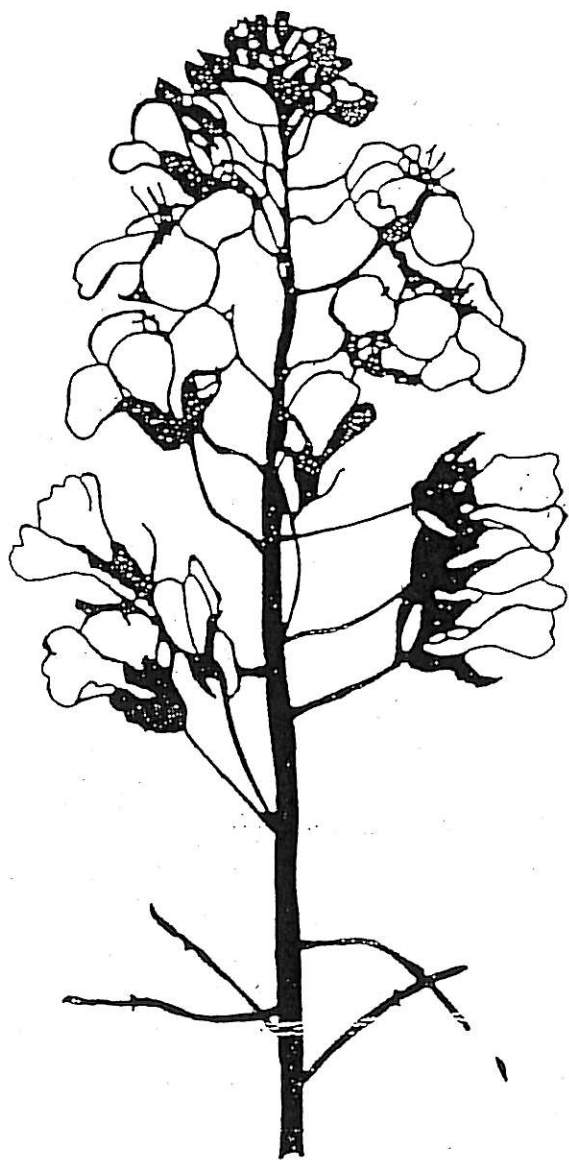


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# VARIABILITY OF SOME MORPHOLOGICAL CHARACTERISTICS IN SOME BREEDING LINES OF BROCCOLI

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## Abstract

The purpose of the research was to determine the genetic and phenotypic variability and broad-sense heritability of six morphological characteristics in selected lines of broccoli.

The investigated lines show the lowest variation in the weight of the lateral curds ( $CV_g=12.20\%$ ,  $CV_f=15.35\%$ ) and in the weight of the primary curd ( $CV_g=14.97\%$ ,  $CV_f=19.26\%$ ). Genetic and phenotypic coefficient of variation is considerably higher for the rest of the morphological characteristics. The highest variability is determined for the number of the additionally formed lateral curds ( $CV_g=54.4\%$ ,  $CV_f=54.98\%$ ). Broad-sense heritability shows the lowest values for the weight of the primary curd (60.37%) and the highest for the central flower head diameter (99.52%). The variation of the morphological characteristics is due mostly to genetic causes.

**Keywords:** *Brassica oleracea* var. *italica* Pl., broccoli, morphological characters, variability, genetic variance, phenotypic variance, coefficient of variation, broad-sense heritability

## Introduction

The characterization of the created genotypes towards the complex of morphological characters is an important element in the breeding of broccoli. According to the degree of variability and heritability of these characters, the possibilities for effective choice towards phenotype in the initial breeding stage, are determined.

The investigations, performed in Bulgaria, on the morphological characters of broccoli genotypes and the studies of their variability and heritability are comparatively limited, and the breeding work with this kind of *Brassica* is still in the beginning.

The purpose of the present study was to be determined the genetic and phenotypic variability and broad sense heritability of six morphological characters in breeding lines.

## Material and methods

During the 1999-2000 period four broccoli breeding lines – 203-21-7, 203-80-6, 203-51-1 and 207-14-07 were studied in the Institute of Horticulture and Canned Foods, Plovdiv.

The experiments were performed by the block method in 4 treatments (lines) with 4 replications /30 plants per a replication/.

The lines were tested according to the technology for late field production with dates of sowing 1-15 June and planting 10-20 July and they were harvested within 1-15 October. The plants were planted according to the scheme 90+70/60 cm on a profiled seedbed area.

The following morphological characters were tested: a central flower head mass (kg), lateral flower head mass (kg), diameter of a central flower head (cm), diameter of a lateral flower head (cm), lateral flower heads (number) and stem height (cm).

The genetic and phenotypic variance, genetic and phenotypic coefficient of variation and broad sense heritability were established. The data were processed mathematically and by two-way analysis of variance (Lakin, 1990; Kik, 1992).

## Results

It is established that the studied bred broccoli have different characters of the morphological characteristics (Table 1). The greatest mass of the central flower head 0.430 kg is recorded for 203-21-7 line, and the smallest mass 0.330 kg for 203-80-6 line. The lateral flower head mass



varies from 0.075 kg to 0.090 kg for lines 203-51-1 and 203-80-6 respectively. The greatest diameter of the central flower head 17.2 cm has line 207-14-07 and the smallest diameter 10.3 cm has line 203-51-1. The lateral flower heads are with diameter from 2.8 cm to 4.8 cm for lines 203-51-1 and 207-14-07 respectively. The variation towards the number of the lateral flower heads is from 2.2 for 203-21-7 line to 4.8 for 207-14-07 line. The plants from 207-14-07 line characterizes with the highest stem 28.7 cm and those from the line 203-51-1 have the smallest stem 17.8 cm.

**Table 1.** Morphological characters of selected broccoli lines

Lines	Central flower head mass kg	Lateral flower head mass kg	Central flower head diameter cm	Lateral flower head diameter cm	Lateral flower heads number	Stem height cm
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
203-21-7	0.430±0.065	0.080±0.006	16.4±1.4	3.5±0.7	2.2±0.5	21.8±4.2
203-80-6	0.330±0.033	0.090±0.008	13.0±0.5	4.5±0.9	4.4±0.7	21.2±3.0
203-51-1	0.360±0.030	0.075±0.010	10.3±0.9	2.8±0.6	3.0±0.5	17.8±1.6
207-14-07	0.390±0.020	0.085±0.008	17.2±1.4	4.8±1.3	4.8±1.0	28.7±3.7
Average	0.378	0.083	14.2	3.9	3.6	22.4

The variability of the studied morphological characters is due to a few factors. The main source of variability, for all characters is a presence of significant genetic differences between the studied broccoli lines (Table 2). The years of study affects only on the variation of the central flower head mass and on the lateral flower head diameter. The interaction between the genotypes and years has significant differences only for the characters central flower head mass and lateral flower head mass.

**Table 2.** Two-way analyses of variance for morphological characters of bred broccoli lines

Sources of variation	Degree of freedom	Central flower head mass	Lateral flower head mass	Central flower head diameter	Lateral flower head diameter	Number of lateral heads	Stem height
Block	3	0.0023	15.532	1.125	2.031	0.167	9.042
Line	3	0.0160***	487.090***	80.875***	6.115***	11.750***	166.375***
Year	1	0.006**	25.920 <sup>ns</sup>	0.125 <sup>ns</sup>	3.781*	0.125 <sup>ns</sup>	2.000 <sup>ns</sup>
Line x Year	3	0.0063***	179.290*	0.375 <sup>ns</sup>	0.948 <sup>ns</sup>	0.208 <sup>ns</sup>	6.083 <sup>ns</sup>
Residual	21	0.0007	57.760	1.4107	0.555	0.619	12.161

\*, \*\*, \*\*\* - significant at  $p \leq 0.05$ ,  $p \leq 0.01$ ,  $p \leq 0.001$

ns – not significant

The phenotypic variance in all morphological characters has higher value than the genotypic variance (Table 3).

The lowest values of the phenotypic and genotypic variance 0.0053 and 0.0032 are established for the mass of the central flower head, and the highest values 55.46 and 53.43 are recorded for the stem height. The phenotypic and genetic coefficients of variation are the lowest for the characters lateral flower head mass (CV<sub>f</sub>=15.35%, CV<sub>g</sub>=12.20%) and central flower head mass (CV<sub>f</sub>=19.26%, CV<sub>g</sub>=14.97%).

The character stem height has the highest phenotypic and genetic coefficient of variation (CV<sub>f</sub>=54.98%, CV<sub>g</sub>=54.48%).



**Table 3.** Genetic ( $S_g^2$ ) and phenotypic ( $S_f^2$ ) variance, genetic (CVg) and phenotypic (CVf) variation coefficient and broad sense heritability ( $H^2$ ) in the examined characters

Morphological characters	$S_g^2$	$S_f^2$	CVg (%)	CVf (%)	$H^2$ (%)
Central flower head mass	0.0032	0.0053	14.97	19.26	60.37
Lateral flower head mass	102.6	162.36	12.20	15.35	63.19
Central flower head diameter	26.83	26.96	36.48	36.57	99.52
Lateral flower head diameter	1.722	2.038	33.65	36.60	84.49
Number of lateral flower heads	3.848	3.917	54.48	54.98	98.23
Stem height	53.43	55.46	32.63	33.25	96.34

The possibility for broad sense heritability has high values for all characters of the morphological characteristics (Table 3). The heritability coefficient has the lowest value 60.37% for the central flower head mass and the highest one 98.23% and 99.52% for the lateral flower head number and central flower head diameter respectively.

### Discussion

The results from the performed study show a presence of considerable variation for every one of the examined morphological characters. Differences between the studied broccoli lines are the main source of the variation. The genetic determination of the variation confirms by the established high values for the heritability in a broad sense.

An indication for comparatively weak effect of the environment is also the closeness between the genetic and phenotypic coefficients of variation about almost all characters from the morphological characteristics of the studied broccoli lines.

These results are in conformity with those, obtained by Mihov and Antonova, 2001 in a similar type of investigation. The presence of considerable variation is a basic criterion for starting of breeding work, and the strict relationship of the variation from genetic factors according to Cervenski et al. (2002), predetermines the successful and effective breeding process. However it has to be taken into consideration that both the differences between the years of examination and proved interactions lines x years effect on the variability of some characters. These sources of variation can take the part of factors with limiting effect on the breeding process (Cervenski et al., 2002; Paul, 1990).

Nevertheless the high possibility for heritability, established for all morphological characters will give a chance for effective choice by phenotypes in the next progenies of the examined broccoli lines. The combination of high heritability in a broad sense with high coefficients of variation can consider as an indication for an action of non-additive genes, controlling the expression of the morphological characters in broccoli.

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# SCREENING OF A ROOT MUTANT IN TURNIP (*BRASSICA RAPA* L.)

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## ABSTRACT

A desirable root mutant was detected in  $M_2$  in turnip (*Brassica rapa* L.) under EMS treatment. It had about three times larger root and was superior in most of the agronomic traits than the parental stock. A promising cultivar of turnip can be raised from this through selection in successive generations.

## INTRODUCTION

Artificial induction of mutation is a major technique of plant breeding. Though radiations are important tools in crop improvement programmes, their handling requires highly specialized personnel and the necessary equipment involve much expenditure. Chemical mutagens are, on the other hand, easy to handle and not expensive. Their effect is so similar to that of radiation that they are called radiomimetic substances and are more efficient in seed propagated crops (1, 3, 11, 12). Ethyl methane sulphonate (EMS) is the most potent mutagen. Present study was undertaken in order to obtain some desirable mutants owing to the effect of EMS.

## MATERIAL AND METHODS

'Rose Red' variety of turnip constituted the material for present study. 300 moist seeds were treated in 1.2%, 1.3%, 1.4%, 1.5% and 1.6% (V/V) EMS solutions separately for 6 hours duration. After that, they were immediately sown in the field to raise  $M_1$  plants along with the Control at Hazaribag. During the period, relative humidity and temperature ranged between 88%-99% and 22°C - 29°C, respectively.  $M_2$  seeds were obtained from  $M_1$  by selfing. The results are presented in Table 1.

## RESULTS AND DISCUSSION

The control plants of turnip had the roots with mean root weight of about 160g. However, in  $M_2$  a plant (P7) under 1.6% EMS treatment had surprisingly very large root which was not truly napiform but had some vertical clefts. The plant had increased number of leaves, stomata/unit area, chloroplasts/guard cell, branches/plant and more plant height with maximum vigour and delay in flowering. On the other hand, it had lesser number of ovules/pistil, seeds/silique, silique/plant and declined fertilization value. The chromocentres/nucleus enhanced. Meiosis was disturbed and pollen viability went down about half of the Control. This plant was isolated from the population and the seeds were collected from this through selfing to raise  $M_3$ . Most of the plants of  $M_3$  had similar large roots.  $M_4$  was also raised in order to isolate the desired superior plants. More balanced meiosis and increased pollen viability was noted in successive generations, along with large root and increased vigour.

The cross - pollinated crops like turnip have enough genetic variability already in nature and hence are not generally subjected to mutagenic agents. However, recently some attention has been paid to increase variability to exploit the mutated genes in the mutational heterosis and also to induce male sterility for use in hybrid seed production (1,2,4-10,11). The root mutant, thus obtained, may be the result of mutational heterosis. It is superior than the parental stock in most of the desirable agronomic traits. Besides it is more suitable for cultivation under the local climatic conditions. A promising cultivar of turnip can be raised from this through selection in successive generations.

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Table 1. : Study of the root mutant

Character	M <sub>2</sub>				M <sub>3</sub>		M <sub>4</sub>	
	P <sub>7</sub> under 1.6% EMS treatment	Other plants under the same treatment		Control	Progeny of P <sub>7</sub>	Control	Progeny of P <sub>7</sub>	Control
		Mean ± SE	Mean ± SE					
No. of leaves/plant	14	11.4 ± 0.46	11.5 ± 0.36	15.3 ± 0.69 **	11.6 ± 0.29	15.5 ± 0.48 **	11.3 ± 0.34	
No. of lobes/leaf	07.9	08.2 ± 0.30	08.7 ± 0.27	07.3 ± 0.30 *	08.2 ± 0.21	07.8 ± 0.30	08.2 ± 0.23	
Area of leaf surface (cm <sup>2</sup> )	436.2	185 ± 13.04	191.7 ± 18.33	368.3 ± 3.66 **	206.67 ± 11.45	382.5 ± 12.92 **	204.2 ± 12.44	
No. of stomata/unit area	27.25 ± 0.43	23.4 ± 0.48 **	25.2 ± 0.28	26.2 ± 0.58 *	24 ± 0.40	26.7 ± 0.49 *	25.3 ± 0.44	
No. of chloroplasts/guard cell	05.5 ± 0.10	04.1 ± 0.15 **	04.6 ± 0.08	05.3 ± 0.13 **	04.7 ± 0.10	05.4 ± 0.19 **	04.7 ± 0.09	
Root weight (g)	375	139.3 ± 13.01	160.0 ± 16.55	316.7 ± 13.61 **	174.0 ± 18.67	339.3 ± 7.08 **	167.3 ± 10.04	
Plant height (cm)	186	129.2 ± 3.36	126.2 ± 4.50	134.0 ± 1.25 **	102.2 ± 2.32	149.3 ± 3.39 **	115.7 ± 3.11	
No. of branches/plant	16	07.8 ± 0.42	07.9 ± 0.33	15.3 ± 2.73 *	09.1 ± 0.61	15.6 ± 0.55 **	08.0 ± 0.47	
Days to flower	112	93	88	110	90	110	87	
No. of ovules/pistil	30.3 ± 0.72	31.2 ± 1.24	32.8 ± 1.04	31.4 ± 1.21	32.1 ± 1.34	31.2 ± 0.73	32.9 ± 1.19	
No. of seeds/silique	15.9 ± 0.89	16.4 ± 0.66	18.0 ± 0.88	15.5 ± 0.51 *	18.5 ± 1.16	15.7 ± 0.42 **	18.4 ± 0.66	
Fertilization value (%)	52.48	52.56	54.88	49.36	57.63	50.32	55.93	
No. of silique/ plant	498	400.0 ± 42.14 *	641.3 ± 83.29	533.3 ± 28.80 **	660.0 ± 24.66	569.3 ± 59.69	641.3 ± 62.37	
No. of chromocentres/nucleus	12.3 ± 0.26	10.1 ± 0.15	10.5 ± 0.20	12.1 ± 0.34 **	10.4 ± 0.19	12.1 ± 0.19 **	10.4 ± 0.22	
Meiotic abnormalities (%)								
i) At metaphase I	8.0 ± 1.92	8.5 ± 1.97	5.0 ± 1.54	7.5 ± 1.86	4.5 ± 1.47	7.5 ± 1.86	5.0 ± 1.54	
ii) At anaphase I	7.5 ± 1.86	8.0 ± 1.92	5.0 ± 1.54	8.5 ± 1.97	5.0 ± 1.54	7.0 ± 1.80	5.0 ± 1.54	
Pollen viability (%)	40.8	60.0 ± 2.19 **	93.0 ± 1.14	42.2 ± 2.21 **	95.0 ± 0.97	56.9 ± 2.22 **	94.6 ± 1.01	

\*\* - Significant at 1.0% level. \* - Significant at 5.0% level.

# EFFECT OF RADIATION AND CHEMICAL MUTAGENESIS ON TURNIP (*Brassica rapa* L.)

## I. ROOT WEIGHT & DAYS TO FLOWER

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### ABSTRACT

Gamma rays and Ethyl methane sulphonate (EMS) cause gradual decrease in root weight of turnip (*Brassica rapa* L.) from lower to higher doses. There is delay in flowering due to radiation, but EMS has negligible effect on it.

### INTRODUCTION

The plant breeders are always interested in experimental modifications of the germplasm of the crop species. Radiation and chemical mutagenesis have been exploited to create variability. The cross-pollinated crops like turnip have enough genetic variability already in nature. Despite this, recently some attention has been paid to increase variability in this root crop to exploit the mutated genes for mutational heterosis and to induce male sterility for use in hybrid seed production (2,4,7). Present study was undertaken to know the effect of gamma rays and EMS on root weight and days to flower of turnip.

### MATERIAL AND METHODS

'Rose Red' variety of turnip constituted the material for present investigation. Five samples, each consisting of 1000 air dried seeds, were irradiated at the doses of 100 KR, 150 KR, 200 KR, 250 KR and 300 KR of gamma rays from  $^{60}\text{Co}$  source at the Bhabha Atomic Research Centre (BARC), Mumbai. For EMS treatment, 300 moist seeds were treated in 1.2%, 1.3%, 1.4%, 1.5% and 1.6% (V/V) solutions separately for 6 hours duration. Then the treated seeds were immediately sown in the field at Hazaribag to raise  $M_1$  plants along with the Control.  $M_2$  seeds were obtained from these through selfing. During this period, relative humidity and temperature ranged between 88-99% and 22°C - 29°C, respectively. The roots of randomly selected plants were pulled out from the soil just before the emergence of the flowering shoot to compute mean root weight. The date of appearance of the first flower and its difference from the date of sowing gave the number of days to flower. All the results are presented in Table 1 and Table 2.

### RESULTS AND DISCUSSION

At the higher doses like 250 KR and 300 KR of gamma rays only a few abnormal seedlings developed which soon turned yellow and ultimately died. A gradual decrease in root weight was observed from lower to higher doses. Some recovery in this trait was noted in  $M_2$  generation at all the doses, but not to the extent of Control. So far days to flower was concerned, there was a progressive delay in flowering owing to the effect of gamma rays from lower to higher doses. Some earliness in this regard was observed in  $M_2$  at all the doses, but not to the level of Control (Table 1). More or less, negligible effect of EMS was noted on days to flower (Table 2).

First and foremost goal of a plant breeder is to achieve higher yield in any crop. Turnip is basically a root crop plant which loses its commercial value after the emergence of the flowering shoot. Hence, delay in flowering is beneficial. There is deleterious effect of radiation and EMS on root weight of turnip. Some earlier workers also noted the similar effects on 'Purple Top White' cultivar of turnip (4, 7). Alteration in root morphology has been reported by some workers in other crops due to radiation (1, 3, 5, 6). Delay in flowering with increasing doses of gamma rays is the direct effect of radiation and is dose dependent (4, 7). It may be attributed to disturbed hormone and enzyme synthesis. The changes induced in the meristem which help in increasing the of vegetative phase, could be the reason for delayed flowering (3). It is possible that the damages caused by higher doses of gamma rays, the meristematic tissue might have delayed the emergence of the flowering shoot.



