliflower B. oleracea var. cauliflower), Broccoli (B. oleracea var. cymosa) and Savoy cabbage (B. oleracea var. sabauda).

Twenty plants of each cultivar, hybrid were inoculated with isolates PPI-B and PPI-C, isolated from Broccoli and Head Cabbage, respectively, from Bugaria. The test was performed at the cotyledon stage after the Kluczewski·s method (1980). The readings was carried out after 10 days according to the Williams· scale (1985).The possibility for identification of the isolates was based on a presence of sporulation and a disease index (Table 1).

The isolates PPI-B and PPI-C do not distinguish by presence of sporulation on the tested plants of Broccoli hybrids. In this case the values of disease index (DI) show the extent of their host adaptation.

The presence of sporulation on the cotyledons in the Cauliflower forms, Savoy cabbage and Head cabbage gives an opportunity for more precise discrimination of the two isolates and determination of their specificity.

High resistant plants were selected in each of the investigated cultivars and hybrids for genetic study of the host specificity in further research works.

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Table 1. **Response of four B. oleracea cultures inoculated with** two isolates of P. parasitica

Brassica oleracea	Isolate			
	PPI-B		PPI-C	
	S	DI	S	DI
var. cauliflower				
Celesta	+	7	+	7
Parti F_1	+	7	+	9
Suprimax	-	5	+	9
Koket F_1	-	5	+	7
var. cymosa				
Shogun F_1	+	7	+	7
Tribute F_1	+	3	+	7
Beaufort F_1	-	5	-	7
Neptune F_1	-	7	-	7
var. sabauda				
Savoy ace F_1	+	5	+	5
Promasa F_1	-	5	+	7
Hamasa F_1	-	5	+	7
Winterton F_1	+	7	+	5
var. capitata				
Hitoma F_1	+	5	-	5
Mustang F_1	+	7	-	7
Hinova F_1	+	5	+	7
Atria F_1	+	5	+	9

S - sporulation; + = present on some individuals population
- = not present

DI - disease index based on 0,1,3,5,7,9 scale where: 0 = immunity
9 = full susceptibility (Williams, 1985)

Quantitative determination of colonization by *Plasmodiophora brassicae* (Wor.) in *Brassica napus* and *Brassica oleracea*

Marion Morgner and María Dolores Sacristán
Institute of Applied Genetics, FU Berlin, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany

Introduction

The life cycle of the fungal pathogen *Plasmodiophora brassicae* was first described by Woronin (1878) and consists of two phases: one occuring in the root hairs and the other in the cortex and stele of roots where the symptoms of the disease can be observed.
The primary phase occurs in resistant and susceptible genotypes (MacFarlane 1955, Karling 1968, Voorrips 1992) while the secondary phase seems to be restricted to the susceptible hosts. The invasion of the pathogen in the root tissue of the susceptible genotypes leads to the development of the typical root galls (clubroot).
Host resistance to clubroot is pathotype-specific and known for a long time in the genus *Brassica* (Karling 1968). However, the resistance expression has not been thoroughly investigated up to now. Resistance could not be generally related to any biochemical, morphological or histological factors.
In this study the degree of secondary phase development of *Plasmodiophora brassicae* in the roots of resistant and susceptible genotypes of *Brassica napus* and *Brassica oleracea* were compared using light microscopy.

Material and methods

Plant material and inoculation: Seven days old seedlings of *Brassica napus* (ECD 07/susceptible, ECD 06/resistant) and *Brassica oleracea* (ECD 14/susceptible, "Böhmerwaldkohl"/resistant) were inoculated with a *Plasmodiophora brassicae* spore suspension (4 ml/plant, 10^8 spores/ml). The different *P. brassicae* isolates ("b" for *B. napus*, "h" for *B. oleracea*) were described by Diederichsen (1992).
Preparation of the test samples: The root systems of 10 plants per genotype were analyzed. Root segments (length 1cm, first lateral root was defined as the middle of the sample) were fixed (12 h, 10% Acrolein in NaH_2PO_4/Na_2HPO_4-buffer, pH8), dehydrated through alcohol series and embedded in glycolmethacrylate (System Technovit 7100, Heraeus Kulzer, Germany). Longitidinal sections through the root axis were cut (7 µm) and stained with Safranin (Gerlach 1984). The sections were examined using light microscopy.
Determination of colonization: Per sample (root segment) 30 tissue sections were analyzed 12 and 22 days after inoculation with the pathogen. The number of colonized cells per section were counted. The mean values of the data (300 data per time and genotype) were calculated and the standard deviations were worked out.

Results and discussion

The temporal development of pathogenesis was studied microscopically. The susceptible and resistant genotypes could be clearly distinguished 12 and 22 days after inoculation with *P. brassicae*.
The results of the quantiative determination of colonization are shown in figure 1. In the susceptible genotypes of *Brassica napus* (ECD07) and *Brassica oleracea* (ECD 14) the pathogenesis followed the same temporal pattern. The number of colonized cells increased significantly during the two analyzed times through further colonization and growth of the root. The mean value of infected cells in a root segment 12 days after inoculation was 2185±1435 in *B. napus* (ECD 07)and 1285±632 in *B. oleracea* (ECD 14). The number of infected cells per root segment was 21100±7616 in *B. napus* (ECD 07) and 27107±3981 in *B. oleracea* (ECD 14) 22 days after inoculation .
In the resistant genotypes of *B. napus* (ECD 06) 12 days after inoculation the pathogen was also observed. However, the number of infected cells was in comparison to the susceptible genotype very low (180±88). *Plasmodiophora brassicae* seemed to be inhibited in its development and further spreading. A similar latent infection without symptom expression was reported by Kroll et al. (1983) for a resistant cultivar of *Raphanus sativus*.

In the root segments of the resistant *B. oleracea* (Böhmerwaldkohl) *Plasmodiophora brassicae* was presented in less than 3 (2.9±3.1) cells 12 days after inoculation and showed therefore almost no colonization. The resistance mechanism of this genotype could not be overcome by the pathogene.
The pathogen could not be detected in any of the resistant genotypes 22 days after inoculation.There were no indications for a hypersensitive response as a reaction to the invasion of the pathogen as reported by Dekhuizen (1979) for a resistant *Brassica campestris* genotype.
The results clearly demonstrate that the development of *P. brassicae* is stopped at the beginning of the secondary phase of the life cycle. It is conceivable that the same resistance mechanism forms the basis of the incompatible interactions in both resistant genotypes. However, it might work more effective in *B. oleraceae* .
The fact that no histological changes were observed could account for an active defense reaction of the host and may refer to a plasmatic defense as described by Akai (1959). A similar reaction was also suggested for blackleg development in a resistant *Brassica napus* cultivar (Xi and Morral 1993). Further histological examinations may lead to a understanding of latent infection in relation to the pathogenesis of this fungal disease and enlighten the way of penetration and spreading of *P. brassicae* in the host root.