**Table 1. Effect of gamma rays on root weight and days to flower in turnip**

Dose	Generation	Root weight (g)		Days to flower
		Mean SE $\pm$	CV (%)	
Control	M <sub>1</sub>	311.0 $\pm$ 14.45	14.70	75
	M <sub>2</sub>	265.0 $\pm$ 20.06	23.94	76
100 KR	M <sub>1</sub>	185.0 $\pm$ 10.61 **	08.11	85
	M <sub>2</sub>	206.0 $\pm$ 11.50 *	17.66	78
150 KR	M <sub>1</sub>	157.5 $\pm$ 22.98 **	20.63	89
	M <sub>2</sub>	131.0 $\pm$ 14.93 **	36.04	80
200 KR	M <sub>1</sub>	120.0 $\pm$ 28.67 **	41.39	101
	M <sub>2</sub>	156.0 $\pm$ 9.19 **	18.62	90

\*\* - Significant at 1.0% level. \* - Significant at 5.0% level.

**Table 2. Effect of EMS on root weight and days to flower in turnip**

Dose (%)	Generation	Root weight (g)		Days to flower
		Mean SE $\pm$	CV (%)	
Control	M <sub>1</sub>	143.3 $\pm$ 9.87	26.68	91
	M <sub>2</sub>	160.0 $\pm$ 16.55	40.05	88
1.2	M <sub>1</sub>	88.0 $\pm$ 10.16 **	44.73	89
	M <sub>2</sub>	154.7 $\pm$ 15.05	37.69	88
1.3	M <sub>1</sub>	86.3 $\pm$ 7.45 **	33.05	96
	M <sub>2</sub>	156.7 $\pm$ 12.46 **	30.83	93
1.4	M <sub>1</sub>	80.0 $\pm$ 8.54 **	41.33	91
	M <sub>2</sub>	120.7 $\pm$ 10.64 *	34.16	91
1.5	M <sub>1</sub>	73.3 $\pm$ 13.02 **	68.76	88
	M <sub>2</sub>	118.7 $\pm$ 8.89 *	29.01	84
1.6	M <sub>1</sub>	70.0 $\pm$ 13.79 **	76.31	96
	M <sub>2</sub>	139.3 $\pm$ 13.01	36.17	93

\*\* - Significant at 1.0% level. \* - Significant at 5.0% level.

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## Taxonomic relationships among *Erucastrum* and *Brassica* species based on flavonoid compounds

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### INTRODUCTION

A comparative study using numerical taxonomy was carried out to determine the relationships among some species of *Erucastrum* and *Brassica* genera using flavonoid compounds, which data, separately, have been previously published (Sánchez-Yélamo 2000, 2001 and 2002).

### MATERIAL AND METHODS

The seeds from plant material used are kept at the germplasm bank of the Departamento de Biología Vegetal de la Escuela Técnica Superior de Ingenieros Agrónomos de Madrid, Spain. The taxa in study and their gametic number are indicated in Table 1. The methodology used to obtain phenolic compounds from leaves, as well as the isolation and identification of flavonoids, was indicated elsewhere (Sánchez-Yélamo, 2000). The data on species relationships in *Erucastrum* and *Brassica* used in this study include Sánchez-Yélamo (2000, 2001, and 2002) The numerical analysis were made with data of presence/absence of compounds of the samples, with those a data matrix was elaborated and a principal components analysis was made using DCENTER and a two-scatter diagram was obtained using MXPLOTG program of NTSYS computer programs (Rolf, 1994).

Table 1. Plant material

TAXON	Gametic number
<i>B. oxyrrhina</i> (Box)	9
<i>B. tournefortii</i> (Bto)	10
<i>B. barrelieri</i> (Bba)	10
<i>B. maurorum</i> (Bma)	8
<i>B. spinescens</i> (Bsp)	8
<i>B. fruticulosa</i> subsp. <i>fruticulosa</i> (Bff)	8
<i>B. fruticulosa</i> subsp. <i>mauritanica</i> (Bfm)	16
<i>B. cossoniana</i> (Bco)	16
<i>E. varium</i> (Eva)	7
<i>E. virgatum</i> (Evi)	7
<i>E. strigosum</i> (Est)	8
<i>E. littoreum</i> subsp. <i>glabrum</i> (Elg)	8
<i>E. littoreum</i> subsp. <i>littoreum</i> (Eli)	16
<i>E. littoreum</i> subsp. <i>brachycarpum</i> (Elb)	24
<i>E. elatum</i> (Eel)	15

### RESULTS AND DISCUSSION

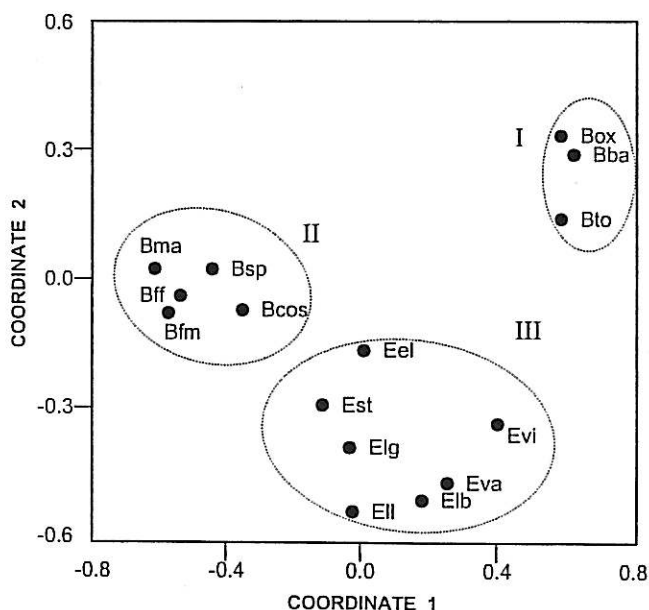


Figure 1. Relationships of taxa based on flavonoid compounds

A total of twenty-three flavonoids were isolated from the foliar extracts of the taxa surveyed. The compounds were glycosides derivatives of kaempferol, quercetin and/or isorhamnetine. The identified flavonoids flavonoid types (O- flavonol-glycosides), the sugars and the glycosylation level (3-O; 7-O; and/or 3-O,7-O - glycosides) which chromatographic characteristics and UV spectral data were reported previously (see Sánchez-Yélamo, 2000, 2001, 2002). Each taxon shows a specific chromatographic pattern, where we can observe a great number of chemical similarities among taxa; however, also some differences were detected, that permit us to establish an inter- specific, intergeneric and even intrageneric differences, specially the distinctive characteristic of *Erucastrum* taxa (at least those studied here) that appears to have incapacity to synthesise isorhamnetin.

Numerical results of present paper are showed in Fig. 1 where the taxa distribution appears in three groups designated as group I, II and III. The relationships among all species reinforces the results obtained when the genera were studied separately (Sánchez-Yélamo, 2001, 2002).

*Brassica* taxa surveyed no shared any compound common to all of them, being the group constitute by *B. maurorum*, *B. fruticulosa*, both  $n=8$ , and *B. fruticulosa* subsp. *mauritanica* and *B. cossoniana*, both  $n=16$  that appear to have the most similar patterns, and clearly different to *B. oxyrrhina* ( $n=9$ ), *B. tournefortii* and *B. barrelieri* (both  $n=10$ ). All taxa of *Erucastrum* share only one compound (kaempferol 3-galactoside-7 rhamnoside), in spite of this fact they appear grouped in the scatter diagram, probably due to the absence of isorhamnetin glycosides.

The tetraploids, *B. fruticulosa* subsp. *mauritanica* and *B. cossoniana* (formerly *B. fruticulosa* subsp. *cossoniana*) were placed into the *B. cossoniana* cytodeme (Harberd, 1972). As can see it in the scatter diagram (Fig. 1), they appear very close to *B. fruticulosa* and *B. maurorum* and *B. spinescens* (*B. fruticulosa* cytodeme), being all of them of Section *Micropodium* DC (Gómez-Campo, 1999). Harberd & McArthur (1976) point out that different species of the same cytodeme generally behave in the same way, but sometimes it might be argued that all these species share a common genic pool, probably from a hypothetical ancestral type, and the tetraploids arose from the common genic stock of the taxa that constitute the *B. fruticulosa* cytodeme, and not necessarily from their diploid subspecies.

*B. oxyrrhina* ( $n=9$ ), *B. tournefortii* and *B. barrelieri* (both  $n=10$ ) constitute a morphological related group that were included in Section *Sinapistrum* Willk. by Salmeen (1979), being also some time ago *B. oxyrrhina* subordinated as subspecies of *B. barrelieri* (Heywood, 1964). The differences, both in chromosome number and their crossability (*B. barrelieri* is allogamous), were enough to consider both taxa with a separate specific status (Salmeen, 1979). Harberd (1972), on the other hand, placed these species in different cytodemes, which in the case of different chromosome number ( $n=9$  vs.  $n=10$ ) is obvious, as well as the allogamy vs. autogamy condition in the case of *B. barrelieri* and *B. tournefortii*. The three taxa show the capacity to synthesise mono-, di- and triglycosides of kaempferol, quercetin and isorhamnetin, but they no share any compound common to the three species. Out of 13 molecules in this group, *B. oxyrrhina* and *B. barrelieri* share only one, and four compounds are shared between *B. barrelieri* and *B. tournefortii* (Sánchez-Yélamo, 2000). The results obtained in present study appear to indicate some genetic affinities among these taxa, however, the results obtained from different calculations clustering methods have show a reticulated taxonomic relationships among them, since based on morphology (Takahata & Hinata, 1986) founded that *B. oxyrrhina* appear nearest to *B. tournefortii*; while on the basis of biochemical (Sánchez-Yélamo *et al.*, 1992), or molecular traits (Warwick & Black, 1993), *B. barrelieri* and *B. oxyrrhina* are the closest.

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## A chemotaxonomic approach to *Moricandia* DC. (Brassicaceae) using seed globulin electrophoretic patterns

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### INTRODUCTION

The use of electrophoretic methods with seed proteins has become one of the most popular techniques for plant characterisation, taxonomy and phylogeny during the last decades. In this sense, seed proteins banding profiles have proved to be useful complementary information to other biological characters. As consequence, a great number of taxonomic investigations have utilised seed storage proteins, since they are unaffected by environmental conditions, are highly stable and very conservative from an evolutionary point of view. In seeds of crucifers, the most abundant storage proteins are globulins, especially cruciferin, a legumin-like 12S storage protein that has been characterised in *Brassica napus* (Dalgarrondo et al., 1986, Rödin et al., 1990). The genus *Moricandia* DC. (subtribe *Moricandiinae* of *Brassicaceae*) is often considered to belong to the *Brassica* coenospecies on the basis of morphological and cytogenetical similarities (Gómez-Campo, 1999). It is comprised by eight species adapted to tolerate drought and arid conditions in the Mediterranean, Irano-Turanian and Saharo-Sindian regions. The distribution of taxa diversity, at specific and infra-specific level, seems to indicate that the origin center of the genus is found in NW Africa or SE of Iberian Peninsula (Sobrinho-Vesperinas, 1997). As a part of a more extensive chemotaxonomic and molecular revision of this genus, in present paper we report preliminary results of the relationships among taxa of *Moricandia* using globulin electrophoretic patterns.

### MATERIAL AND METHODS

**Plant material** - Seeds of 13 studied accessions were collected from their natural habitats and stored under long-term preservation conditions (Gómez-Campo, 1990) at the germplasm bank of the Dept. de Biología Vegetal, E. T. S. de Ingenieros Agrónomos de Madrid (Spain) (Table 1).

Table 1. Plant material studied in the electrophoretic analysis of globulins.

Accession number(*)	TAXON	ORIGIN
GC-9304-96	<i>Moricandia arvensis</i> (L.) DC.	Tahal-Tabernas (Almería, Spain)
GC-0863-66	<i>M. arvensis</i> (L.) DC.	Jumilla (Murcia, Spain)
GC-6665-84	<i>M. arvensis</i> (L.) DC.	Catania (Sicily, Italy)
GC-3660-75	<i>M. arvensis</i> (L.) DC. f. <i>robusta</i>	El Kantara (Algeria)
GC-3670-75	<i>M. arvensis</i> var. <i>garamantum</i> Maire	Tamanrasset (Algeria)
GC-4073-76	<i>M. foetida</i> Bourgeau	Tabernas (Almería, Spain)
GC-5549-80	<i>M. foleyi</i> Batt.	Merzuga (Morocco)
GC-9414-97	<i>M. moricandioides</i> (Boiss.) Heywood	Benamejí (Córdoba, Spain)
GC-2276-77	<i>M. moricandioides</i> (Boiss.) Heywood	Alcoy (Alicante, Spain)
GC-2129-72	<i>M. nitens</i> (Viv.) Durd. & Barr	Botanical Garden of Tohoku Univ. (Japan)
GC-3748-75	<i>M. sinaica</i> Boiss.	Behbahan (Iran)
GC-1845-70	<i>M. spinosa</i> Pomel	Drahu (Algeria)
GC-9274-96	<i>M. suffruticosa</i> (Desf.) Coss. & Dur.	Missur (Morocco)

(\*) In the germplasm bank of Departamento de Biología Vegetal, Escuela Técnica Superior de Ingenieros Agrónomos de Madrid (Spain).

**Protein extraction and electrophoresis** - Five seeds per accession were homogenised and salt soluble proteins (globulins) were extracted in a 0.4M NaCl buffer with 3% mercaptoethanol during 18 h at room temperature. After centrifugation, supernatant was boiled for 4 min under reducing conditions as in Sánchez-Yélamo et al. (1992). Protein electrophoresis (SDS-PAGE) was carried out following Laemmli (1970) system. Three replications were made.

**Data analysis** - The banding patterns obtained were analysed to estimate the relationship among taxa. Each polypeptide (band on the gel) was considered as a qualitative character, and treated as a binary character in a data matrix (coded presence or absence as 1 and 0 respectively). Clustering analysis were made by UPGMA method using the Jaccard' coefficient, and a dendrogram was also obtained using NTSYS computer programs (Rohlf, 1993).

## RESULTS AND DISCUSSION

Electrophoretic analysis of *Moricandia* taxa globulins showed two banded-zones on gels corresponding with heavy  $\alpha$  chains (aprox. 30 KDa) and light  $\beta$  chains (aprox. 20 KDa) groups of cruciferins, founded by Rödin et al. (1990) in *B. napus*. In our case, as many of 15 different and clearly resolved bands of polypeptides were detected on gels of *Moricandia* taxa studied, and several qualitative differences have been observed among some of them. *M. foleyi* showed the most distinctive electrophoretic pattern; all *M. arvensis* accessions showed identical pattern, and the same is true with respect to *M. nitens*, *M. spinosa* and *M. suffruticosa*. A great similarity between *M. arvensis* f. *robusta* and *M. arvensis* patterns was observed. However, they could be observed quantitative and qualitative differences between the two accessions of *M. moricandioides*. They were detected specific patterns in the rest of taxa. None quantitative differences respect to the width of the bands were detected respect to ploidy level of the species.

The dendrogram (Fig. 1), shows the relationships of taxa on the basis of their globulin profiles. In a general way, these results are in agreement with the work of Sobrino-Vesperinas (1983) from a morphological and caryological point of view. The endemic taxon *M. foleyi* appears to be the most distinctive respect to the rest of species. Its lowest value of similarity compared to the other taxa in globulins polypeptides (very conservative) together with other caryological and morphological features, as well as geographic distribution (it is endemic in Morocco), possibly point it this species involved in the origin of this genus.

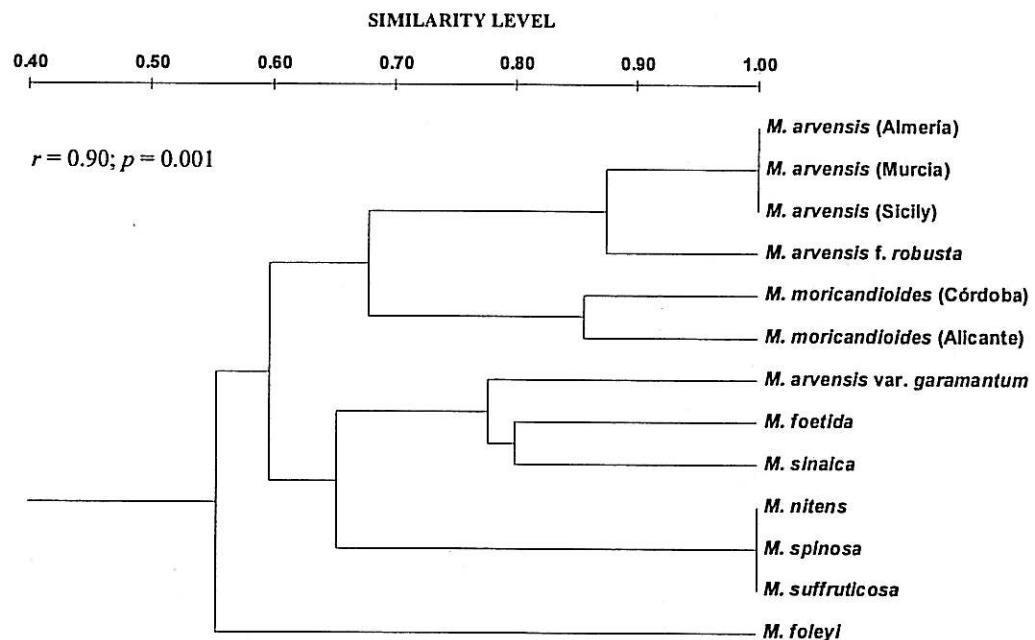


Figure 1. Dendrogram derived from a UPGMA cluster analysis, using Jaccard's coefficient based on the globulin patterns.

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# INDUCED PODDING MUTANTS OF INDIAN MUSTARD (*BRASSICA JUNCEA* L. CZERN & COSS)

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## ABSTRACT

The performance of two induced mutants, viz., bunching and appressed pods of *Brassica juncea* cv. RH 30 was studied. One of them, i.e. with bunching pods is dwarf, has more seed weight and oil content. The mutant with appressed pods is superior in respect of plant height, number of primary and secondary branches and seed yield though it is as good as parental stock in respect of oil content.

## INTRODUCTION

Seeds of *Brassica juncea* cv. RH 30 were exposed to different doses of gamma rays (source  $^{60}\text{Co}$ ) ranging from 20-60 kR each followed by treatment with 0.25% EMS, Ethyl methane sulphonate (freshly prepared in 1 M phosphate buffer, pH 7.4) for 16 h and from the M<sub>3</sub> generation of treatment with 20 kR gamma rays + 20% EMS various mutants including interesting ones viz., bunching pods and appressed pods were selected and their true breeding stocks prepared. The present communication reports the data on preliminary studies conducted on the performance of these mutants.

## MATERIALS AND METHODS

Seeds of mutant RH 30 M6 (Bunching pods) and RH 30 M13 (Appressed pods) were grown in field following standard cultivation practices. After 50% flowering, the branches of unopened buds of the plants were covered with parchment paper bags and kept thus till pod formation to avoid any cross-pollination. These were retested for any segregation in the preceding four rabi seasons in a plant to row system. As there was no further segregation, the plants were marked as true breeding and hence pure true breeding stocks were prepared. These mutants were grown along with the parental genotype cv. RH 30 in three replications in RBD and various observations were recorded.

## RESULTS AND DISCUSSION

The data recorded on various parameters in two mutant stocks i.e. RH 30 M6-1 (bunching pods) and RH 30 M13 (appressed pods) along with the parental stock cv. RH 30 of *Brassica juncea* are presented in Table 1. Both the mutants were shorter in height, matured earlier, had more secondary branches and seed yield/plant. One of these, RH 30 M6-1 (bunching pods) was very dwarf (116.2 cm) and had more 1000-seed weight (7.16 g) and oil content (45%). The other mutant with appressed pods was superior to the parental variety in respect of plant height (60.8 cm), number of primary branches (6.8), secondary branches (29.8) and siliquae/plant (954.8) and the seed yield/plant (36.1 g). Thus, the mutant with bunching pods is dwarf, early and has higher oil content while the other one with appressed pods has higher seed yield per plant. These mutants are being exploited in further programme of *Brassica juncea* improvement.