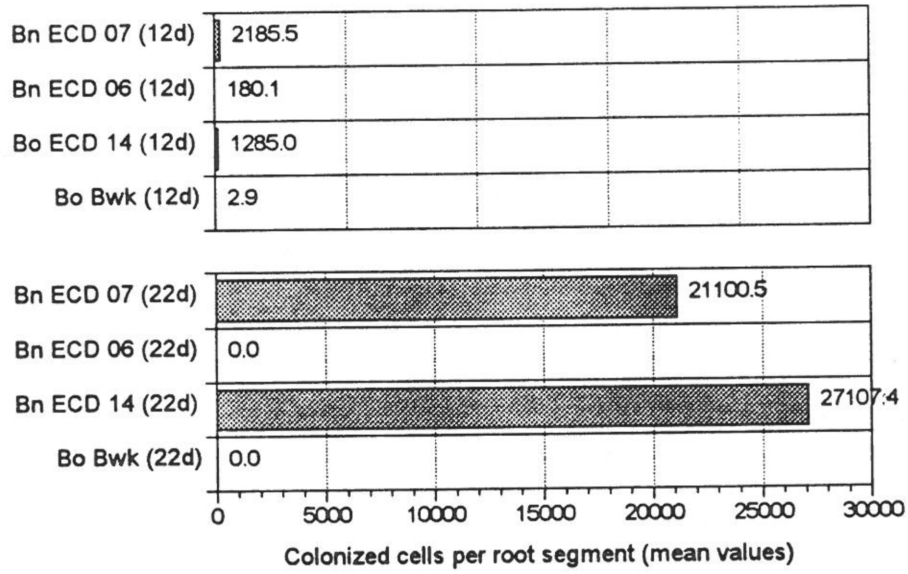

Figure 1: Quantitative determination of colonization by *Plasmodiophora brassicae* 12 and 22 days after inoculation (Bn: *B. napus*, Bo: *B. oleracea*, Bwk: Böhmerwaldkohl)

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INOCULATION METHOD FOR TESTING RADISH SEEDLINGS FOR RESISTANCE TO *RHIZOCTONIA*

M. Nieuwhof and S. Giezen

Centre for Plant Breeding and Reproduction Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands

1. Introduction

Radish can be heavily attacked by *Rhizoctonia solani* Kuehn. Seedlings may be killed before or after emergence and surviving plants may show brownish or black lesions on the roots. To study prospects to develop resistant cultivars a seedling test was developed. For this purpose effects of temperature and inoculum density on attack of radish seedlings by *Rhizoctonia* were studied.

2. Material and methods

The *Rhizoctonia* isolate used belonged to the anastomosis group AG4 and was maintained on a PA medium at 5°C. Inoculum was prepared by growing the fungus in a mixture of 10% oat meal and 90% potting soil at 20°C for 3 to 4 weeks.

The experiments were carried out according to a split plot design with 3 replications, in glasshouses under natural light conditions. Seeds were sown in a potting soil with a pH of 5.8, on plots of one row, with a row length of 30 cm, a row distance of 10 cm, and with 20 seeds per plot.

Plants which were killed after emergence by damping-off, were counted periodically. Remaining plants at the end of the experiment were checked for root attack. Pre-emergence killing was calculated by substracting the number of emerged seedlings from the number of seeds sown out.

Differences between treatments were tested on binomial probability paper (Ferguson, 1956).

3. Results

In Exp. 1 seeds of two early round red cvs ('Helro' and 'Novired') and two long white cvs ('IJskegel' and 'Tokinashi') were pregerminated for 2 days at 22°C on moist paper, and sown at 10, 14, 18 and 22°C in soils with inoculum densities of 1:50; 1:100; 1:200 and 1:500. At 10 dates after emergence plants were checked for damping-off. Surviving plants were harvested 26 days after sowing and checked for root attack. Results presents Table 1. As the cvs reacted similarly the results of them are pooled.

At 10°C almost no diseased plants occurred. At 14°C a number of the plants were killed by damping-off, and a number of the surviving plants showed root attack. At 18 and 22°C almost all seedlings were killed by damping-off, while most surviving plants showed root symptoms. At all temperatures the percentages of pre-emergence killing increased with higher inoculum concentrations. At 18 and 22°C all plants died independent of the inoculum concentration. At 14°C the percentage of healthy plants decreased with higher inoculum concentration.

In Exp. 2 the cvs Helro and Flamboyant (long white) were sown at 10, 14, 18 and 22°C in soils with inoculum densities of 1:50 and 1:100. Half of the seeds were pregerminated. Plants were checked at 9 dates for damping-off symptoms and surviving plants were harvested 24 days after sowing and checked for root attack.

The effects of inoculum concentration and pregermination were small. Significant differences in attack occurred between temperatures and cultivars. These effects shows Table 2, in which results of both inoculum densities and of pregerminated and not-pregerminated seeds are taken together. Between 14, 18 and 22°C no important differences occurred. At 10°C the attack was less severe, most seedlings emerged, but after emergence always more plants became diseased, resulting in almost 100% disease incidence. This was true for both cvs, though 'Helro' was more severly attacked at first than 'Flamboyant'.

4. Discussion

By sowing radish in a soil with a high inoculum density at about 20°C many seedlings are killed already before emergence, and the remaining plants just after emergence. Two to four weeks after sowing screening tests can already be finished. Pre-emergence killing descreased slightly by

Table 1. Effect of temperature and inoculum concentration (1/500, 1/200, 1/100, 1/50) on attack by *Rhizoctonia*.

Temp. (°C)	% preemergence killing				% postemergence killing			
	1/500	1/200	1/100	1/50	1/500	1/200	1/100	1/50
10	3	6	+ [1)]	3	+ [1)]	0	0	+ [1)]
14	6	14	38	36	11	36	38	36
18	29	58	75	77	59	40	25	21
22	32	77	94	98	62	20	5	1

Temp. (°C)	% root attack				% healthy plants			
	1/500	1/200	1/100	1/50	1/500	1/200	1/100	1/50
10	0	0	0	0	97	94	100	97
14	28	14	14	5	55	36	10	23
18	13	4	0	4	0	0	0	0
22	1	0	0	0	5	3	1	1

1) + : < 0,5%

Table 2. Effect of temperature and cultivar (H = Helro, Fl = Flamboyant) on attack by *Rhizoctonia*.

Temp. (°C)	% preem. killing		% postem. killing		% root attack		% healthy plants	
	H	Fl	H	Fl	H	Fl	H	Fl
10	23	5	63	55	13	33	1	8
14	79	38	21	60	0	2	0	0
18	84	30	16	69	0	+ [1)]	0	+ [1)]
22	72	42	28	58	0	0	0	0

1) + : < 0,5%

pregermination of the seeds. In other experiments it was found that it also decreases by a high germination energy of the seeds and by seed treatment (Nieuwhof, 1988). The retarted attack of 'Flamboyant' in Exp. 2 may be due to the strong disinfection of the seeds.

Attack of seedlings was favoured by high inoculum concentrations, but temperature is a more decisive factor. High temperatures can compensate low inoculum densities (in an experiment, at about 20°C, 100% disease incidence could be obtained in a soil with a 1:1000 inoculum density), but high inoculum concentrations cannot compensate low temperature effects as Exp. 1 shows.

For each test a freshly inoculated soil is recommended, as the infection potential may decrease, as was found in successive tests for which the same inoculated soil was used (Nieuwhof and Giezen, 1988). In an experiment in which 2% oat meal was added pre-emergence killing could be raised again from 4 to 85%.

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[dnhf0712.hue]

REACTION OF DIFFERENT OILSEED BRASSICAS SPECIES AND SOME OF THEIR HYBRIDS TO INFECTION OF Alternaria LEAF BLIGHT DISEASE

B. RAI AND H. KUMAR
Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi 221 005, INDIA

Incorporation of genetic resistance to Alternaria leaf blight in the commercial cultivars of oilseed Brassicas is now being sought after for developing long term strategy in controlling this disease. Crop losses due to this disease alone were noted to vary from 40 to 70%. 2,3,8. Some observations on the reaction of different oilseed Brassicas species to this disease have been reported. 1,5,7,10. In this study, effort was made to evaluate the comparative reactions of the different oilseed Brassicas species and some of their hybrids to this disease.

The experimental materials comprised of six oilseed Brassicas species and four of their hybrids. The F_1's alongwith their parental species were grown at the Crop Research Centre, BHU, Varanasi. Three rows of each entry were planted in 5 m long row; spaced 45 cm apart. Plant to plant distance was 10 cm. Severe disease infestation was created. Observations were recorded at peak disease development stage during the 1st week of February at adult plant stage using 0 -5 scale. In these ratings, the leaves totally free from infection were taken as 0, those with 1 to 4, 5 to 10, 11 to 25, 26 to 50 and 51 to 100 % infections as 1, 2, 3, 4,and 5, respectively. Ten leaves from each plant were taken at random and were visually scored for disease reaction. The rating score of 1.0 to 2.0 was considered as Resistant (R), 2.1 to 2.5 as Moderately Resistant (MR), 2.6 to 3.5 as Susceptible (S) and 3.6 to 5.0 as Highly Susceptible (HS).