TABLE 1

Mean performance of podding mutants of *Brassica juncea* cv. RH 30

Genotype	Days to 50% flowering	Plant height (cm)	No. of primary branches/plant	No. of secondary branches/plant	No. of siliquae/plant	No. of seeds/silique	Silique length (cm)	Seed yield/plant (g)	1000-seed weight (g)	Oil content (%)
RH 30 (Control)	69.6 ±0.24	179.0 ±12.08	5.4 ±0.50	20.2 ±3.46	566.0 ±87.09	12.6 ±0.30	5.9 ±0.17	31.12 ±4.85	6.78 ±0.24	42.5
RH 30 M6-1	59.6 ±0.24	116.2 ±3.32	5.0 ±0.54	23.2 ±2.74	696.4 ±81.71	11.8 ±0.36	5.2 ±0.13	32.7 ±5.20	7.1 ±0.58	45.0
RH 30 M13	60.8 ±0.37	165.0 ±6.70	6.8 ±0.80	29.8 ±3.51	954.8 ±121.05	12.7 ±1.49	5.1 ±0.23	36.1 ±5.67	4.4 ±0.36	42.6

# Intergeneric hybridization between *Brassica rapa* and *Coincya pseuderastrum*, and the meiotic behavior

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## Introduction

The wild relatives are often variable source of useful genes for crop plants and the studies on the phylogenetic relationships between crop plants and wild relatives are important. Many interspecific and intergeneric hybrids had been obtained by conventional pollination and embryo rescue techniques and their meiotic chromosome associations had been reported (Harbert and McArther 1980; Inomata 1997; Glimelius 1999; Prakash et al. 1999). This study produced intergeneric hybrid between *Brassica rapa* and *Coincya pseuderastrum* and showed the behavior of meiotic chromosome associations of the F<sub>1</sub> hybrid. The crossability of the progeny was further investigated.

## Materials and Methods

The materials used in the experiment were *Brassica rapa* (syn. *campestris*) ssp. *chinensis* cv. Seppakutaina (2n=10; AA) and *Coincya* (syn. *Heutera*) *pseuderastrum* (2n=24; CpCp) which seed was provided by Gómez-Campo. When emasculated flowers of *B. rapa* bloomed, the conventional pollination was made with fresh pollen grains of *C. pseuderastrum*. The crossed ovaries were cultured in according to the previous paper (Inomata 1990). The medium used in the experiment was MS medium (Murashige and Skoog, 1962) with 300mg/l of casein hydrolysate (CH). When growing embryos were obtained in the ovaries, the embryos further cultured in the 1/2 MS medium with 300mg/l of CH. Somatic chromosomes and chromosome associations in the first meiotic division were checked by using the method of Inomata (1994).

## Results and Discussion

One hundred and twenty-six ovaries were cultured at 3 days after pollination. One seed and one late torpedo-shaped embryo were obtained. They were further cultured in the 1/2MS medium with 300mg/l of CH. One seed did not germinated and one late torpedo-shaped embryo developed into matured in the field. The hybrid plant showed 34 chromosomes. It might consisted of normal female gamete of *B. rapa* and unreduced male gamete of *C. pseuderastrum*. The leaves and flowers of the F<sub>1</sub> hybrid were intermediate in morphology between the parents. Table 1 shows the



call it a “super-long silique” line. We also named this line (H218) as “foot long silique rape”. In 2002, the super long silique line was further observed on a whole plant basis. The average silique length was 16.72cm and the extreme length remained 25.2cm(table 1). In all the 3 years, the super long silique line appeared genetically stable.

The super long silique line is a unique novel *B. napus* line. It was derived from an interspecific hybridization between a semi-wild landrace of *B. campestris* L. from Eastern Tibet and a vegetable cabbage “Bai-Hua-Jie-Lan” (*B. oleracea* var. *alboglabra*, Bailey) from Yunnan Province, P. R. China. Its extremely long silique character can be of high values in rapeseed breeding and genetic studies. Initial investigations have shown that the average silique lengths of the F1 hybrids between the super long silique line and the normal rapeseed lines were near the middle parent values, and the F2 progenies showed a continuous distribution in Silique length, characteristic of a quantitative trait. The F1 hybrids also showed higher numbers of seeds per silique and larger 1000-seed weights than the normal parent lines. As a result, the F1 hybrids had a higher seed yield per plant. These results were consistent with those observed by Chay P. et al(1989) and Wei W. L.(2000). However, this super long silique line was per se low in seed setting rate in present( lower than 80%), but its hybrid with normal rapeseed lines appeared normal in seed setting..

The super-long silique line (H218) is also useful in the genetic studies. It is a unique novel material for studies on the mechanism of silique elongation and development, the rules of inheritance of silique lengths, the modes of gene actions, and the molecular basis of rapeseed silique length control. This material has enriched the genetic resources of rapeseed.

Tab. 1 The Silique Lengths of H218 observed in 2000-2002<sup>1)</sup>

Year	Popu- Lation/ plants	Sample/ plants	Silique length/cm			Body length/cm			Beak length/cm		
			Range	Mean	Longest	Range	Mean	Longest	Range	Mean	Longest
2000	40	5	16.58-19.34	18.12	30.5	13.72-16.20	15.25	25.0	2.73-3.12	3.07	5.5
2001	40	5	16.59-24.36	19.34	31.5	13.97-20.73	16.26	26.1	2.50-3.63	2.98	5.4
2002	120	10	13.84-18.76	16.72	25.2	11.43-15.95	13.95	21.3	2.41-3.24	2.76	3.9

1) In 2000 and 2001, 5 siliques were taken as a sample from the middle place of the main raceme of each plant; in 2002, 50 sample siliques were randomly taken as a sample from the mixed siliques of each whole plant.

# A super-long silique variant developed from the resynthesized rapeseed

(*Brassica napus* L.)

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**Abstract:** From resynthesized *B. napus* lines a group of specially long silique lines were indentified. Among them a super long silique line was selected, i.e. H218. The average silique length reached 19.34cm, with a body length of 16.26cm. The extreme length of siliques in this line reached 31.5cm, with a body length of 26.1cm. This is the longest rapeseed silique ever found in Brassica. This super long silique line, named "foot long silique rape", is of high values in rapeseed breeding and genetic studies.

**Key word:** *Brassica. napus* L.; Resynthesized line; Super long silique

In existing *Brassica napus*, the lengths of siliques are normally 5-8 cm, with body lengths of 4-6 cm. A few scientists have reported rapeseed materials with silique lengths of more than 10 cm long (Chay P. et al., 1989; Liu D. F. et al., 1994). Since 1980's, we have collected a number of landraces, wild and semi-wild forms of *B. cmpestris* L. and *B. chinensis* (L.) Makino, including oilseed types and vegetable types from SW China. Reciprocal crosses were made with these materials and a local vegetable cabbage called "Bai-Hua-Jie-Lan" (*B. oleracea* var. *alboglabra* Bailey). A large group of new *B. napus* lines were resynthesized (Niu Y. Z. et al., 1999).

In 2000, over 160 resynthesized lines were investigated in field with 30-40 plants each. Among the lines, 21 specially long silique lines were identified, with an average silique length of 10-18cm (Niu Y. Z. et al., 2002). The line of the longest siliques was H218 (field code in 2000), showing an average silique length of 18.12cm, with a body length of 15.25cm. Its longest silique was 30.5cm, with a body length of 25cm (Table 1). All the 21 specially long silique lines were further investigated in 2001. Eighteen of them remained specially long in silique lengths, with average silique lengths between 10-19cm. The line of the longest siliques remained to be "H218", with an average silique length of 19.34cm and a body length of 16.26cm. In this line the extreme length of siliques reached 31.5 cm, with a body length of 26.1cm (Fig. 1, Table 1). This was the longest rapeseed silique ever found in *Brassica*. We

results for pollen fertility and chromosome associations in the first meiotic division. No development of filaments and anthers was obtained in the flowers. No pollen fertility was observed. The mode of chromosome associations was  $14_{II}+6_I$ . The bivalent association ranged from 11 to 15, with a mean of 13.12. A few trivalent was also observed. Partial chromosomal homology might exist in allosyndesis of the  $F_1$  hybrid. The type of microspore mother cell of the  $F_1$  hybrid showed dyad and tetrad. Most of them were tetrad. No another types of microspore mother cell were observed. No seeds were obtained from the  $F_1$  hybrid backcrossed with *B. rapa* in 94 flowers.

Table 1. Pollen fertility and chromosome associaiton in the first meiotic division of the  $F_1$  hybrid of *Brassica rapa* x *Coincya pseudorcastrum*

Chromosome number in root tip (2n)	Pollen fertility (%) <sup>a</sup>	Number of PMCs observed	Mean chromosome associations per cell at metaphase I (range in parenthesis)		
			III	II	I
34	0	42	0.05 (0-1)	13.12 (11-15)	7.62 (4-12)

<sup>a</sup>: 500 pollen grains were counted.

#### Acknowledgement

I would like to thank Dr. C. Gómez-Campo, at Universidad Polytecnica, Madrid, Spain for providing the seed of *Coincya pseudorcastrum*.

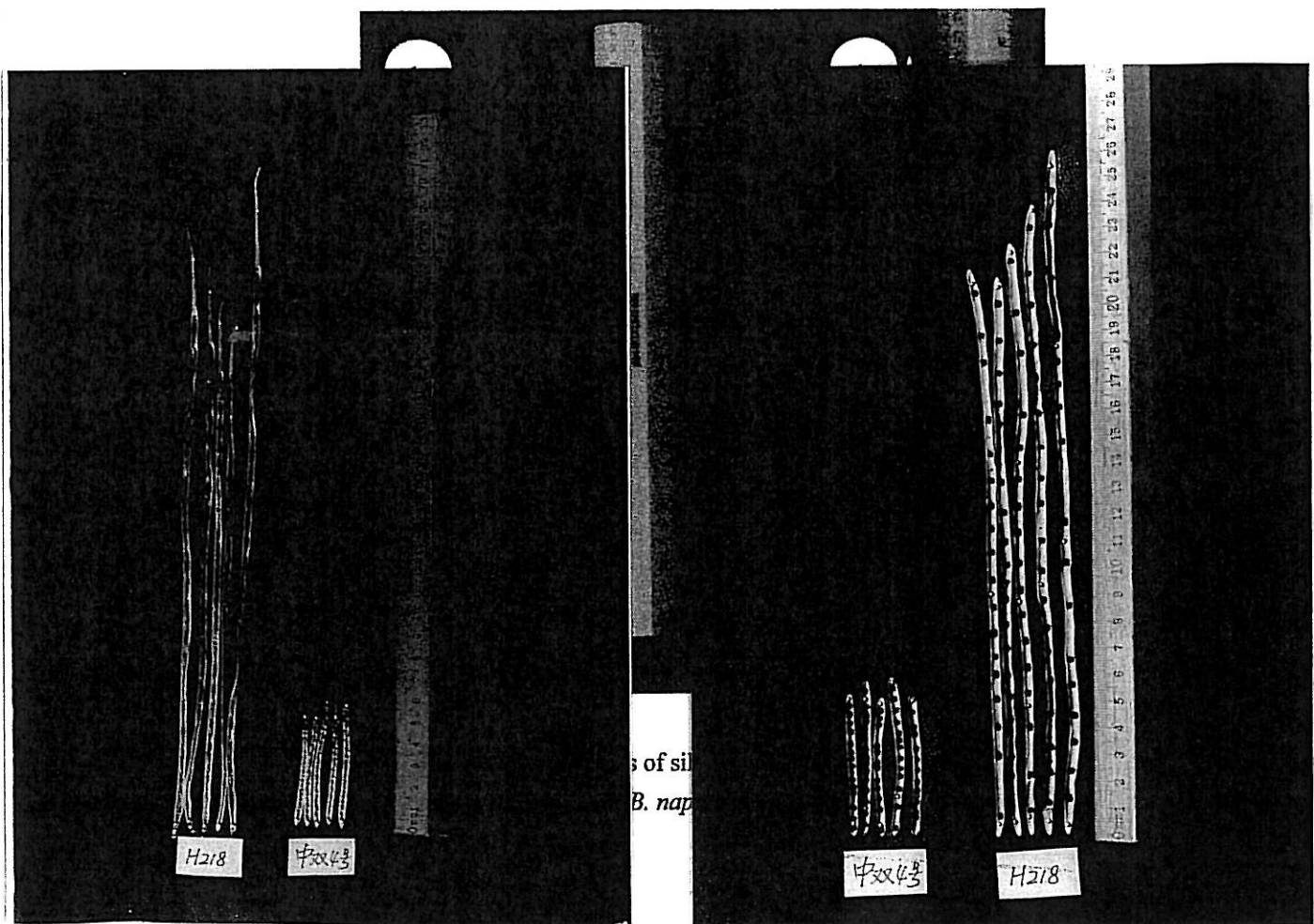
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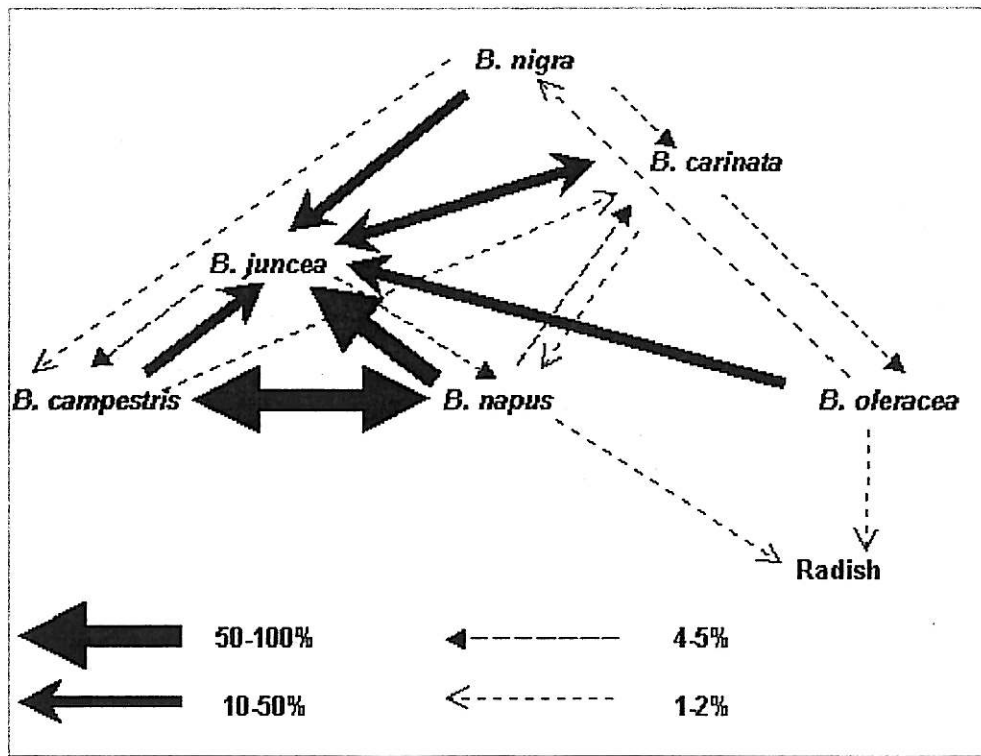
## A Review of crossing relationship between cultivated Brassica species

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After reviewing the literature on the hundreds of handcrossing experiments published the following diagram was created (Stewart 2002).

The arrows represent the direction of pollen flow and the size of the arrow represents the likely seedset in the cross using hand pollinated emasculated flowers. However, it must be understood that although these results represent what has been generally reported any specific cross could be different, usually, but not always in the direction of lower seedset.

Arrows have not been included where the reported seedset is below 0.1% as all combinations have been crossed. The seedset of crosses with *S. alba*, *S. arvensis*, *Eruca sativa* and many other Brassica relatives are almost all less than 0.1% so they are not included in this diagram. It should be noted that for the plant breeder the rate of hybrids obtained can be increased significantly by using embryo and ovule culture techniques.



In this diagram the allotetraploid *B. juncea* is placed closer to *B. campestris* than *B. nigra* and *B. carinata* is placed closer to *B. nigra* than *B. oleracea* because of their

closer affinity. *B. napus* is placed in an intermediate position between *B. campestris* and *B. oleracea* (Song et al 1994).

The rate of natural crossing in the field will be orders of magnitude less than this. For example crops of *B. juncea* adjacent to *B. napus* which can have near normal seedset on handcrossing will typically only have 3% contamination in the field (Bing 1991, Jorgensen et al. 1994). Although radish can be handcrossed with *B. oleracea* or *B. napus* with a 1% seedset there are very few reports of crossing between adjacent crops in the field.

It is interesting to note in this diagram of the promiscuous nature of *B. juncea* having a reasonable seedset in crosses with all the other Brassica species, this offers considerable potential to act as a bridge between species.

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# MONOSOMY ASSOCIATED WITH APETALOIDY IN *BRASSICA JUNCEA*

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Apetaloidy is a valuable trait in *Brassica* breeding since it contributes to reducing sclerotinia stem rot incidence and can be associated with enhanced photosynthetic activity (Singh *et al.* 1991). Apetalous breeding lines have been developed in *B. napus* (AACC,  $2n=38$ ) (Buzza 1983) and *B. juncea* (AABB,  $2n=36$ ) (Singh *et al.* 1991). The apetalous characteristic is controlled by two loci in *B. napus* (Buzza 1983; Fu *et al.* 1990) and *B. juncea* (Singh *et al.* 1991). In the present paper, we report on meiotic studies on a spontaneous apetalous plant from Canadian condiment mustard cultivar Cutlass and on plants of its selfed progeny.

## Materials and Methods

An apetalous plant was identified in a population of plants of *B. juncea* cv. Cutlass growing in the greenhouse. This plant was virtually apetalous at the early stage of development but produced some petalous flowers as it matured. The plant was selfed and self-progeny was raised under greenhouse conditions. The average number of petals on the first 25 flowers was determined on 34 self-progeny plants.

Meiotic preparations were made according to Cheng *et al.* (2002). The chromosome number and meiotic behaviour of the apetalous plant and selfed progeny were examined in a minimum of 10 pollen mother cells (PMCs) at metaphase I (MI) and/or anaphase I (AI).

## Results and Discussion

The apetalous plant was monosomic with  $2n=35$  chromosomes. Seventeen bivalents and one small univalent were observed in a total of 47 PMCs. At AI, chromosome segregation was 18-17 chromosomes.

Twelve self-progeny plants (35.3%) were virtually apetalous with petal number ranging from 0 to 1 petal per flower, as observed in the parent plant at the same stage of development. The remaining 22 plants (64.7%) had a high petal number ranging from 3 to 4 petals per flower.

All apetalous plants were monosomic with  $2n=35$  chromosomes forming 17 bivalents and 1 univalent at MI, while all petalous plants had  $2n=36$  chromosomes forming 18 bivalents. This suggests that genes for petal production are present on the monosomic chromosome and that there is an additive effect. Based on the frequency of apetalous plants in the self-progeny, the monosomic chromosome was transmitted at a frequency of 35.3% through the female and the male gametes.

*Brassica juncea* is an amphidiploid species and has a high tolerance for aneuploidy. Monosomic plants, nullisomic plants and plants with additional chromosomes have been observed in breeding lines derived from interspecific hybridization (Cheng *et al.* 2001; Cheng *et al.* unpublished). In the present study, the apetalous flower trait was found to be

correlated with a missing chromosome. This finding underlines the importance of conducting cytogenetic studies in variants used for *Brassica* research and breeding.

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## Genetic Correlation of Chromocentres and Chiasmata in some cultivated Crucifers

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**Introduction:** Recently chromocentres have been identified as dark staining heteropycnotic bodies in the interphasic nuclei representing pericentric constitutive heterochromatin with varying number and size. (Lima de Faria and Jaworska, 1968; Nagl, 1976). The chromocentres have now acquired special significance in chromosome pairing gene regulation, genetic recombination and evolution. Earlier studies on chromocentres demonstrated that the amount and distribution of constitutive heterochromatin are under polygenic control and depend on the heterozygosity of the material (Kumar, 1997). The present study gives a comparative account of number and distribution of chromocentres and chiasmata in the cultivated crucifers in order to know whether an interrelationship exists between these nuclear characters.