Among the Brassica species (Table 1), B. carinata had the lowest rating followed by B. napus, B. nigra, B. oleracea var botrytis, B. juncea and B. campestris. The cultivated species B. juncea and B. campestris were in the 'S' and 'HS' ratings, respectively; similar results have been also noted by others. 6,7. B. carinata has been reported to show better tolerance to Alternaria laef blight than either B. campestris or B. juncea. 1. In general, the two digenomic species (B. carinata and B. napus) showed comparatively better tolerance to this disease than that of the monogenomic species (B. campestris and B. oleracea var botrytis), which could be due to favourable intergenomic interactions of the AA, BB or the CC genomes.

In the crosses Table(1), in which one parent was as 'R' and the other was as 'HS', in one case (B. carinata x B. juncea), the F_1 reaction was as 'R', in the other case (B. juncea x B. napus), the F_1 reaction was as 'MR' or almost intermediate. In case of B. napus x B. campestris, the F_1 reaction was towards the susceptible parent. However, in cross B. napus x B. carinata, where P_1 was as 'MR' and the P_2 was as 'R', the F_1 was resistant. Incidently in these two crosses, where BB genome was present in the resistant parent, the F_1 reaction was resistant. Both monogenomic and as well as polygenic control of resistance to this disease have been reported. 4, 9.

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Table 1. Characterization of the six oilseed Brassica species and their crosses with respect to their reactions to Alternaria leaf blight disease at B.H.U., Varanasi (Rabi, 1992-93)

Species/Cross	Genomic Constitution	Average score for the leaf blight disease score (0-5)			Level of Resistance		
		P_1	P_2	F_1	P_1	P_2	F_1
B. campestris	AA	4.20	—	—	HS	—	—
B. oleracea var *botrytis*	CC	3.00	—	—	S	—	—
B. nigra	BB	2.80	—	—	S	—	—
B. napus	AA CC	2.28	—	—	MR	—	—
B. juncea	AA BB	3.35	—	—	S	—	—
B. carinata	BB CC	1.50	—	—	R	—	—
Crosses:							
B. napus x *B. carinata*		2.20	1.39	1.50	MR	R	R
B. juncea x *B. napus*		3.85	1.96	2.29	HS	R	MR
B. carinata x *B. juncea*		1.07	3.85	1.80	R	HS	R
B. napus x *B. campestris*		1.92	4.35	3.68	R	HS	S

R = Resistant, MR = Moderately Resistant, S = Susceptible
HS = Highly Susceptible

BIOCHEMICAL ASSAYS AND YIELD PERFORMANCE OF SPROUTING BROCCOLI GENOTYPES IN MID HILLS OF HIMACHAL HIMALAYAS

PRITAM KALIA, Department of Vegetable Science, Himachal Pradesh Agricultural University, Palampur 176 062, India.

INTRODUCTION

Broccoli (*Brassica oleracea var. italica*) is an important cole vegetable which has sneaked into Indian market in the recent years . It is the most nutritious of the cole crops, especially in vitamin content,calcium, iron etc.(Pierce,1987) . The present communication reports certain biochemical constituents of edible portion and marketable yield in 18 sprouting broccoli genotypes.

MATERIALS AND METHODS

Eighteen genotypes of broccoli introduced from HRI , Wellesbourne (UK) were grown in non-replicated plots at the Vegetable Research Farm ,Department of Vegetable Science, Himachal Pradesh Agricultural University,Palampur during rabi season, 1990- 91 . Each genotype was planted at 45 x 45 cm spacing in a plot measuring 3. 60 x 1 . 80 sq . m . Recommended cultural practices and fertilizer applications for cauliflower were followed to raise this crop under Palampur conditions(HPAU,1987) . Yield data were recorded on 10 randomly selected plants when the terminal head reached the marketable maturity followed by one or more harvest of spears which arose from the leaf axils along the main stem . Composit samples of fresh edible portion were drawn from 10 randomly selected plants in each genotype at the time of harvest for biochemical analysis . Moisture , protein , total carotenoides , total chlorophyll, and ascorbic acid were estimated by using standard methods of AOAC(1976) . The dried samples of broccoli were wet digested with di- acid mixture(1950) to estimate calcium,phosphorus and sodium . The digested samples were analysed for calcium and sodium by using Flame Photometer and phosphorus was assayed by colorimeteric method .

RESULTS AND DISCUSSION

The data on the variations in biochemical parameters and marketable yield are presented in Table 1 . The average values for various characters studied in 18 diverse genotypes are given in Table 2 and 3 .Variability observed in the moisture content roughly indicates the degree of maturity and fibre accumulation pattern in the crop which is an important criterion contributing towards consumers acceptability of the crop harvest . Genotypes BI80760 and BI74003 possessed higher moisture content .Varietal variation in moisture content at edible stage might be due to rapid variability in accumulation of photosynthates and other nutrients in terms of dry matter . Variation in total chlorophyll content indicates the maturity as well as succulence status of the edible parts of the vegetable crops in particular . Genotypes BI80334 and BI80347 recorded high total chlorophyll content . The highest protein content were observed in BI76029 and BI76044 . Although, BI80336 gave the highest marketable yield , but the heads of BI76042 had high cosmetic appeal . The overall carotene content ranged from 0.102 to 0.567 mg/g in edible portion . Genotypes BI76026 and BI76044 showed its highest content . The highest value of ascorbic acid was noticed in genotypes BI76008 and BI80334 . Significant differences were also observed among genotypes for calcium and phosphorus content . Genotype BI76044 showed their highest values . Variation in sodium content was ranging from 13 .56 to 26 .23 mg/100g with its highest content in BI80347. Considerable variations in vitamins , minerals and marketable yield among the genotypes suggest a possible scope for depending on the screening of further genotypes of broccoli with a view to select a specific improved genotype(s) .

TABLE 1 VARIATION IN BIOCHEMICAL ATTRIBUTES AND MARKETABLE YIELD IN BROCCOLI GENOTYPES

Character	Range	Mean	S.D.
Moisture (%)	83.53 - 88.70	86.192	1.32
Carotene (mg/g)	0.102- 0.567	0.369	0.15
Ascorbic acid (mg/100g)	86.67- 186.67	137.928	35.08
Calcium (mg/100g)	27.84- 55.51	39.627	8.41
Phosphorus(mg/100g)	53.36 - 93.57	70.885	11.81
Sodium (mg/100g)	13.56- 26.23	20.137	3.19
Total Chlorophyll (mg/g)	0.050- 0.232	0.142	0.06
Protein (%)	2.26 - 3.30	2.74	0.28
Marketable yield (q/ha)	95.18 - 271.45	194.842	57.33

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TABLE 2 VITAMIN AND MINERAL CONTENT* OF THE EDIBLE PART OF DIFFERENT BROCCOLI GENOTYPES

S. No.	Genotype	Carotene (mg/g)	Ascorbic acid (mg/100g)	Calcium (mg/100g)	Phosphorus (mg/100g)	Sodium (mg/100g)
1.	BI 74003	0.109	93.33	27.84	53.36	16.24
2.	BI 74005	0.408	170.67	32.20	77.28	19.32
3.	BI 74015	0.543	120.00	47.23	64.17	22.22
4.	BI 76008	0.431	186.67	42.44	85.56	20.53
5.	BI 76013	0.369	134.67	36.17	68.73	17.36
6.	BI 76015	0.353	94.67	42.87	63.62	16.60
7.	BI 76018	0.519	120.00	49.42	67.07	25.42
8.	BI 76026	0.567	130.67	43.10	65.30	22.20
9.	BI 76029	0.310	133.34	54.35	90.58	21.41
10.	BI 76042	0.361	128.00	35.02	66.55	18.21
11.	BI 76044	0.567	128.00	55.51	93.57	20.62
12.	BI 80334	0.532	186.67	35.62	78.37	19.39
13.	BI 80335	0.354	178.67	40.09	81.67	19.30
14.	BI 80336	0.329	181.34	33.07	66.15	18.52
15.	BI 80345	0.260	132.00	37.23	75.18	22.91
16.	BI 80347	0.422	184.00	43.71	69.21	26.23
17.	BI 80741	0.109	86.67	29.16	55.89	21.87
18.	BI 80760	0.102	93.33	28.25	53.67	13.56
	Check	0.369	137.928	39.627	70.885	20.137
	index	± 0.059	±3.562	± 3.249	± 4.564	± 1.232