**Materials and methods:** The materials used in the present investigation include five cultivated species of Brassicaceae such as *Iberis amara* L., *Brassica rapa* L., *Brassica campestris* L. var *sarson* Prain., *Brassica oleracea* L. var *botrytis* L. and *Raphanus sativus* L. Their flower buds were fixed in acetic alcohol mordanted with a few drops of  $FeCl_3$  and usual squash technique was adopted. The number of chromocentres was noted from 100 interphasic nuclei of stigmatic papillae cells while the number of chiasmata was noted from 25 PMCs at diakinesis in each material. The data were analysed statistically by applying correlation coefficient.

**Results and discussion:** The chromocentre frequency and chiasma frequency were studied from the buds of the same plants. It was interesting to note that *B. oleracea* var *botrytis* had the minimum frequency ( $10.03 \pm 0.16$ ) while *R sativus* had the maximum value ( $14.25 \pm 0.11$ ). It was further noted that in the distribution pattern of chromocentres *B rapa* and *B oleracea* var *botrytis* resembled each other and *B campestris* var *sarson* resembled to *R sativus* while *I amara* had a distinguished pattern of distribution. The values of chiasma frequency were expressed per bivalent. The results revealed that *B oleracea* var *botrytis* had the maximum value of chiasma frequency ( $1.76 \pm 0.04$ ) while *R sativus* had the minimum value ( $1.34 \pm 0.04$ ) (Table-1). The analysis of correlation coefficient revealed that the chromocentre frequency and chiasma frequency are negatively correlated ( $r = -0.98$ ) and highly significant (Table-2). The significant variation of chromocentre and chiasma frequency between species indicates different levels of heterozygosity of the cultivated crucifers. The results confirm that *B rapa* and *B oleracea* var *botrytis* with almost identical level of heterozygosity are genetically more closer and more evolved than *B campestris* var *sarson*, *R sativus* and *I amara*. The pattern of chromocentres distribution also demonstrates the genetic correlation among the cultivated crucifers and confirms the earlier finding in the varietal populations of radish (Dayal *et al.*, 1982). It is also obvious that *B rapa* and *B oleracea* var *botrytis* with narrow spread of chromocentre distribution might have been evolved parallel to each other from a common ancestor.



**Table-1. Chromocentre frequency and chiasma frequency in some cultivated crucifers.**

Species	Number of chromocentres																				Chromocentre frequency		Chiasma frequency	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	M	+ SE	M	+ SE						
Iberis amara	-	7	9	11	8	10	12	13	12	11	6	-	-	-	12.07	+ 1.26	1.40	+ 0.03						
Brassica rapa	5	13	22	22	15	12	9	1	1	-	-	-	-	-	10.12	+ 0.17	1.65	+ 0.03						
B campestris var sarson	-	-	-	8	23	27	16	12	7	4	2	1	-	-	12.41	+ 1.16	1.51	+ 0.03						
B oleracea var botrytis	5	13	23	32	7	9	7	2	2	-	-	-	-	-	10.03	+ 0.16	1.76	+ 0.04						
R sativus	-	-	-	-	6	24	25	19	14	4	2	2	2	2	14.25	+ 0.11	1.34	+ 0.04						

**Table-2. Correlarion coefficient of chromocentre frequency(X) and chiasma frequency(Y) in some cultivated crucifers.**

Species	X	Y	X <sup>2</sup>	Y <sup>2</sup>	X.Y	r	P
I A	12.07	1.40	145.68	1.96	16.89		
B R	10.12	1.65	102.41	2.72	16.69		
BCS	12.41	1.51	154.00	2.28	18.73	-0.98	0.001
BOB	10.03	1.76	100.60	3.09	17.65		
R S	14.25	1.34	203.06	1.79	19.09		

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# Effect of genome dosage and cytoplasm on petal size in *Brassica*

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## Introduction

Petal size is one of the most obvious differences among various plant organs in *Brassica* species. The flowers and petals of individual plants grown under the same conditions are remarkably uniform in size, showing high heritability. Often, polyploid plants are simply larger than their diploid cousins but the effects of polyploidy in plants are more complex (Soltis and Soltis, 2000). The developmental mechanisms that regulate the inherent petal size of plants in *Brassica* have rarely been investigated until recently. This presentation gives a first evidence for the effect of genome composition and cytoplasm on petal size.

## Materials and methods

Two varieties of *Brassica rapa* ( $2n=20$ , AA), i.e. var. *chinensis* cv. Kosaitai ( $A_{15}A_{15}$ ), var. *pekinensis* cv. Kanzakihanana ( $A_{16}A_{16}$ ) and three varieties of *Brassica oleracea* i.e. var. *alboglabra* cv. Kairan ( $C_{11}C_{11}$ ), var. *gongylobes* cv. KDBF ( $C_{13}C_{13}$ ) and KDWF ( $C_{14}C_{14}$ ) were used in this study to produce amphidiploid and sesquidiploid lines. Amphidiploid lines of *B. napus* ( $2n=38$ , AAC or CCAA) were resynthesized through reciprocal interspecific crosses between *B. oleracea* and *B. rapa* by means of ovule culture and colchicine treatment. Correspondently, six sesquidiploid lines ( $2n=29$ , AAC and CAA) were produced by reciprocally backcrossing the above resynthesized *B. napus* ( $2n=38$ ) with *B. rapa* ( $2n=20$ ). In flowering stage, petal length and width (mm) were investigated. Ten freshly opened flowers for each plant and four plants for each line were measured. After averaging the data from ten flowers of each plant, a data array of  $6 \times 3 \times 4$  was placed to analysis of variance, covariance and correlation for petal length and width (Liu et al., 1984).

## Results and discussions

Accessions of *B. oleracea* showed obviously bigger petal size ( $21.9 \pm 1.3$  mm) than *B. rapa* ( $11.9 \pm 0.8$  mm). Petal length of the amphidiploid lines (AAC or CCAA) ranged from 19.4 to 23.6 mm with no significant difference from *B. oleracea* (Table 1). The sesquidiploid lines ranged from 14.8 to 18.5 mm being intermediate between *B. rapa* and the resynthesized *B. napus* (Table 1). Analysis of variance showed highly significant differences among genomic compositions ( $df=5$ , mean square= $192.3^{**}$ ),

among cross combinations (df=2, mean square=17.7\*\*) and in interaction between genomic compositions and cross combinations (df=10, mean square=6.4\*\*). The heritability of petal length is 96.3%. Results indicated that C-genome enlarged the petals in an additive mode as AA (11.9mm) — AAC(16.9mm) — AACC(21.5mm). Each dosage of C-genome added about 5mm in petal length. However, no significant difference was found between CC (21.9mm) and AACC (21.5mm), indicating that C-genome is epistatic over A-genome. Results also indicated that cytoplasm of *B. oleracea* had positive effect on petal size with a range of 0.6-2.0mm and an average effect being 1.4mm.

In another experiment, we measured the petal length of 15 cross combinations between natural *B. napus* and *B. rapa* (Lu et al. 2001). The average petal length of natural *B. napus* (AACC), *B. rapa* (AA) and their sesquidiploids (AAC) were 14.0mm, 10.4mm and 12.5mm, respectively. Obviously, the petal length of the resynthesized *B. napus* (21.5mm) is generally larger than natural ones (14.0mm), but the tendency of petal change with genome composition was the same. This evidence may indicate that the existing *B. napus* has originated from a certain *B. oleracea* which had a petal size at about 14mm rather than about 22mm or has lost a dosage of C-genome genes for petal length during evolution.

Table 1. Petal length (mm) in various genome compositions

Parental Source	Genome composition					
	CCAA	AACC	CAA	AAC	AA	CC
C <sub>11</sub> C <sub>11</sub> and A <sub>15</sub> A <sub>15</sub>	22.6	20.6	17.7	16.4	11.4	23.3
C <sub>13</sub> C <sub>13</sub> and A <sub>15</sub> A <sub>15</sub>	23.6	23.0	18.5	17.0	13.1	20.0
C <sub>14</sub> C <sub>14</sub> and A <sub>16</sub> A <sub>16</sub>	23.1	21.8	18.1	16.7	12.2	21.6
Mean	23.1	21.8	18.1	16.7	12.2	21.6

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# CYTOGENETIC STUDIES ON SYNTHETIC *BRASSICA JUNCEA* LINES PRODUCED BY SEXUAL AND SOMATIC HYBRIDIZATION

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*Brassica juncea* (genome AABB,  $2n=36$ ) is an amphidiploid species derived from interspecific hybridization between *B. rapa* (AA,  $2n=20$ ) and *B. nigra* (BB,  $2n=16$ ) (U 1936; Song *et al.* 1988). Analyses of chloroplast and mitochondrial DNA restriction patterns have indicated that *B. rapa* is the cytoplasm contributor (Erickson *et al.* 1983; Palmer 1988). The elucidation of the origin of *B. juncea* has opened up the possibility of broadening the genetic diversity of this species by means of resynthesis. Resynthesized plants of *B. juncea*, however, may be cytologically unstable due to intergenomic pairing, as reported in resynthesized *B. napus* (Heneen *et al.* 1995). In this paper, we report on the chromosome number and meiotic behaviour of plants of synthetic *B. juncea* lines produced by sexual and somatic hybridization.

## Materials and Methods

F<sub>2</sub> seed from self-pollinated F<sub>1</sub> hybrid plants of five synthetic *B. juncea* lines developed at the AAFC Saskatoon Research Centre, Canada, was used in the study. Lines 15041 and 15042 were produced by sexual hybridization of *B. rapa* cv. AC Parkland x *B. nigra* accession R1819 and *B. rapa* cv. Tobin x *B. nigra* accession R890, respectively. Lines 15049, 15050 and 15051 were produced by protoplast fusion between *B. nigra* R890 and *B. rapa* Tobin (Campbell 1993). Plants of synthetic lines 15049 and 15050 possess the chloroplast and mitochondrial genomes of the *B. rapa* parent and plants of line 15051 possess the mitochondrial genome of the *B. rapa* parent and the chloroplast genome of the *B. nigra* parent (Campbell 1993).

Meiotic chromosome preparations were made according to Cheng *et al.* (2002). A minimum of 10 pollen mother cells (PMCs) at metaphase I (MI) and/or anaphase I (AI) were studied to determine chromosome number and pairing behaviour. Pollen fertility was inferred from pollen stainability of 300 pollen grains per plant in 1.5% acetocarmine.

## Results and Discussion

Chromosome number, meiotic pairing and pollen fertility were investigated in 19 plants from five resynthesized *B. juncea* lines.

### Synthetic lines of *B. juncea* produced by sexual hybridization

A single F<sub>2</sub> plant of line 15041 was examined and found to have  $2n=35$  chromosomes forming 17IIs + 1I at MI, indicating that the plant was monosomic. The plant had high pollen fertility (95.5%), suggesting that the missing chromosome had little effect on male fertility. In line 15042, six F<sub>2</sub> plants contained  $2n=36$  chromosomes (18IIs) and had high pollen fertility (89.7-97.8%) as expected, while three plants were monosomic ( $2n=35$  chromosomes, 17IIs + 1I) and had low to good pollen fertility (12.9-74.3%).

### Synthetic lines of *B. juncea* produced by somatic hybridization

In line 15049, two plants had the expected chromosome number ( $2n=36$ , 18IIIs) but reduced pollen fertility (43.6-49.8%). A third plant was aneuploid, possibly double monosomic, with  $2n=34$  chromosomes (16IIIs + 2Is). Pollen fertility in this plant was reduced (57.8%). In line 15050, three plants had  $2n=36$  chromosomes (18IIIs) with pollen fertility ranging from 53.6 to 89.0% and two plants were monosomic ( $2n=35$  chromosomes, 17IIIs + 1I) with high pollen fertility (79.0- 91.3%). A single plant of line 15051 was examined. The plant had  $2n=36$  chromosomes (18IIIs) and high pollen fertility (94.1%), suggesting no influence of the novel cytoplasm (*B. nigra* chloroplast genome and *B. rapa* mitochondrial genome) on male fertility.

That various types of aneuploid plants were observed in the  $F_2$  progeny of the resynthesized *B. juncea* lines suggests the occurrence of meiotic instability in the  $F_1$  generation. In addition, monosomic plants ( $2n=35$  chromosomes) had pollen fertility ranging from 12.9 to 95.5%, suggesting that the plants were missing different chromosomes.

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# Effect of Basal Media, Growth Regulators and Putrescine on Callus Induction and Proliferation from Seedling Explants of *Brassica juncea* RH-781

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## INTRODUCTION

Two of the possible strategies considered to overcome freeze injury are transfer of genes responsible for tolerance and selection of variant cells using low temperature as selection pressure. Both of these require large amounts of callus. Here we present efforts made to optimize callus induction and proliferation in *Brassica juncea* RH-781, a genotype developed for frost tolerance. As a part of the ongoing work in this laboratory, putrescine has been compared with NAA and BAP for its effect on proliferation of callus.

## MATERIALS AND METHODS

Seeds of *Brassica juncea* RH-781 were washed with detergent for five min and with tap water for 8 to 10 min. These were then sterilized with 0.1%  $\text{HgCl}_2$  for one min and excess  $\text{HgCl}_2$  removed by washing with sterilized water. One week old seedlings raised from these seeds on MS + 0.8% Agar provided explants for callus induction.

Readymade Murashige and Skoog (1962) media (MS) was dissolved in deionised distilled water. Different concentrations of BAP (benzyl amino purine), NAA (naphthalene acetic acid) and putrescine were added and pH adjusted to 5.8.

Different basal media were purchased from Centron Chemicals Ltd. Sucrose (3%) and agar-agar (0.8%) were added to each medium, apart from 2,4-D (0.5) in case of cotyledon and KIN (0.5) + BAP (0.5) + NAA (0.5) in case of hypocotyls explants.

## RESULTS AND DISCUSSION

MS was the best medium for callus induction from both the explants (Table 1). Although, NM basal medium also showed a significant response, but the callus became hard on proliferation and also did not respond well in regeneration experiments. The explants dried on Knudson's basal medium after turning chlorotic. A few explants survived on LS basal medium, but the amount of callus induced from both explants was comparatively less. Therefore, MS basal medium was used for further studies in selection of freeze tolerant cell lines.

Table - 1 Effect of different Basal media on callus induction from seedling explant of *Brassica juncea* RH-781. Values are per cent explants that responded  $\pm$  S.E.

Explant	KM	LM	NM	MS
Cotyledons	0.00	55.7 $\pm$ 0.07	71.0 $\pm$ .07	83.4 $\pm$ 0.1
Hypocotyls	28.7 $\pm$ 0.04	63.9 $\pm$ 0.2	94.0 $\pm$ 0.03	94.5 $\pm$ 0.06

KM: Knudson's Medium; LM: Linsmair Bedner and Skoog's Medium; NM: Nitsch Medium

Further experiments involving MS medium with different hormones (Fig. 1) showed that MS + 1.0 mg/l BAP was the best medium for raising callus from hypocotyl explants of *Brassica juncea* var RH-781 and hence this medium was used for producing large quantity of callus.

Effect of different concentrations of BAP, NAA and putrescine on callus proliferation (Tables 2) in *Brassica juncea* var RH-781 was studied at 27, 38 and 49 days after inoculation of callus previously initiated from hypocotyl explants as above. Low concentration of BAP ( $5 \times 10^{-6}$  M) showed better response compared to the higher concentrations in all the observations. For NAA, the response was better at 27 days in  $5 \times 10^{-5}$  M concentration, however at 38 and 49 days, the effect of  $10^{-5}$  M was



equally significant. Response of *Brassica* calli for proliferation to the three concentrations of putrescine was poor in all the observations. Thus, of the three chemicals, NAA was the best and at  $10^{-5}$  M it showed highest callus proliferation. Contrary to commonly used BAP, better response to NAA for *Brassica* callus proliferation is an interesting observation.

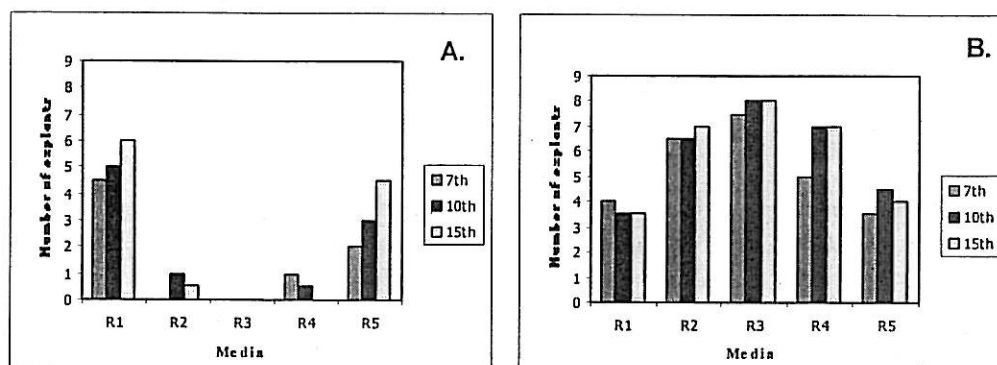


Fig. 1. Effect of different media on callus formation in *Brassica juncea* RH-781. Cotyledon (A) and hypocotyls (B) explants were inoculated in different media and the number of explants out of 8 that formed callus was recorded on 7<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> day after inoculation. R<sub>1</sub> = 2,4-D (4.0 mg/l), R<sub>2</sub> = NAA (1.0 mg/l), R<sub>3</sub> = BAP (1.0 mg/l), R<sub>4</sub> = 2,4-D (2.0 mg/l)+NAA (0.5 mg/l) +BAP (0.5 mg/l), R<sub>5</sub> = 2,4-D (4.0 mg/l)+NAA (1.0 mg/l) +BAP (1.0 mg/l). MS+agar (0.8%) was a part of each medium.

Putrescine showed lesser effect in causing callus proliferation in *Brassica*, compared to BAP and NAA. Polyamines such as putrescine, spermidine and spermine affect a variety of molecular and cellular functions, thereby affecting physiological processes. Bajaj and Manchikatla (1995) have shown that cell regeneration in rice could be improved with spermidine treatment. A higher level of putrescine in comparison to other polyamines was reported in embryonic cell cultures of wild carrot (Feirer et al. 1984). High levels of conjugated and bound polyamines have also been noted in embryo forming callus in *Vigna* species. Meijer and Simmonds (1988) reported that free putrescine level in *Medicago sativa* increases up to 32 folds during cultures on embryonic medium.

Table 2. Effect of BAP, NAA and putrescine on callus proliferation in *Brassica juncea* RH-781. Callus growth was recorded on a 0 to 6 scale. Values are averages  $\pm$  S.E.

Media	27th day	38th day	49th day
MS+Agar+BAP ( $5 \times 10^{-6}$ )	2.50 $\pm$ 0.022	3.41 $\pm$ 0.223	4.00 $\pm$ 0.327
MS+Agar+BAP ( $10^{-5}$ )	1.83 $\pm$ 0.202	1.91 $\pm$ 0.253	3.17 $\pm$ 0.507
MS+Agar+BAP ( $5 \times 10^{-5}$ )	2.00 $\pm$ 0.169	2.00 $\pm$ 0.240	2.00 $\pm$ 0.816
MS+Agar+NAA ( $5 \times 10^{-6}$ )	2.58 $\pm$ 0.280	2.92 $\pm$ 0.388	3.16 $\pm$ 0.440
MS+Agar+NAA ( $10^{-5}$ )	3.00 $\pm$ 0.120	4.00 $\pm$ 0.157	5.65 $\pm$ 0.900
MS+Agar+NAA ( $5 \times 10^{-5}$ )	5.16 $\pm$ 2.385	3.66 $\pm$ 0.249	5.00 $\pm$ 0.465
MS+Agar+PUT ( $5 \times 10^{-6}$ )	1.83 $\pm$ 0.109	2.41 $\pm$ 0.320	2.0 $\pm$ 0
MS+Agar+PUT ( $10^{-5}$ )	1 $\pm$ 0	1.17 $\pm$ 0.143	1.0 $\pm$ 0
MS+Agar+PUT ( $5 \times 10^{-5}$ )	1.83 $\pm$ 0.223	2.25 $\pm$ 0.212	2.33 $\pm$ 0.277

From the above study it is clear that hypocotyls inoculated on MS + 1.0 mg/l BAP are the best for callus induction and MS medium with BAP or NAA is suitable for further proliferation of callus in *Brassica juncea*, RH-781.

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# EFFECT OF SEED TREATMENT WITH SALTS OF Mg ON PHOSPHORUS CONTENT OF GROWING PLANT OF MUSTARD

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## Abstract

Phosphorus content of the growing crops of mustard varieties, Vaibhav and Kranti were found to improve with the application of Mg-salts as 24h - seed treatment before sowing. In between the salts  $Mg(NO_3)_2$  was found more effective than  $MgSO_4$  and among concentrations, 10 mM of both the salts showed maximum enhancement in phosphorus content in shoots.

Seed treatment with salts of Mg was found to improve the germination, seedling vigour, amino acid, chlorophyll and nitrogen contents of leaves and finally the yield in mustard crop (Bose and Mishra, 1997, 1999, and 2001). Kumar *et al.* (1981) observed that application of 30 ppm Mg increased the concentration and uptake of phosphorus in wheat plant. Mg was also found to improve the rate of transport and accumulation of phosphorus in grain of spring barley (Fecenko, 1981) With these points in mind in the present investigation trials have been made to find out the effect of seed treatment with salts of Mg on status of phosphorus in growing crops of mustard.

Seeds of *Brassica juncea* L. Czern & Coss (Var. Vaibhav and Kkranti) were obtained from Oil seed section of the Department of Genetics and Plant Breeding of this Institute. Surface sterilized seeds were placed in petridishes (32 cm diameter), lined with single layer of Whatmann No. 1 filter paper, moistened with either 7.5 ml of distilled water or solutions (concentrations ranging from 5 to 10 mM) of  $Mg(NO_3)_2$  and  $MgSO_4$  for 24 h for soaking. The experiment was conducted in room illumination ( $10 W m^{-2}$ ) at a temperature of  $18 \pm 2^\circ C$ . For each of the treatment 3 sets of petridishes were maintained. After proper soaking, the seeds were transferred to pots containing 20 kg of garden soil added with 1.7 g urea, 3.7 g super phosphate and 0.66 g potassium chloride corresponding to the recommended doses of NPK @ 80, 60 and 40 kg/ha respectively. Ten days after sowing (DAS) thinning was done by keeping three plants/pot. Five pots were maintained for each treatment. Pots were irrigated as per their requirement. The phosphorus contents of the shoots of mustard crop were estimated at 5, 15, 30 and 45 DAS by using the method of Bhargava and Raghupathi (1993).

The cultivars, Vaibhav and Kranti showed significant level of difference in the content of P at 15 and 30 DAS of study period, and the former variety had more P content than the later one (Table 1).

In between the salts  $Mg(NO_3)_2$  was found significantly more influential than  $MgSO_4$  and both salts were able to raise the P content in the mustard crop. Among the concentrations of salts 10 mM was found most effective in this regard. The interaction studies showed that all the concentrations of  $Mg(NO_3)_2$  were found to have a greater values than the concentrations of  $MgSO_4$  at all observations. It was also noted that in variety Vaibhav the P content increased upto 15 DAS and then it declined whereas in variety Kranti the maximum P content was noticed on 5 DAS and then it declined gradually upto 45 DAS (Table 1)



**Table 1 : Influence of pre-sowing soaking treatment to the seeds of different varieties (Vaibhav (Va) and Kranti (k) of mustard with Mg(NO<sub>3</sub>)<sub>2</sub> and MgSO<sub>4</sub> on Phosphorus content (% dry weight) in shoots**

Treatment	Salt Conc. (mm)	Days After Sowing							
		5		15		30		45	
		Va	K	Va	K	Va	K	Va	K
Control	0.0	0.50	0.50	0.50	0.50	0.50	0.30	0.20	0.30
Mg(NO <sub>3</sub> ) <sub>2</sub>	5.0	0.70	0.70	0.80	0.60	0.70	0.60	0.50	0.40
	7.5	0.80	0.80	0.80	0.80	0.80	0.70	0.50	0.60
	10.0	0.80	0.90	0.90	0.80	0.90	0.70	0.60	0.50
	5.0	0.60	0.60	0.70	0.60	0.60	0.50	0.30	0.40
MgSO <sub>4</sub>	7.5	0.70	0.60	0.70	0.60	0.60	0.50	0.40	0.40
	10.0	0.70	0.60	0.70	0.60	0.50	0.60	0.40	0.40
	Mg(NO <sub>3</sub> ) <sub>2</sub>	0.70	0.72	0.75	0.67	0.72	0.57	0.45	0.45
MgSO <sub>4</sub>	0.62	0.57	0.65	0.57	0.55	0.47	0.32	0.37	
Control	0.0	0.50	0.50	0.50	0.50	0.50	0.30	0.20	0.30
	5.0	0.65	0.65	0.75	0.60	0.65	0.55	0.40	0.40
	7.5	0.75	0.70	0.75	0.70	0.70	0.60	0.45	0.50
	10.0	0.75	0.75	0.80	0.70	0.70	0.65	0.50	0.45
Mean		0.66	0.65	0.70	0.62	0.64	0.52	0.39	0.41
Factors		C.D. at 5%		C.D. at 5%		C.D. at 5%		C.D. at 5%	
V		NS		0.048		0.051		NS	
S		0.046		0.048		0.051		0.047	
C		0.065		0.068		0.072		0.067	

V = Variety, S = Salt, C = Concentration

The overall results as regard to P content, suggested that although its content was not found statistically significant at some places, but its content was found more in most of the treated sets as compared to control. Therefore, it is to be manifested that Mg salt has certainly some effect in increasing the uptake of P and it is well in agreement with the results of Tiwari and Mandal (1972) and Fecenko (1984) who suggested that Mg has direct effect on the uptake of P in plants. However, it is also found that Mg improves germination via improving the synthesis of GA and ATP formation (Kiss, 1979). Therefore it can be predicted that when Mg is applied in form of seed soaking treatment, influxes to seed (Bose and Mishra, 1999) and consequently improved the utilization of P in the growing crops. This further necessitates the uptake of P by the plants.

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# Study on Biology Characteristics and Nutrition of Chinese Cabbage CMS96 and Maintenance Lines, Hybrids of CMS96 and Hybrids of Related Maintenance Lines

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## Materials and method

26 different Chinese cabbages (*Brassica campestris* L. ssp. *pekinensis*) had been crossed and back-crossed with a *B.napus* cytoplasmic male sterile material which was stable in sterility, normal in nectary, strong in vigor and 100% male sterile since 1996 and attained successfully 5 different stable Chinese cabbage CMS96 types (including orange and yellow heading color Chinese cabbage) which were cytoplasmic male sterile via artificial multi-backcrossing for 8-9 generations until 2001. We had crossed 70 new F1 hybrids using these materials in 2001 and cultivated them at open and tunnel field on August 8, 2001. Some biology characteristics and nutrition were tested including plant height, outer leaves expansion, number of outer leaves, heading height, heading width, length of internal stem, rough weight, net weight, vitamin C,  $\beta$ -carotin, crude fiber, calcium, terrum and thiocyanic acid redicel comparing CMS96 cytoplasmic male sterile with maintenance lines, hybrids of CMS96 with hybrids of related maintenance lines. Nutrition was tested using common chemical analysis. Data were tested using variance analysis. 5 different stable Chinese cabbage CMS96 cytoplasmic male sterile types were showed at table1.

**Table 1. Characteristics of 5 Chinese cabbage CMS96 cytoplasmic male sterile types**

name	code	characteristic	resource
CMS96-1	3-178	orange heading color, half over-lapping type	Beijing vegetable research center
CMS96-2	3-304	high late-maturity over-lapping type	same as above
CMS96-3	3-307	same as above	same as above
CMS96-4	205	yellow heading color over-lapping type	same as above
CMS96-5	C27	same as above	same as above

## Results and discussion

It was easy to transfer *B.napus* cytoplasmic male sterile to Chinese cabbage [1]. 5 Chinese cabbages which had been transferred cytoplasmic male sterile were stable in sterility, normal in nectary, strong in vigor and fast in transfer. The rate and degree of Chinese cabbage CMS96 male sterile plants were 100%. They are new and better cytoplasmic male sterile materials now in China, and they had showed distinct predominance. Chinese cabbage CMS96 and hybrids of CMS96 had showed similar or higher results of minutus or significance using F0.05 level comparing with maintenance lines and hybrids of related maintenance lines in biology characteristics, such as plant height, outer leaves expansion, heading height, etc. and nutrition such as vitamin C,  $\beta$ -carotin, crude fiber, etc.(Table 2,3,4). Chinese cabbage CMS96 had retained better characteristics of maintenance lines, and did not degenerate by multi-backcrossing. It made better combinations and would have wide breeding value in the future. Our results showed 3 advantages of these new Chinese cabbages CMS96 compared with other male sterile materials in China [2]. One, they showed better characteristics as stable sterility, strong vigor, easy and fast transfer, and uniform plants. They did not degenerate through multi-backcrossing and had better combination power. Two, they were entire male sterile. The rate and degree of male sterile plants were 100%. These Chinese cabbage CMS96 materials were normal in nectary with white degenerative anthers. Three, they were new and better Chinese cabbage cytoplasmic male sterile in China, and would show wide value for breeding new Chinese cabbage varieties and protecting breeder's rights in the future.

**Table 2. Comparison of CMS96, maintenance lines and related F1 hybrids in means**

item name	code	plant height (cm)	F0.05 value	outer leaves expansion (cm)	F0.05 value	No. of leaves (sheet)	F0.05 value	Length of internal stem(cm)	F0.05 value
fertile line	3-176②	36.6	a	62.4	a	8.4	a	3.2	a
CMS96-1	3-178③	36.6	a	62.6	a	9.0	a	3.4	a
fertile line	3-306③	47.0	a	68.6	a	9.1	a	4.5	a
CMS96-3	3-307	50.0	a	72.6	a	10.8	a	4.7	a
fertile line	3-303③	43.4	b	73.2	b	10.0	a	3.7	a
CMS96-2	3-304	53.6	a	81.2	a	12.0	a	5.9	a
fertile line	194	39.8	b	59.8	a	10.4	a	3.0	a
CMS96-4	205	42.8	a	66.2	a	9.4	a	3.3	a
CMS96(F1)	3-378	38.0	b	63.8	b	9.4	a	5.6	a
fertile F1	2-198	45.4	a	76.2	a	8.4	a	5.3	a
CMS96(F1)	3-383	41.6	a	69.4	a	7.8	a	5.1	a
fertile F1	2-366	37.4	b	65.8	a	8.2	a	5.5	a
CMS96(F1)	3-395	50.2	a	82.0	a	10.2	a	5.4	b
fertile F1	2-319	43.8	b	70.0	a	7.8	b	6.9	a
CMS96(F1)	3-398	53.2	a	83.6	a	11.2	a	6.1	a
fertile F1	2-361	54.0	a	74.8	b	7.0	b	5.3	a

**Table 3. Comparison of CMS96, maintenance lines and related F1 hybrids in means**

item name	code	heading height(cm)	F0.05 value	heading width(cm)	F0.05 value	Crude weight (kg)	F0.05 value	pure weight(kg)	F0.05 value
fertile line	3-176②	32.0	a	16.4	a	2.2	a	1.8	a
CMS96-1	3-178③	32.8	a	18.0	a	2.7	a	2.1	a
fertile line	3-306③	32.8	b	17.8	a	3.8	a	2.6	a
CMS96-3	3-307	35.2	a	18.6	a	3.8	a	2.5	a
fertile line	3-303③	37.0	a	18.4	a	3.5	b	2.3	b
CMS96-2	3-304	37.6	a	21.0	b	5.5	a	3.6	a
fertile line	194	25.0	a	13.3	b	2.7	a	2.1	b
CMS96-4	205	28.3	a	18.3	a	3.4	a	2.6	a
CMS96(F1)	3-378	28.6	b	23.6	a	5.1	a	4.1	a
fertile F1	2-198	32.4	a	23.0	a	4.8	a	3.7	a
CMS96(F1)	3-383	29.6	a	20.2	b	4.2	a	3.2	a
fertile F1	2-366	27.4	a	21.2	a	4.1	a	3.2	a
CMS96(F1)	3-395	36.2	a	26.8	a	6.3	a	4.1	a
fertile F1	2-319	30.0	b	23.0	a	4.7	a	3.8	a
CMS96(F1)	3-398	39.8	a	18.2	a	5.5	a	4.1	a
fertile F1	2-361	41.6	a	19.0	a	5.1	a	3.7	a

**Table 4. Comparison CMS96 with maintenance lines of some nutrition in means**

item name	code	vitamin C (mg/100g)	F0.05 value	β-carotin (mg/kg)	F0.05 value	crude fiber (%)	F0.05 value	Calcium (mg/kg)	F0.05 value	Terrum (mg/kg)	F0.05 value	thiocyanic acid redicel (mg/kg)
CMS96-1	3-178①	20.0	b	--	--	0.42	a	412	a	2.29	a	13.52
fertile line	3-176①	25.4	a	--	--	0.50	a	452	a	2.51	a	11.86
CMS96-5	C27	19.6	a	0.70	a	0.50	a	576	a	5.71	a	12.15
fertile line	P1	16.8	b	0.30	a	0.46	a	434	a	3.15	b	13.42
CMS96-3	3-307	25.4	a	2.20	a	0.49	a	571	a	5.92	a	17.44
fertile line	3-306①	22.5	b	1.90	a	0.48	a	626	a	4.88	b	14.70

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# Photothermal unit requirement of mustard (*Brassica juncea L.*) under dryland conditions

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## Introduction

Any physiological and morphological development occurrence in plants is markedly influenced by temperature and day length. The application of Photothermal indices provide a scientific basis for determining the effect of temperature and photoperiod as phenological behaviour of crop. Photothermal units has been used by Hundal and Kingra (2000) to evaluate the phenophasic model of soybean.

## Materials and Methods

Field study on mustard was conducted under rainfed condition during *rabi* 1999 - 2000 at Dryland Research Station, SKUAST-J, Dhiansar ( 32°-39' N, 74°-58' E and 332 meters above mean sea level). The treatments comprised of 3 dates of sowing (9<sup>th</sup> October- D1, 24<sup>th</sup> October-D2 and 8<sup>th</sup> November-D3) and two cultivars Varuna and Pusa Bahar were laidout in random block design with four replications. Three phenophases viz , emergence to flower bud initiation (PS1), flower bud initiation to siliqua formation (PS2), and siliqua formation to maturity (PS3) were identified. The photothermal units (PTU) were computed as follows:

$$\text{Accumulated PTU} = \sum_{de}^{dm} (\text{GDD}) \times \text{Day length}$$

$$\text{GDD} = \sum \frac{\text{MaxT} + \text{MinT}}{2} - T_b$$

GDD- Growing degree days ( °Cd)

MaxT and MinT- Daily maximum and minimum ( °C), respectively

T<sub>b</sub>- Base temperature i.e 5 °C (Kar and Chakravarty,1999)

de- date of emergence or starting date of the phenophase of interest

dm- date of maturity or end of the phenophase of interest.

## Results and Discussion

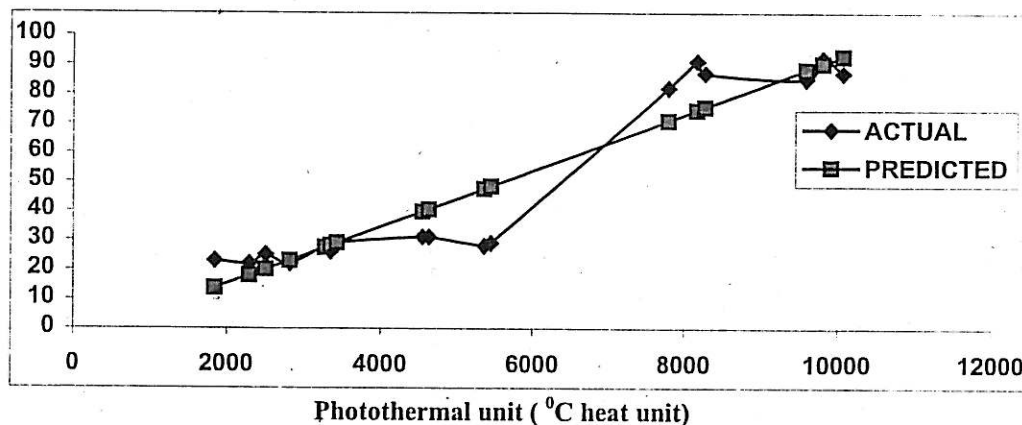
Photothermal unit of different phenophases of mustard were workedout and presented in Table 1. It is observed from the results that PTU showed decreasing trend during the periods from emergence to flower bud initiation (PS1) and flower bud initiation to siliqua formation (PS2) with delay in sowing in both cultivars, however, no consistent trend was found in days taken to attain the different phenophases. The variation in accumulation of photothermal unit may be due to receding soil moisture under late sown conditions. The relationship between actual days taken for particular phenophase and PTU corresponding to that phenophase of crop was developed and depicted graphically in Fig 1.

**Table1: Phothermal unit and days taken to attain various phenophases in mustard**

Treatment	Days taken (Phenophase)			Photothermal units (Phenophase)		
	PS <sub>1</sub>	PS <sub>2</sub>	PS <sub>3</sub>	PS <sub>1</sub>	PS <sub>2</sub>	PS <sub>3</sub>
V1 (Varuna)						
D1	28	26	92	5369	3352	9812
D2	31	22	87	4638	2297	8270
D3	29	23	87	3434	1850	10069
V2 (Pusa Bahar)						
D1	29	22	91	5455	2820	8166
D2	31	25	82	4554	2511	7795
D3	28	23	85	3284	1850	9505

PS<sub>1</sub>:- Emergence to flower bud initiation PS<sub>2</sub>:- Flower bud initiation to siliqua formation  
 PS<sub>3</sub> :- Siliqua formation to maturity

**Days taken**



**Fig 1. Relationship between days taken and Photothermal unit required to attain various phenophase.**

The regression equation so obtained  $y = - 4.11 + 9.61 \times 10^{-3} \times \text{PTU}$  ( $R^2 = 0.884^{**}$ ) indicates that the rate of development of mustard was 9.61 days per 1000 PTU and same is accounted 88.4 per cent variation in onset of different phenophase

**Reference**

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## Increment in Seed Germination, Growth and Nitrate Reductase Activity in Seedlings of $Mg(NO_3)_2$ Hardened Seeds of Mustard

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### ABSTRACT

The seeds of mustard while hardened with 7.5 mM of  $Mg(NO_3)_2$  solution for 12h showed higher % of germination, root-shoot length, fresh weight and nitrate reductase activity of seedlings as compared to non-hardened seeds. Distilled water-hardened seeds also showed higher rate of germination than non-hardened one.

### INTRODUCTION

Soaking of seeds with  $Mg(NO_3)_2$  before sowing improves germination, vegetative growth, nitrate reductase activity of seedlings/leaves and yield of various crops. (See, Bose and Pandey, 2003). Hardening of seeds with water and  $KNO_3$  also found to improve germination and growth of seedlings of wheat and rice (Mandal and Basu, 1984; Pfahler *et al.* 1991, Andoh and Kobata, 2000). But there is hardly a report where the hardening of seeds is done with  $Mg(NO_3)_2$  to an important oil crop like mustard. Hence, the present study has been conducted.

### MATERIALS AND METHODS

Healthy and bold seeds of mustard (*Brassica juncea* L. Czern and Coss. Var. Varuna), taken from Genetics and Plant Breeding Department of same Institute, were surface sterilized by keeping them in 0.1%  $HgCl_2$  solution for 2-3 minutes and then washed thoroughly with distilled water. These seeds were allowed for soaking either in distilled water (d.w) ( $T_1$ ) or in 7.5 mM of  $Mg(NO_3)_2$  ( $T_2$ ) for 12h. The seeds then washed twice gently with distilled water and dried back to its original weight at room temperature by placing them under fan. After drying, seeds were packed in paper bags and kept in dry cupboards. After a week these seeds were sown in plastic pots (10 cm diameter and 10 cm height), filled upto 7.5 cm with thoroughly washed sand (acid treated). For control ( $T_3$ ) the seeds were sown to pots without any hardening treatment. Each and every pots were supplied with only distilled water as per requirement. Observations regarding germination, root and shoot length, fresh weight and nitrate reductase activity (NRA) of the seeds/seedlings were taken upto 5 days after sowing (DAS). NRA was estimated by using the method of Srivastava (1974). Before enzyme assay, seedlings were kept for 2h in 10mM  $KNO_3$  for induction of NR. All experiments were repeated thrice with three replicates and Randomised Block Design was adopted for statistical analysis.