* Values on fresh weight basis

TABLE 3 MOISTURE, TOTAL CHLOROPHYLL, PROTEIN CONTENT AND MARKETABLE YIELD FOR DIFFERENT BROCCOLI GENOTYPES

S.NO.	Genotype	Moisture (%)	Total Chlorophyll (mg/g)	Protein (%)	Marketable Yield (q/ha)
1.	BI 74003	88.40	0.053	2.27	258.91
2.	BI 74005	87.12	0.161	2.42	202.47
3.	BI 74015	86.11	0.141	2.73	101.24
4.	BI 76008	86.31	0.174	2.78	222.02
5.	BI 76013	85.53	0.204	2.85	95.18
6.	BI 76015	86.17	0.156	2.78	152.19
7.	BI 76018	85.88	0.096	2.70	209.60
8.	BI 76026	86.94	0.181	2.63	248.56
9.	BI 76029	83.53	0.116	3.30	96.30
10.	BI 76042	85.99	0.179	2.80	216.26
11.	BI 76044	84.14	0.181	3.24	245.68
12.	BI 80334	85.75	0.232	2.79	208.56
13.	BI 80335	85.15	0.184	3.05	241.64
14.	BI 80336	86.77	0.116	2.72	271.45
15.	BI 80345	85.68	0.050	2.85	243.50
16.	BI 80347	85.43	0.222	2.76	171.89
17.	BI 80741	87.85	0.053	2.39	184.78
18.	BI 80760	88.70	0.058	2.26	136.92
	check	86.192	0.142	2.74	194.842
	index	±0.511	± 0.023	±0.110	±22.160

A SOMACLONE OF *BRASSICA JUNCEA* IS PROCESSED INTO A VARIETY AND IS RELEASED FOR COMMERCIAL CULTIVATION IN INDIA

R.K. KATIYAR and V.L. CHOPRA

A high-yielding, bold seeded, shattering resistant variety (named Pusa Jai Kisan) of mustard (*Brassica juncea*(L) Czern & Coss) was developed by tissue culture(somaclonal variation). A leading variety of Indian mustard (*Brassica juncea*) CV Varuna(Type-59) was used as parental material for the culture to generate variability (Anuradha et al. 1992).

The variability thus generated was field evaluated for the stability and performance. Among many somaclone lines, one with very bold siliqua and seeds, medium plant height and much higher yield than controls (on an average of 17.3 percent) was identified. The somaclone was tested in the national multilocation test done by All India co-ordinated Research Project on Oilseed Improvement, for three consecutive years,i.e. 1991-1993, for seed yield, oil content, seed weight(1000 seeds) and days to maturity against the best National checks (Varuna and Kranti). The somaclone(Pusa Jai Kisan) registered superiority in all the four above said characters. It outyielded the checks, Varuna and Kranti by 17.3 and 19.7 % respectively(Table-1). In seed size also the somaclone was superior by 10.2 and 42.1 percent respectively.

Based on alround superior performance of the somaclone over best existing varieties, it was released for the commercial cultivation by Government of India in 1993, for the states of Gujarat, Rajasthan and parts of Maharashtra. Rajasthan is the largest mustard producing state in India and accounts for over 32 percent of the total area sown under this crop. The performance of Pusa-Jai Kisan, in comprison with the best available other varieties, was assessed on farmer's field by the Technology transfer and the agricultural extension agency of the Institute, under the adopted villages Programme. The results from 14 demonstrations, each grown on one acre plot, are given in table-2. It is seen that Pusa Jai-Kisan outyielded all varieties. The yield advantage over the traditionally cultivated local variety in the state was seen over 56 percent. The average yield of the somaclone was 2.35 tonnes per hectare and the maximum recorded to be 3.2 tonnes per hectare.

National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi- 110 012(India)