### RESULT AND DISCUSSION

Germination of d.w. ( $T_1$ ) and  $Mg(NO_3)_2$  ( $T_2$ ) hardened seeds were higher in respect to non-hardened control ( $T_3$ ) seeds at 3 and 5 DAS. Root and Shoot length, fresh weight and NRA of seedlings all were more in  $T_2$  in respect to  $T_1$  and  $T_3$  at 5 DAS. The root length and NRA both were lowest in  $T_1$ . However, NR activity was quite more in  $T_3$  as respect to  $T_1$  (Table 1).

It is well established that when  $Mg(NO_3)_2$  is given as seed treatment prior to sowing improves germination by increasing the Mg content (Bose and Mishra, 1999), electrical conductivity and NRA of seedlings (Bose and Pandey, 2003) in various crops. However, seed hardening by hydration-dehydration technique is used to induce resistance in crops towards various stresses (see review by Kar, 1997). Some reports also suggested that hardening of

seed with  $GA_3$  and  $KNO_3$  increases the % germination, coleoptile length and leaf length in wheat (Pfahler *et al.* 1991). All these reports are at par with the observations, taken in this work (Table 1). However, in the present case the seeds were sown in sand therefore the NRA of the seedlings represented the presence of constitutive NR enzyme in seeds and this might be induced/activated by the application of  $Mg(NO_3)_2$  as hardening treatment, which may generate NO by an alternate path way in  $T_2$  seedlings, as a result plants showed an increment in the length of root, a character towards induced resistant (See review by Salgado *et al.* 2002). The study, therefore concludes that hardening of mustard seeds with  $Mg(NO_3)_2$  may induce resistance in seedlings against various stresses.

**Table 1** : Effect of seed hardening with distilled water and  $Mg(NO_3)_2$  Salt on germination percentage, root and shoot length (cm) fresh weight (mg) and nitrate reductase activity (NRA) ( $\mu\text{mole of } NO_2 \cdot h^{-1} \cdot g^{-1}$  fresh weight) of seeds/seedlings of mustard.

Treatments	PARAMETERS					
	Germination %		Root length	Shoot length	Fresh Weight	NRA
	3 DAS*	5 DAS	5 DAS	5 DAS	5 DAS	5 DAS
$T_1$	85.0	95.0	2.615	6.73	50.8	1826
$T_2$	78.3	93.3	5.23	7.66	62.6	2222
$T_3$	35.0	80.0	2.83	5.9	46.22	20.46
S.E.M.	4.407	9.051	0.5186	0.2323	4.270	36.07
C.D. at 5%	12.235	25.126	1.439	0.645	11.855	100.14

$T_1$  = Distilled water hardened seeds.  $T_2$  =  $Mg(NO_3)_2$  hardened seeds.

$T_3$  = non hardened seeds (Control). \*Days after sowing

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## Germplasm release of two sporophytic incompatible lines of Topas summer rape (*Brassica napus* L.)

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Two sporophytic incompatibility alleles were introgressed into the summer rape  
(*Brassica napus* L.) cultivar Topas.

### Breeding

Both lines are BC5 Topas (*B. napus* L.) heterozygous for the S-allele developed  
by backcrossing Topas as the recurrent parent with the sporophytic incompatible  
(SI) line. The W1 S-allele (courtesy of Paul Banks) was developed by  
introgressing a SI allele from the turnip rape (*B. rapa* L.) cultivar Candle into  
rape. The T2 S-allele (courtesy of Paul Banks) was developed at the Scottish  
Crops Research Institute (identified as Z in their program) using SI Swede  
(*Brassica napus* ssp. *rapifera* {Metzg.} Sinsk.). The BC5 Topas W1 and T2 lines  
are designated 11938 and 11968 respectively.

### Performance

In our experiments, 11938 (Topas, W1) averaged 73% outcrossing in the field and  
11968 (Topas, T2) averaged 65% (Lewis et al. 2000).

### Availability

Seed may be obtained from the authors, or from Plant Gene Resources of Canada,  
Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan,  
Canada S7N 0X2, under the designations CN19188 for 11938 and CN19189 for  
11968.

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Lewis, L. J., Woods, D. L., and Klein-Gebbinck, H. W. 2000. Evaluation of two  
self-incompatibility alleles in three summer rape (*Brassica napus* L.) cultivars by  
UV fluorescence microscopy, seed set and outcrossing rates.  
Can. J. Plant Sci. 80: 255-260.



## Unknown cause of malformations in canola crops in Western Canada in 2002 and 2003

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In the spring and summer of 2002 and 2003, malformations were observed in Alberta and Saskatchewan on canola seedlings, plants at the rosette stage and mature plants. Seedlings and plants at the rosette stage showed large, swollen and brittle cotyledons and a growing point transformed into callus-like tissue. Examples of malformed seedlings and plants at the rosette stage can be viewed on the Canola Council of Canada website:

[http://www.canola-council.org/production/agronomy\\_july3.html](http://www.canola-council.org/production/agronomy_july3.html)

Malformations on mature plants included twisted and purple leaves and stems, and shortened internodes. The plants bore a few small, pale yellow flowers that sometimes produced partially filled pods.

Malformed plants were observed in patches (5-30 m in diameter) in the affected fields; two fields in north-western Saskatchewan had 60 and 85% malformed plants, respectively. Despite the fact that most of the affected fields had only small areas of malformed plants, the plants were quite noticeable and their presence alarmed producers. It was initially suggested that the malformations were the result of herbicide injury (Funke 2003, Whetter 2003), but the symptoms were not completely typical of herbicide injury. Because of the similarity of symptoms caused by aster yellows (AY) phytoplasma infection and the malformations (purple leaves and stems, short internodes, plant stunting), it was further suggested that malformed plants were infected with AY phytoplasmas or another FPA (fastidious prokaryotic agent).

Canola crops are affected by phytoplasmas belonging to AY strains 16SrI-A and 16SrI-B (Wang and Hiruki 2001). The disease is very common in canola crops in western Canada, but its incidence is usually very low, on average less than 1% (Pearse et al. 2002, 2003). Infected plants are scattered in the field, but are more common at the edges of fields. AY phytoplasmas are presumed to be transmitted by the leafhopper *Macrostelus quadrilineatus* Forbes. The incubation period before leafhoppers are infective is temperature dependent. At 20°C, the incubation period is 5 to 6 weeks (Murrall et al. 1996). Once a canola plant has become infected, symptoms appear within 4 to 6 weeks, depending of temperature. Therefore AY symptoms generally appear on canola plants at the end of July at the earliest.

The malformations were observed from seedling emergence on, appearing too early to be caused by AY phytoplasmas. Also, most of the symptoms were not typical of AY infection. For instance, the flowers of malformed plants were morphologically normal and no bushy inflorescences of aborted flowers were observed. Bladder-like pods that fail to set seed, the most common AY symptom, were absent. In 2002, phytoplasma DNA was detected in 16 out of 20 malformed plants harvested in August at Alberta, but in 2003, fewer than 1% of 200 malformed plants harvested in July in Alberta and Saskatchewan tested positive for phytoplasma DNA, leading to the

conclusion that phytoplasmas are not the causal agent of the malformations. Further experimentation will be required to solve the problem.

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# Heterosis in cytoplasmic male sterile lines of cabbage (*Brassica oleracea* L. var. *capitata*)

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Commercial exploitation of heterosis in any crop improvement programme largely depends upon the presence of substantial magnitude of heterosis and the economic method of producing the hybrid seed. In the present investigation, cytoplasmic male sterility system has been utilized to produce the hybrids in cabbage. As this type of system of male sterility can be effectively used in crops, like cabbage, where not the seed but vegetative part is of economic importance and fertility restorer is not required to make the F<sub>1</sub> fertile. Keeping the above points in consideration and the importance of hybrids in cabbage, the proposed study was undertaken.

## Materials and Methods

Four cytoplasmic male sterile (CMS) lines of cabbage viz., cms-1, cms-2, cms-3 and cms-4 were crossed with five testers viz., MR-1, EC-240613, AC-204, AC-236 and AC-208 in a line x tester design of mating as proposed by Kempthorne (1957). The resulting 20 F<sub>1</sub> hybrids were evaluated along with their 9 parents in a randomized block design with 2 replications during *rabi* 2001-2002. Three rows of each entry were planted in a plot of size 3m x 3m maintaining row to row and plant to plant spacings at 45 cm each. Data were recorded on 5 randomly selected plants for six quantitative characters and heterosis were calculated over better parent (BP) and mid parent (MP).

## Results and Discussion

Heterosis in the negative direction is always desirable for the development of characters viz., days to harvest, stalk size index and number of outer leaves while a positive heterosis is required for the characters such as head size index, gross weight per plant and yield per plant. A wide range of heterosis (%) over both better parent and mid parent were observed for all the characters under study (Table 1).

Among the 20 crosses, the top three best heterotic crosses over the better parent for each character are presented in Table 2. For days to first harvest, the crosses cms-3 x MR-1 and cms-3 x EC-240613 exhibited highest negative heterosis (-17.35% each) over the better parent followed by cms-2 x AC-204 (-6.36%). Only two crosses cms-3 x MR 1 and cms-1 x EC-240613 showed negative heterosis of -21.91% and -2.43%, respectively, for stalk size index. The magnitude of negative heterosis over the better parent for number of outer leaves was rather lower in the three crosses, cms-3 x MR 1, cms-2 x EC-240613 and cms-2 x MR 1 (-10.62%, -9.43% and -8.96%, respectively).

For head size index, the crosses cms-1 x AC-204, cms-2 x AC-208 and cms-2 x EC-240613 were the highly heterotic with heterosis percentages of 56.79, 56.49 and 28.51, respectively. The top three best heterotic crosses for gross weight per plant and yield per plant were the common although they differ with respect to their magnitude and relative ranking. Cms-4 x MR-1 was the highly heterotic cross (62.69%) for gross weight per plant followed by cms-1 x MR-1 (55.47%) and cms-1 x AC-204 (48.68%) while for yield per plant cms-1 x AC-204 possessed highest heterosis (63.68%) followed by cms-4 x MR 1 (58.32%) and cms-1 x MR 1 (50.20%). Hybrid vigour of significant magnitude has also been reported by More and Wallace (1987) for different characters in cabbage and Joshi and Verma (1998) has developed few cabbage hybrids by utilizing the self-incompatibility and cytoplasmic male sterility systems.

The three crosses, cms-1 x AC-204, cms-4 x MR 1 and cms-1 x MR 1 along with cms-3 x MR-1 can be further exploited for the development of superior high yielding varieties of cabbage.

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Table 1. Range of heterosis (%) over BP and MP

Character	Range of heterosis (%) over	
	BP	MP
Days to first harvest	-17.35 to 41.98	-41.41 to 37.31
Stalk size index	-21.91 to 146.82	-39.16 to 77.00
No. of outer leaves	-10.62 to 13.44	-12.47 to 10.18
Head size index	-37.71 to 56.79	-27.15 to 79.18
Gross wt/plant	-21.90 to 62.69	-12.23 to 81.74
Yield/plant	-22.10 to 63.68	-14.94 to 95.72

BP- better parent, MP- mid parent

Table 2. Best heterotic crosses (top three) showing maximum heterosis over better parent for different characters

Character	Best heterotic crosses	Heterosis (%)
Days to first harvest	cms-3 x MR 1	-17.35
	cms-3 x EC-240613	-17.35
	cms-2 x AC-204	-6.36
Stalk size index	cms-3 x MR 1	-21.91
	cms-1 x EC-240613	-2.43
	cms-3 x MR 1	-10.62
No. of outer leaves	cms-2 x EC-240613	-9.43
	cms-2 x MR-1	-8.96
	cms-1x AC-204	56.79
Head size index	cms-2 x AC-208	56.49
	cms-2 x EC-240613	28.51
	cms-4 x MR-1	62.69
Gross wt/plant	cms-1 x MR-1	55.47
	cms-1 x AC-204	48.68
	cms-1 x AC-204	63.68
Yield/plant	cms-4 x MR 1	58.32
	cms-1 x MR 1	50.30



## WATER STRESS RELATED PROTEINS IN LEAVES OF *BRASSICA JUNCEA* CULTIVARS DIFFERING IN DROUGHT TOLERANCE

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Stress induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with stress conditions. Water stress causes both quantitative reduction in the rate of protein synthesis and qualitative changes in the quality of protein produced. *Brassica juncea* is an important oil seed crop whose yield is affected under drought condition. *Brassica juncea* cultivars differing in drought tolerance viz Varuna (tolerant), PPMS and Prakash (sensitive) were obtained from the Department of Plant Breeding and SR-3 (salt-tolerant somaclone of Prakash) from the Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar. The plants were raised using Hoagland nutrient solution in river sand filled earthen pots. Irrigation was done at 2d interval. Rapid water stress was created by complete withhold of water at five week old stage and observations were recorded at 8, 9, 10, 11 days after withholding water (DAWW). Measurement of the osmotic potential ( $\pi$ ) and relative water content (RWC) was made concurrently on the same youngest leaf (Turner, 1981). Osmotic potential ( $\pi$ ) was measured with Vapour Pressure Osmometer model 5100-B (Wescor, USA) and expressed in -MPa. RWC was measured by taking 10-12 discs of 5mm diameter each from the middle portion of leaves. Both RWC and  $\pi$  have been used for the assessment of osmotic adjustment (Morgan, 1984). Rapid water stress resulted in sharp reduction in RWC and the decline was maximum in cv PPMS in which it decreased from 85.9% to 45.9%. Osmotic potential also declined progressively with the increase in the length of stress. The magnitude of decrease in leaf osmotic potential was maximum in cv Varuna in which it decreased from -0.99 MPa to -2.80 MPa (Table 1). Differential behaviour in RWC and  $\pi$  in cultivars differing in stress tolerance has also been reported by Asharaf and Mahmood (1990) and Phutela *et al.* (2000).

The leaf proteins were extracted in Tris-HCl buffer (pH 6.8) and characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions following the method of Laemmli (1970) using 10-20% linear acrylamide gradient. The stress effected changes in polypeptide pattern revealed that a 57 kDa polypeptide was present in leaves of SR-3 and Varuna (Fig. 1) under both stress as well as non-stress conditions while it disappeared in cv Prakash and PPMS on water withholding. Likewise a polypeptide of 53.2 kDa did not alter under stress in SR-3 but got converted into two components in cv Prakash and PPMS (Fig. 1). Reviron *et al.* (1992) also reported the increased synthesis of Bn22 polypeptide by rapid water stress in *B. napus* that disappeared on rehydration. Presence and/or absence of polypeptides upon imposition of water stress suggest their role in drought tolerance.

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Table 1. Relative water content (RWC) and osmotic potential ( $\pi$ ) of leaves of *Brassica juncea* cultivars subjected to rapid water stress.

Genotypes	Days after withholding water				
	0	8	9	10	11
	RWC (%)				
Prakash	88.7±1.6	77.4±0.2	72.3±0.5	67.9±1.7	61.8±0.3
SR-3	85.5±0.6	76.0±0.5	70.2±1.1	69.6±1.6	51.6±0.5
PPMS	85.9±0.5	72.4±1.2	65.2±0.7	57.2±1.0	45.9±0.8
Varuna	86.4±1.7	75.3±1.3	64.7±2.3	58.7±1.4	51.4±0.3
	Osmotic potential (-MPa)				
Prakash	0.99±0.11	1.10±0.19	1.32±0.23	1.87±0.34	2.22±0.18
SR-3	0.94±0.18	1.19±0.20	1.35±0.15	1.83±0.23	2.24±0.39
PPMS	1.06±0.32	1.19±0.16	1.47±0.51	1.73±0.29	2.61±0.44
Varuna	0.99±0.09	1.22±0.31	1.36±0.90	1.65±0.24	2.80±0.55

Values are mean of four replicates  $\pm$  S.D.

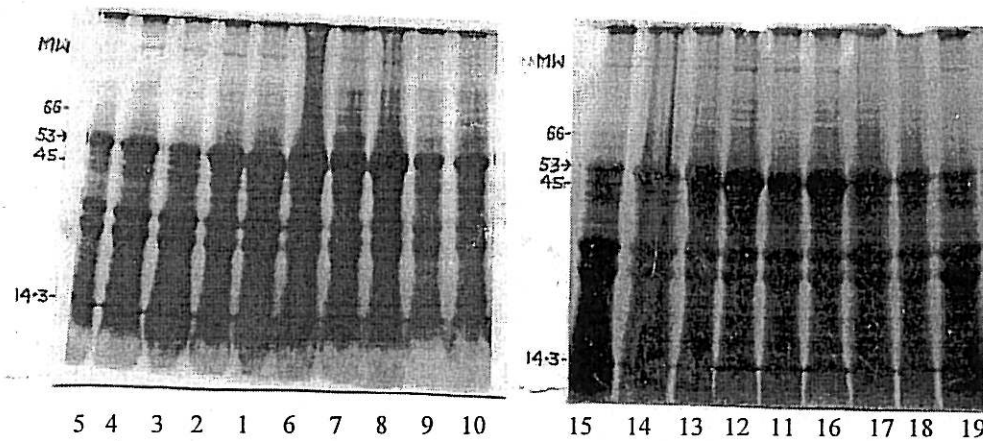


Fig.1. SDS-PAGE of leaf proteins of *B. juncea* genotypes after rapid water stress, Prakash; lane 1 control, lane 2-5 stressed samples of 8, 9, 10, 11 days: SR-3; lane 6 control, lane 7-10 stressed samples of 8, 9, 10, 11 days: Varuna; lane 11 control, lane 12-15 stressed samples of 8, 9, 10, 11 days: PPMS; lane 16 control, lane 17-19 stressed samples of 8, 9 and 10 days:

# INFLUENCE OF LEAF EXTRACTS ON CULTIVATED TURNIP

## VIII. PLANT HEIGHT, BRANCHES/ PLANT & DAYS TO FLOWER

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### ABSTRACT

Lower doses of aqueous leaf extract of neem (*Azadirachta indica* A. Juss.) and moderate higher doses of periwinkle (*Catharanthus roseus* Don.) have enhancing effect on plant height and number of branches/ plant of turnip (*Brassica rapa* L.). Carrot weed (*Parthenium hysterophorus* L.) extract has deleterious effect on both the traits. All the three extracts cause delayed flowering.

### INTRODUCTION

Study of plant height, branches/ plant and time of flowering bear special significance in relation to yield in any crop. They are positively or negatively correlated (9, 11). Time of flowering determines the earliness or lateness of a cultivar. Artificial induction of mutation remains a major technique to create variability in any crop. The commonly used chemical mutagens are not only expensive but also injurious to health and environment. Hence search for safe and effective mutagens of botanical origin can be an important approach (1-8). Periwinkle has antitumour effect (14). Carrot weed exhibits allelopathic activity (12). Neem elaborates a vast array of biologically active and chemically diverse constituents due to which it is antimicrobial, antiulcer and anti-inflammatory (13). Keeping all these in mind, present study was undertaken to know the effect of aqueous leaf extracts of periwinkle, carrot weed and neem on the plant height, branches/plant and days to flower of turnip.

### MATERIALS AND METHODS

The material used, method of preparing the solutions of different concentrations from the mother solution, mode of seed treatment and raising the plants from them, were the same as described earlier (3). Turnip is a suitable material for such studies as it is annual, grows rapidly, economic on space and is an important root crop. Height of 25 randomly selected plants in each concentration under all the treatments were taken separately with the help of a scale and thread. For branches/ plant, the secondary branches developing from the primary axis were counted. Date of appearance of the first flower, and its difference from the date of sowing, gave number of days to flower. All the results are presented in Table 1.

### RESULTS AND DISCUSSION

Due to the effect of periwinkle leaf extract, a gradual decrease in plant height and number of branches/ plant was seen from 20% to 60% doses, followed by a sudden increase at 80% dose, and again a fall at 100% in  $M_1$  generation. Noticeable recovery or enhancement took place in  $M_2$  at all the doses. Carrot weed extract caused a gradual decrease in both the traits from lower to higher doses in  $M_1$  generation, though significant recovery took place in  $M_2$  but not to the extent of control. On the whole, enhancing effect of neem extract was noted on the plant height and branches/ plant at all the doses (except 100%) in  $M_1$  generation which further improved in  $M_2$ . So far days to flower was concerned, delayed flowering was noted under all the treatments in  $M_1$ . Some earliness in this regard was observed in  $M_2$  under periwinkle and carrot weed treatments, but not to the extent of control. Remarkably, a further delay in flowering was noted at all the doses in  $M_2$  under neem extract treatment.

Only a few reports are available on the effect of botanical extracts on yield and yield components of turnip (1-8). Several alkaloids have been isolated, purified and determined chemically from periwinkle, and two most active among them are vinblastine and vincristine which have antitumour effect (14). At the cellular level they provide similar effect to those observed after colchicine treatment and are known to interfere with the metabolic reactions related to DNA and RNA synthesis (15-17). Prolonged exposure in relatively high concentration of vincristine does not produce gross morphological or cytochemical changes in the nucleus or cytoplasm of the resting cells and does not prevent these cells from initiating normal prophase. The moving forward through  $G_1$ , S and  $G_2$  is not altered and vincristine does not inhibit the onset of mitosis (18). Probably, this is one of the reasons behind the rapid recovery and back to normality in  $M_2$ .

Carrot weed yields a non-alkaloidal, non-glycosidic substance called parthenin which is a sesquiterpene lactone pseudoguaienolide with abnormal carbon skeleton (12). It is found in varying concentrations in different parts of plant body being highest in the leaves (19). Besides it has some water soluble inhibitors like caffeic acid, P-coumaric acid (20) and anedic acid, vanillic acid and P-hydroxybenzoic acid. The carrot weed plant material inhibit seed germination, growth and yield of wheat (20), turnip (1,3,6), cowpea and ragi (19). It is suggested not to use carrot weed as green manure since it shows the allelopathic activity (19,21). Biochemical interactions occurs when allelochemicals (secondary plant metabolites) produced by *Parthenium* escape into environment and influence the growth and development of organisms growing in the vicinity. Due to this, it is a threat to yield productivity of turnip (10).

Neem leaf extract has nimbin, nimbenene, 6 - desacetabimbinene, nimbadiol, mimbolide and quercetin (13). Besides appreciable amount of protein, minerals, carotenes and trace elements (except zinc) are also present (22). N, P and C balances are positive. Under the present work, enhancing effect of neem extract was noted on plant height and branches/ plant, particularly at the lower doses.

Turnip is basically a root crop plant. It loses its commercial value after the emergence of flowering shoot. All the three leaf extracts cause delay in flowering which is beneficial from agricultural point of view. Quick reversion to normality in  $M_2$  may be attributed to the presence of a strong DNA repair mechanism in this radio-resistant plant. At present it is difficult to ascertain out of various constituents present in the leaf extracts

which one, or a group of these, is the causal factor for the retardation or enhancement of the yield components of turnip. Obviously, it requires further biochemical studies.

Table 1. Effect of botanical extracts on plant height, branches/ plant and days to flower of turnip.

Leaf extract	Dose	Gen-eration	Plant height (em)		No. of branches plant		Days to flower	
			Mean $\pm$ SE	CV (%)	Mean $\pm$ SE	CV (%)		
Periwinkle	Control	M <sub>1</sub>	89.2 $\pm$ 1.16	6.48	16.7 $\pm$ 0.22	6.65	75	
		M <sub>2</sub>	89.8 $\pm$ 1.11	6.18	16.4 $\pm$ 0.22	6.71	76	
	20%	M <sub>1</sub>	85.4 $\pm$ 1.09 *	6.39	15.5 $\pm$ 0.20 **	6.58	79	
		M <sub>2</sub>	91.2 $\pm$ 0.95	5.21	16.5 $\pm$ 0.21	6.42	78	
	40%	M <sub>1</sub>	60.8 $\pm$ 1.66 **	9.51	14.2 $\pm$ 0.23 **	8.03	80	
		M <sub>2</sub>	80.2 $\pm$ 0.96 **	5.97	16.4 $\pm$ 0.24	7.26	78	
	60%	M <sub>1</sub>	54.8 $\pm$ 0.96 **	8.74	14.8 $\pm$ 0.22 **	7.43	83	
		M <sub>2</sub>	67.6 $\pm$ 0.98 **	7.28	17.3 $\pm$ 0.23	6.65	81	
	80%	M <sub>1</sub>	74.6 $\pm$ 1.02	8.81	27.7 $\pm$ 0.23	4.15	86	
		M <sub>2</sub>	81.2 $\pm$ 1.07 **	6.58	28.0 $\pm$ 0.21 **	3.71	84	
	100%	M <sub>1</sub>	72.0 $\pm$ 0.94	6.51	17.5 $\pm$ 0.25	7.26	88	
		M <sub>2</sub>	74.2 $\pm$ 0.97 **	6.52	20.1 $\pm$ 0.21 **	5.12	85	
	Corrot weed	Control	M <sub>1</sub>	115.8 $\pm$ 0.88	3.80	12.2 $\pm$ 0.20	8.03	76
			M <sub>2</sub>	114.8 $\pm$ 1.05	4.59	12.4 $\pm$ 0.20	7.90	74
20%		M <sub>1</sub>	97.0 $\pm$ 0.89 **	4.61	11.6 $\pm$ 0.21 *	9.14	83	
		M <sub>2</sub>	106.4 $\pm$ 0.99 **	4.66	12.2 $\pm$ 0.25	10.08	62	
40%		M <sub>1</sub>	92.6 $\pm$ 1.01 **	5.44	11.2 $\pm$ 0.21 **	9.46	86	
		M <sub>2</sub>	107.2 $\pm$ 1.00 **	4.66	12.0 $\pm$ 0.23	9.42	63	
60%		M <sub>1</sub>	84.8 $\pm$ 0.92 **	5.45	10.8 $\pm$ 0.27 **	12.31	92	
		M <sub>2</sub>	108.4 $\pm$ 0.98 **	4.50	11.0 $\pm$ 0.25 **	11.45	68	
80%		M <sub>1</sub>	53.6 $\pm$ 0.82 **	7.61	10.2 $\pm$ 0.16 **	7.84	95	
		M <sub>2</sub>	106.6 $\pm$ 0.98 **	4.58	11.2 $\pm$ 0.19 **	8.39	69	
100%		M <sub>1</sub>	44.4 $\pm$ 1.16 **	13.11	8.4 $\pm$ 0.20 **	12.14	97	
		M <sub>2</sub>	102.8 $\pm$ 0.83 **	4.06	12.0 $\pm$ 0.25	10.50	70	
Neem		Control	M <sub>1</sub>	71.6 $\pm$ 0.44	4.37	9.6 $\pm$ 0.19	14.60	76
			M <sub>2</sub>	71.5 $\pm$ 0.24	2.40	9.0 $\pm$ 0.22	17.40	75
	20%	M <sub>1</sub>	78.4 $\pm$ 0.28 **	2.51	11.2 $\pm$ 0.25 **	15.80	78	
		M <sub>2</sub>	79.2 $\pm$ 0.56 **	4.96	12.3 $\pm$ 0.19 **	11.12	79	
	40%	M <sub>1</sub>	92.2 $\pm$ 0.63 **	4.81	14.9 $\pm$ 0.21 **	9.83	87	
		M <sub>2</sub>	94.6 $\pm$ 0.56 **	4.22	15.3 $\pm$ 0.23 **	10.41	88	
	60%	M <sub>1</sub>	88.9 $\pm$ 0.44 **	2.47	12.4 $\pm$ 0.33 **	13.10	85	
		M <sub>2</sub>	90.8 $\pm$ 0.50 **	3.93	13.0 $\pm$ 0.29 **	11.43	86	
	80%	M <sub>1</sub>	74.6 $\pm$ 1.16 *	10.96	10.9 $\pm$ 0.28 **	13.04	80	
		M <sub>2</sub>	83.1 $\pm$ 0.54 **	4.63	12.5 $\pm$ 0.26 **	10.41	82	
	100%	M <sub>1</sub>	66.5 $\pm$ 0.53 **	5.68	8.6 $\pm$ 0.23 **	13.14	78	
		M <sub>2</sub>	75.2 $\pm$ 0.52 **	4.93	9.8 $\pm$ 0.27 *	14.02	79	

\*\* Significant from the respective control at 1.0% level. \* Significant from the respective control at 5.0% level

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# Studies on the effect of siliqua position and alignment on seed number and weight in *Brassica campestris* var. Yellow sarson

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## Introduction:

Of the three common ecotypes (i.e. Toria, Yellow sarson and Brown sarson) of *Brassica campestris* found in India, *Brassica campestris* var. Yellow sarson is reported to bear the lowest number of total siliquae per plant and per square meter, where as 1-grain weight and seeds per siliqua are exceedingly higher (Bhargava, 1996). This suggests to emphasize on these two primary yielding attributes over the whole plant model in general and over the main raceme which in yellow sarson is the major contributor to total siliquae on a plant, in particular. In yellow seeded rape, flowers open acropetally on the main (indeterminate) raceme leaving considerable time interval between the opening of first and last flower, which results in non-synchronous flowering within the raceme. Hence, it becomes imperative to study the effect of this differential developmental pattern of both flowering and pod setting within the raceme on the seed weight and seed number per siliqua.

## Materials and methods:

A study was carried out using genotypes with three varying siliqua alignments on the basis of which they were categorized into Pendant (down turned pod inclination), Horizontal (with respect to land surface) and Upright (erectly oriented) groups. Total of 10 Pendants, 10 Upright and 9 Horizontal genotypes were laid in split-plot design with three replicates during the rabi 2001-02. Inclination feature, only secondarily important was treated as main plot factor, which in turn as a group was studied for pod samples from three different zones of main raceme (upper, middle and lower raceme lengths) collected from 3 sub-plots respectively. Leaving besides a terminal and the lower most siliqua on the raceme (to reduce sampling error), a random sample of 1 siliqua per zone (or per sub-plot) from each of 10 competitive plants was collected to study seed weight per 10 pods and seed number per siliqua. The difference among the three nodal positions as well as among the three inclination types was worked out through Least significant difference and Mean-squared deviations from mean, following Gomez and Gomez (1983).

## Results:

In yellow seeded rape seed weight per pod revealed indifferent and consistent mean phenotypic values across the three inclination groups. Seeds were significantly lighter in the apically positioned siliquae, where as, middle and lower lengths of main raceme bore pods with comparable test-weights (Table 1). This discrepancy as well leads to significant mean square deviations when studied over these three zonations. As therefore expected, variance among orientations and orientation  $\times$  pod position was recorded to be non-significant at both 5% and 1% levels (Table 2). Unlike seed weight, seed number per pod varied much among the inclination groups with Upright siliqua type bearing more seeds per pod than both Horizontal and Pendant types. As regards particular pod positions, upper, middle and lower lengths of raceme were noted to bear pods with similar seed numbers, thus showing non-significant variance.

## Discussion:

Owing to a rapid decline in leaf area during pod filling (Chauhan and Bhargava, 1984) the assimilate supply to the pods developing at basal, middle and apical positions of the raceme is likely to differ. Our results are in consonance with the findings of Chauhan and Bhargava (1986) in *B. juncea* cv. Pusa Bold. Like ours these observations show a little variation for seed numbers per pod possibly because seed number per pod gets fixed as early as at flower initiation. Since, seed weight is dependent upon pod wall photosynthesis thus suffers more at later stages of development in apically positioned pods.

### Conclusion:

In conclusion it can be suggested that Upright siliqua forms out -perform other two inclination groups in terms of seed weight per siliqua as because of higher seed weight per siliqua in middle of the main raceme. Conversely, selection for genotypes which retain leaves for longer periods in post flowering phase could enhance seed yield further, by supplementing pod wall photosynthesis in apically positioned siliquae.

**Table 1. Simultaneous mean value comparisons over 3 siliqua inclinations and 3 pod positions as regards seed number per pod and seed weight per 10 pods.**

Siliqua orientation	Trait	Pod position			Mean over inclination groups
		Upper pod	Middle pod	Lower pod	
Pendant	Seed weight per 10 pods (gm)	0.848	1.066	0.995	0.970
	Seed number per pod	25.910	26.563	27.776	26.750
Horizontal	Seed weight per 10 pods (gm)	0.922	1.068	1.092	1.027
	Seed number per pod	26.583	26.650	27.126	26.786
Upright	Seed weight per 10 pods (gm)	0.940	1.1097	1.064	1.038
	Seed number per pod	30.843	32.740	29.376	30.986
Mean over pod positions	Seed weight per 10 pods (gm)	0.903	1.081	1.050	-
	Seed number per pod	27.778	28.651	28.093	-

**Table 2. Mean Sum of Squares involving 3 siliqua orientations and positions as regards traits seed weight per 10 pods and seed number per siliqua.**

Source of variation	d.f.	Mean Sum of Squares	
		Seed weight per 10 pods	Seed number per pod
Replicates	2	0.067	14.655
Orientation group	2	0.012	53.386 **
Error (A)	4	0.033	5.540
Pod position	2	0.081 **	1.756
Orientation × Pod position	4	0.002	4.864 **
Error (B)	12	0.002	0.531

CV

5.26%

2.58%

\*\* exceeds 1% level of significance.

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# Effect of siliqua angle on seed yield and its component attributes in tetra -locular *Brassica campestris* var. Yellow sarson

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## Introduction

This study was inspired by *a priori* works and established theories of some authors who suggest that apart from inherent seed yield attributes, the yield per plant can be taken into account in relation to any morpho -physiological property of the plant type itself. With yellow seeded genotypes of oilseed rape (*Brassica campestris* var. yellow sarson), one such study was initiated owing to the perceptible variation of 3 discrete siliqua orientations viz. Upright, Horizontal and Nodding pod (plant) types. Thus it becomes intelligible and imperative to study that having been differing in the angle of inclination of their siliquae, if the same genotypes must show as a group a significant contrast for seed yield and its attributes when compared to one another.

## Materials and methods

A total of 30 advanced true breeding lines of yellow seeded rape classed into 3 orientation groups of 10 each (which correspond to upright, horizontal and down turned inclination of their pods) were line sown in RCB design for 2 consecutive winters of 2000-01 and 2001-02. Randomization was practiced between and not within groups. Comparisons were made among 3 inclination groups as regards seed yield and its component attributes by working out Least Significant Difference for mean phenotypic values. To authenticate the mean value contrasts for different traits, the squared deviations from mean were calculated. A total treatment variance was further partitioned into contributing sources of within and among group differences as per the method followed by Rangaswamy (1995).

## Results and discussion

For the sake of brevity only the results pooled over years one and two are discussed. As evident from the Table 1, Upright plant types as a group out -performed the horizontally aligned pod forms which in turn revealed greater mean values than nodding fruited types as regards traits plant height (PH), length of main shoot (LM), height up to first fruiting branch (HB), total siliqua per plant (TS), siliqua on main raceme (SM), days to 50% flowering (DF), its completion (DC), days to maturity (DM), seeds per siliqua (SS) and seed yield per plant (SY). Pendants exceeded Horizontals for number of total branches per plant (NB), siliqua borne over them (SB) and 1000 -seed weight (SW). However, this out -range doesn't help to enhance seed yield in nodding pod forms, as in yellow sarson it is the main raceme which is the major contributor to total siliqua on a plant (Labana *et al.*, 1980). Also, can be argued otherwise that longer length of main shoot and hence large pod bearing surface on it and more so the highest number of seeds per siliqua makes Upright pod forms best yielder group of all the three plant types. Moreover, number of seeds per siliqua seems to play an important role in governing seed yield, which being significantly low for Horizontal group, degress it from competitive ability with Upright class and by which reason inspite of superseding for other characters makes Horizontals comparable yielder to Pendants. The same can be authenticated by studying among group variances (Table 2), where again Uprights collectively as a group significantly differ with respect to other two siliqua types for most of the seed yielding parameters. SM, SB, PH and DM showed significant Genotype  $\times$  Year interactions, indicating their differential response over the two environments.

## Conclusion

As the experimental material was never selected for any character except for the sole inclination feature of the pod. The significant excess as per seed yield and its attributes coming out by default in favor of Upright plant types against Horizontals and Pendants, presumably may be attributed, linked to the siliqua angle, although passively. However, this needs to be judged strictly for some physiological process related to pod inclination and in light of the inheritance pattern assumed by the angular types as rightly studied by Woods (2000).

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Table 1. Mean value contrasts among the three siliqua inclination groups over the years pooled.

Siliqua group	PH	LM	HB	NB	TS	SM	SB	SY	SS	SW	DF	DC	DM	OC
Pendant	87.23	38.45	31.53	6.11	49.33	30.46	18.75	5.22	27.54	3.84	41.37	69.25	87.96	46.27
Horizontal	101.5	49.51	44.16	5.27	56.8	43.98	13.32	5.84	28.21	3.64	45.26	72.16	89.28	44.19
Upright	124.7	67.21	51.62	5.88	77.59	61.75	15.84	6.73	31.37	2.76	54.31	76.74	89.57	43.88
CD at 5%	9.36	5.32	4.21	0.73	5.84	8.82	2.98	0.76	2.98	0.54	6.37	4.13	2.73	1.96

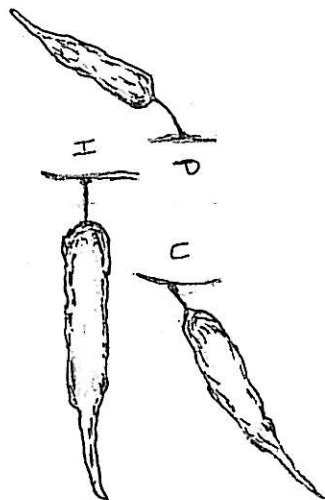


Table 2. Squared deviations from mean for three siliqua orientation classes over the years pooled.

Source	d.f	Mean sum of squares															
		PH	LM	HB	NB	TS	SM	SB	SY	SS	SW	DF	DC	DM			
Years	1	394.25 <sup>NS</sup>	31.89 <sup>NS</sup>	125.28 <sup>NS</sup>	17.39*	278.11 <sup>NS</sup>	154.61 <sup>NS</sup>	124.34 <sup>NS</sup>	21.27**	57.64**	0.006 <sup>NS</sup>	7.22 <sup>NS</sup>	24.38**	1.09 <sup>NS</sup>			
Genotype	29	1607.20**	845.18**	564.37**	19.64*	654.36**	361.16**	293.72**	4.28**	146.35**	0.91**	43.86**	20.12*	16.49 <sup>NS</sup>			
Pendant	9	145.63 <sup>NS</sup>	127.32 <sup>NS</sup>	111.63 <sup>NS</sup>	6.75 <sup>NS</sup>	134.21*	24.31 <sup>NS</sup>	110.72*	3.50**	28.67**	0.009 <sup>NS</sup>	8.64 <sup>NS</sup>	5.38 <sup>NS</sup>	12.69 <sup>NS</sup>			
Horizontal	9	319.47*	93.95 <sup>NS</sup>	151.24 <sup>NS</sup>	3.52 <sup>NS</sup>	426.35**	139.33**	284.27**	3.79**	37.24**	0.03 <sup>NS</sup>	7.41 <sup>NS</sup>	6.86 <sup>NS</sup>	11.08 <sup>NS</sup>			
Upright	9	895.56**	106.12**	137.15**	4.26 <sup>NS</sup>	495.73**	272.60**	223.35*	4.32**	58.61**	0.11*	7.88 <sup>NS</sup>	4.31 <sup>NS</sup>	12.50 <sup>NS</sup>			
Upright Vs Rest	1	15937.5**	127.20**	1158.27**	12.72 <sup>NS</sup>	3560.09**	2610.32**	952.11**	36.47**	452.18**	1.16**	115.62**	12.71*	15.40 <sup>NS</sup>			
Pendant Vs Horiz	1	1754.83*	976.39**	924.48**	21.61**	168.41*	95.25**	72.35 <sup>NS</sup>	3.89 <sup>NS</sup>	7.14 <sup>NS</sup>	0.05 <sup>NS</sup>	11.41 <sup>NS</sup>	9.86*	11.68 <sup>NS</sup>			
Genotype x Years	29	61.76*	20.45 <sup>NS</sup>	18.32 <sup>NS</sup>	1.73*	72.44*	22.62**	49.24**	0.96*	9.34 <sup>NS</sup>	0.04 <sup>NS</sup>	7.61*	3.24 <sup>NS</sup>	12.61**			
Pooled Error	116	28.24	8.67	7.27	0.56	23.82	8.45	21.64	0.27	4.26	0.02	3.24	2.83	4.33			
Error Combined	145	-	14.32	12.34	-	-	-	-	-	5.39	0.03	5.71	-	7.94			

\*\* exceeds 1% level of significance  
 \* exceeds 5% level of significance

PH: Plant height(cm); LM: Length of main raceme(cm); HB: Height up to first fruiting branch(cm); NB: Number of primary branches; TS: Total number of siliqua on a plant; SM: Siliqua on main raceme; SB: Siliqua on branches; SY: Seed yield per plant(gm); SS: Seed number per siliqua; SW: 1000-seed weight(gm); DF: days to 50% flowering; DC: Days to flowering completion; DM: Days to maturity; OC: Oil content(%)

# GENETICS OF COMPONENT OF VARIATION FOR SEED YIELD, HARVEST INDEX AND OIL CONTENT IN INDIAN MUSTARD

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Indian mustard (*Brassica junica* (L) Czern & Coss) is grown as an oilseed crop. Selection after hybridization were generally followed for funneling out for concentrating the desirable gene(s) governing the characters of economic importance of segregating generation. The success of selection programme usually needs the basic information on the type of gene action involved in the expression of these characters, the extent of heritability and the magnitude of advanced that could be obtained by utilizing a particular scheme of selection. A diallel crossing system involving 10 selected parents was initiated for this purpose and comparative performance of  $F_1$  hybrids of these characters were studied and results are reported in this paper.

The material for the present investigation comprised of 10 varieties/ strains of mustard viz. Kranti, Pusa bold, Pusa barani, RLM-198, PHR-2, Divya, Zem-1, EC-322090, EC-322092 and Domo of diverse origin and their all possible  $F_1$  crosses (excluding reciprocals). The parents and 45  $F_1$  hybrids were evaluated in a randomized complete-block design with three replications in single row of 5m long at a distance of 30 and 10 cm between rows and plants, respectively. Observations on yield / plant, harvest index and oil content. The oil content of seed was determined by NMR (Nuclear Magnetic Resonance spectroscopy) and harvest index was computed as the ratio of seed yield and biological yield x 100. Components of variance were computed following Hayman (1954).

Significant variability among parents for seed yield harvest index and oil content was observed in both the years. The significant value of  $t^2$  revealed the presence of non-allelic interaction for harvest index and oil content in 1998 and 1999, respectively. On the other hand, non significant  $t^2$  values for seed yield, oil content and harvest index 1998 and 1999, respectively indicated the validity of diallel cross analysis (Table-1) thus, these results are discussed. The higher magnitude of H component than D indicated predominance of dominance effects for these characters. The ratio of  $\sqrt{H_1}/D$  ( $>1.0$ ) confirm these results and indicated the presence of over dominance. Similar results

were reported by Govil *et.al* 1983, Thakral *et.al* 1985 Ram Bhajan *et.al* 1997. Asymmetry in the distribution of genes with positive and negative effects was indicated by  $H_2 / 4H_1$  ratio ( $<0.25$ ) for all these characters. The estimates of KD/KR ( $>1.0$ ) indicated the excess of dominant genes in the parents for all the traits in both the years for yield / plants in 1999, where excess of recessive genes was evident ( $<1.0$ ).

Heritability (narrow sense) was high for oil content (47.5) in 1998 and moderate for seed yield (20.26 % and 27.8%) and harvest index (26.6 %). More or less similar results have been reported by Wang and Wang 1986, Malik *et.al* 1995 and Hussain *et.al* 1998. On the basis of these results, it is concluded that selection procedure which allow intermating of improved genotypes in successive generation may be adapted.

**Table 1: component of genetic variance and their ratio for seed yield /plant, Harvest index and oil content mustard.**

Genetic Component	Seed yield /Plant		Harvest index		Oil content	
	1998	1999	1998	1999	1998	1999
D	1.01**	1.17*	0.34**	0.17**	0.47 **	0.05
H <sub>1</sub>	3.21**	10.43**	0.53**	0.55**	0.86 **	7.18**
H <sub>2</sub>	2.88**	9.17**	0.46**	0.45**	0.73**	1.60**
h <sup>2</sup>	0.59	9.89**	0.20**	0.35**	0.81**	0.05
F	0.95	-0.24	0.31**	0.07**	0.07	-0.21
E	0.14	0.24	0.04	0.03	0.11	0.34
$\sqrt{H_1/D}$	1.78	2.98	1.26	1.81	1.35	6.13
$H_2 / 4 H_1$	0.22	0.22	0.22	0.24	0.21	0.22
KD/KR	8.72	0.93	2.14	1.26	1.12	0.48
h <sup>2</sup>	20.26	27.80	15.53	26.60	47.50	20.38
t <sup>2</sup>	NS	NS	Sig	NS	NS	Sig

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## LAVEL OF SELF- INCOMPATIBILITY IN SPROUTING BROCCOLI (*Brassica oleracea* L. var. *italica* Plenck)

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This is among very few studies conducted about the level of self-incompatibility in sprouting broccoli in India. This investigation deals with self-incompatibility aspects in 12 lines of sprouting broccoli.

The 12 lines used in present study were Palam Samridhi, Calabrese Sutton, BR-76018, BI-80345, DPGB-5, EC-10356. Broccoli Green Head, BI-80167, BI-80336, BR-1, Punjab Broccoli-1 and Broccoli Purple. The crop was raised during 2000-2001. The inflorescence at bud stage was partitioned in to four parts. The first part was labelled and left to allow open pollination by insects for natural cross pollination. The second part was used for selfing by bud pollination. The third part was left for self pollination by hand in freshly opened flowers and fourth part was left for natural self pollination by covering with muslin cloth bag. Twenty five siliqua from each part / plant were taken randomly and seeds were counted.

The compatibility status of the genotypes were assessed by following the methods advocated by Watts (1963) and Nieuwhof(1974) by determining fertility index. Mean seed number per pod in bud pollination expressed as percentage of mean seed number per pod under open pollination gave percentage self-compatibility(Watts,1968).Bud pollination was done in support to the results obtained and further to see its feasibility for producing  $S_1$  lines in case there is self-incompatibility.

The average number of seeds per siliqua under different types of pollination and fertility index are presented in Table-1.

The average number of seeds per siliqua produced in different types of pollination varied between the lines as well as within a line. In the lines Palam Samridhi, BR-76018, BI-80345, DPGB-5, EC-10356, Broccoli Green Head, BI-80167, BI-80336, BR-1 and Pb. Broccoli-1, seed set under natural cross-pollination was, in general higher than that of bud pollination.

Under self-pollination in freshly opened flowers, Palam Samridhi, Calabrese Sutton, BR-76018, BI-80345, DPGB-5, EC-10356, Broccoli Green Head, Pb. Broccoli-1 produced on an average 2.3, 0.54, 2.6, 11.6, 8.9, 5.3, 3.1 and 6.33 seeds per siliqua respectively, which was much lower than the seed set under bud pollination and natural cross-pollination. The average seed set in BI-

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80167, BI-80336, BR-1 and Broccoli purple was 13.8, 12.5, 17.8 and 18.0, respectively. It was higher than that under bud pollination lines. BR-1, Br-76018, Broccoli purple and BI-80345 gave highest seed set under natural self-pollination.

The fertility index (average number of seeds per siliqua in natural cross-pollination divided by average number of seeds per siliqua under self-pollination in freshly opened flowers) of parental lines varied from 1.77 (compatible) to infinity (fully incompatible). The maximum number of plants with fertility index more than 2.00 were in the lines Palam Samridhi, Calabrese Sutton, BR-76018, DPGB-5, EC-10356, Broccoli Green Head, BI-80167, BI-80336 and Pb. Broccoli-1.

Out of 12 genotypes nine genotypes listed for self-incompatibility testing nine genotypes showed fertility index more than two and hence were classified as self-incompatible on the basis of fertility index but showed comparatively good seed setting i.e., one or more seed per pod, upon selfing. Genotypes Calabrese sutton, BI-80336, DPGB-5, Broccoli Green Head, BR-76018, Palam samridhi showed high fertility index and very less seed set upon selfing and thus highly self incompatible. Level of self incompatibility depends on a number of factors such as expressivity of S-alleles, modifier genes on physiological factors (Haruta, 1972). Environmental factors such as high temperature and high humidity also weaken the level of self-incompatibility (Carter and McVeilly, 1975, Ockendan, 1978, Stern *et al.*, 1982). In the present study varied level of expression of self-incompatibility was observed that could be due to above mentioned factors. The results obtained in this investigation suggested that a wide range of incompatibility from complete self-incompatible to self-compatible lines are present in this crop. For utilization of self-incompatibility in hybrid seed production, it is essential that level of self-incompatibility should be high so as to avoid the selfed seed. Consequently, while using these lines for breeding, one should maintain acceptable amount of heterozygosity for the production of synthetics/composites or F<sub>1</sub> hybrids. For the material exhibiting strong self-incompatibility reaction, mass selection and mass pedigree method of breeding could also be effective.

This investigation has demonstrated high level of self-incompatibility in six varieties of sprouting broccoli along with success of bud pollination which offer a promise to exploit heterosis in this crop.

### **Summary**

Twelve varieties of sprouting broccoli were tested for level of self-incompatibility. Out of twelve, six lines exhibited high level of self-incompatibility along with success of bud-pollination which offer a promise to exploit heterosis in this crop.



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Table 1. Seed set in lines/var. of broccoli under different types of pollination and fertility index

Lines	No. of seeds per siliqua					Fertility index
	NCP	BP	SP	NSP		
Palam Samridhi	12.0	10.80	2.3	1.35	5.2	
Calabrese Sutton	11.0	12.0	0.54	1.0	20.37	
Br-76018	19.8	11.6	2.6	12.5	7.61	
BI-80345	20.6	15.3	11.6	5.9	1.77	
DPGB-5	20.9	11.1	8.9	2.0	10.45	
EC-10356	11.50	9.5	5.3	0.0	Infinity	
Br. Green	16.20	8.67	3.1	1.9	8.52	
BI-80167	20.12	11.10	13.8	5.3	3.79	
BI-80336	10.45	10.32	12.5	1.0	10.45	
BR-1	22.80	13.87	17.8	14.8	1.54	
Pb. Broccoli-1	15.60	12.33	6.33	4.0	3.90	
Broccoli purple	22.30	14.80	18.0	12.1	1.87	

NCP = Natural cross pollination, BP = Bud pollination, SP = Self-pollination in freshly opened flowers, NSP = Natural self pollination

# Overcoming self-incompatibility in *Eruca sativa* via chemical treatment

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## INTRODUCTION

*Eruca Mill.* is self incompatible (SI)<sup>[1]</sup>. Research has shown that SI plants produce an "isolating layer" of proteins on the surface of the stigma 1-2 days before flowering. This "isolating layer" prevents pollens of the same genotype from germination on the stigma<sup>[2][3][4]</sup>. SI helps the plants maintain heterozygous condition, which is advantageous for the plant's survival and adaptation. In plant breeding, however, we often need to overcome SI in order to obtain inbred lines. The present study was to identify the effective chemicals and the effective concentrations for overcoming SI in *Eruca*.

## MATERIALS AND METHODS

1. Plant materials: Five *Eruca sativa* varieties, "Yuzhong", "Huachi", "Tianshui", "Zhuoni", and "Yongjing", were used. The plants of these varieties were inbred lines obtained from generations of forced self pollination and therefore had uniform characteristics within each line.

2. Methods: 140 inflorescences were bagged in each variety. Eight buds were kept on each inflorescence. The six chemicals were used at five concentrations specified as follows: sucrose, 0.5, 1, 4, 7, 14%; table salt, 1, 3, 8, 10, 15%; gibberellin, 20, 40, 60, 80, 100 ppm; alcohol, 30, 50, 75, 95, 100%; urea, 5, 10, 12, 15, 20%; ammonium sulphate, 10, 15, 20, 25, 30%. Distilled water was used as the control. Pollination was conducted either immediately or 0.5 hours after the chemical treatment. Statistics of the pods and seeds produced from the treated buds were collected. The pod-setting rates and seed-setting rates were calculated using the following formula: Pod-setting rate (%) = (number of pods / number of treated buds) X 100; seed-setting rate = number of plump seeds / number of treated buds.

## RESULTS

1. Effects of different chemicals: The effectiveness of a chemical in overcoming SI can be measured by pod- and seed-setting rates of chemical-treated self-pollinated buds. The data in Table 1 show that chemical treatment could effectively overcome SI in *Eruca sativa*. Gibberellin, urea, and ammonium sulphate significantly ( $p < 0.01$ ) increased the pod-setting rates and the seed-setting rates. The average pod- and seed-setting rates of alcohol, sucrose and table salt were not significantly different from that of the control ( $p > 0.05$ ). (Table 1)

Table 1. Pod- and seed-setting rates of chemical-treated self-pollinated buds, showing the effects of different chemicals, averaged across five concentrations, in overcoming SI in *Eruca sativa*

Chemicals	Pod-setting rate (%)	Seed-setting rate	Significance of difference	
			P=0.05	P=0.01
Gibberellin	40.2	3.30	A	A
Ammonium sulphate	14.3	2.88	A	A
Urea	19.2	2.94	A	A
Sucrose	12.5	1.40	B	B
Table salt	16.6	1.61	B	B
Alcohol	11.3	1.07	B	B
Distilled water	12.2	1.42	B	B

2. Effects of plant varieties and chemical-genotype interaction: Seed-setting rates differed significantly among *Eruca sativa* varieties. Tianshui and Huachi had significantly higher seed-setting rates than the other three varieties ( $p < 0.01$ ). The variation among these varieties suggests that these varieties differ in their degrees of SI. Gibberellin, ammonium sulphate, and urea generally had significant effects in overcoming SI in all varieties. Gibberellin treatment consistently resulted in the highest seed-setting rates in all five varieties tested, and alcohol treatment consistently resulted in the lowest seed-setting rates in all five varieties tested. The chemical-genotype interaction was not significant, suggesting that a chemical that is effective on one variety is likely to be effective on another variety in overcoming SI. (Table 2).

Table 2. Effects of plant varieties and chemical-genotype interaction in overcoming self incompatibility in *Eruca sativa* as indicated by seed-setting rates of self-pollinated buds

Chemical	Genotype				
	Zhuoni	Yuzhong	Tianshui	Yongjing	Huachi
Sucrose	1.06	1.04	2.1	1.20	1.60
Table salt	1.41	1.12	2.41	1.34	1.80
Gibberellin	2.67	2.10	4.42	2.70	4.61
Alcohol	0.82	0.97	1.41	1.14	1.02
Ammonium sulphate	2.65	2.04	4.27	1.81	3.61
Urea	2.51	2.04	4.39	1.80	3.97
Distilled water	1.25	1.02	2.21	1.26	1.34
Mean	1.76	1.48	3.03	1.61	2.56

3. Effects of chemical concentrations: The effects of chemical concentrations in overcoming SI of *Eruca sativa* was highly significant ( $p < 0.01$ ). Sucrose, for example, had little effects on seed-setting rates comparing to the water control when seed-setting rates were averaged across all five concentration levels (Table 1). At 1% concentration, however, sucrose had a seed-setting rate of 2.73, which was significantly higher than that of the control (1.42) (Tables 1 & 3). Even the most effective chemicals such as gibberellin and urea were not effective at certain concentrations. The most effective concentrations for the six chemicals tested were: 1% sucrose, 8% table salt, 100 ppm gibberellin, 50% alcohol, 20% ammonium sulphate, and 15% urea (Table 3).

Table 3. Effects of chemical concentrations (values in the brackets) in overcoming self incompatibility in *Eruca sativa* as indicated by seed-setting rates of self-pollinated buds

Chemical	Concentration				
	Level 1	Level 2	Level 3	Level 4	Level 5
Sucrose	0.18 [0.5%]	2.73 [1%]	0.93 [4%]	1.70 [7%]	0.60 [14%]
Table salt	0.39 [1%]	1.39 [3%]	1.99 [8%]	0.97 [10%]	1.91 [15%]
Gibberellin	2.04 [20ppm]	2.73 [40ppm]	1.59 [60ppm]	0.95 [80ppm]	4.95 [100ppm]
Alcohol	0.79 [30%]	1.64 [50%]	1.46 [75%]	0.78 [90%]	1.06 [100%]
Ammonium sulphate	1.34 [10%]	0.98 [15%]	1.20 [18%]	3.21 [20%]	1.38 [25%]
Urea	1.73 [5%]	1.68 [10%]	3.23 [15%]	1.67 [17%]	0.69 [20%]

4. Effect of pollination timing: Pollination immediately following the chemical treatment was not significantly different from pollination 0.5 hours after the chemical treatment.

## SUMMARY

Certain chemicals can dissolve, deposit, and/or denature proteins so that these proteins no longer have their designated biochemical activities. Such effects of chemicals on proteins are not protein-specific. The fact that chemical treatment significantly increased seed production of self pollinated buds suggests that in *Eruca sativa* as in many other plant species<sup>[2]</sup>, the mutual recognition of the proteins on the pollen surface and those on the stigma surface plays a key role in generating the SI. Among the six chemicals tested, gibberellin, urea, and ammonium sulphate were highly effective in overcoming the SI in the five *Eruca sativa* varieties tested. The concentrations of the chemicals had significant effects. If the concentration was not right, even the most effective chemical may show no effect, which can lead to a false conclusion that this chemical is not effective. Different plant varieties differed significantly in their seed productivity from self pollination. The SI of Tianshui and Huachi was relatively weaker than that of Zhuoni, Yuzhong, and Yongjing. Chemical-genotype interaction was not significant suggesting that chemicals that are effective in one variety are also likely to be effective in another variety.

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## Variability in Quality parameters in Indian Mustard (*Brassica juncea*)

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The emphasis is being laid upon improvement in seed size, colour, fiber content, oil content, fatty acid profile, protein content and glucosinolate content i.e quality of oil and oilcake, because these character are directly associated with nutritional and market value of produce and product. Development of variety with good quality of oil & cake i.e desirable fatty acid profile and low fiber, glucosinolate, high protein, these information about variability are prime importance in deciding breeding. Therefore present study was conducted to observe variability between different quality parameters.

### Materials and methods

Experiment was conducted with forty genotypes of *Brassica juncea* (Indian mustard). The genotypes were planted in randomized block design with two replication at CRC. Pantnagar during 2000-2001. Harvested material was analyzed in oil quality laboratory for quality parameters viz., protein, fiber, glucosinolate content and fatty acid compositions. Oil content was estimated by NMR (nuclear magnetic resonance), fatty acid composition by gas liquid chromatograph, protein by micro Kjeldhal method, glucosinolate by tetrachloro pallodate with help of ELISA reader and fiber content by AOAC (1984) were estimated the data recorded on all traits subject to statistical analysis. Coefficient of variability as calculated according to **Burton and Davane (1953)**; heritability and genetic advance under selection were estimated according to **Lush (1949)**.

### Results and discussion:

Significant variation was observed for all parameters Table 1. Widest range was recorded for glucosinolate (29.26 to 176.66 micro mole/gm of fat free meal) followed by oil content (33.26 to 44.89%), protein content (33.26 to 42.44%) and fiber content (9.70 to 15.34%). Among the fatty acids profile, erucic acid exhibited widest range (0.10 to 53.33%) followed by oleic acid (7.92 to 60.43%), Linoleic acid (1.73 to 43.40%), GCV for all the characters were almost equal to PCV, suggesting that environment had no major influence on them. Highest coefficient of variations (GCV and PCV) were recorded for glucosinolates followed by protein content. Among the fatty acids, erucic acid observed highest coefficient of variations followed by erucic, oleic, stearic acids.

All the characters reflected high heritability (> 98%) except oil content and palmitic acid. High heritability coupled with high genetic advance for glucosinolate and erucic acid suggested preponderance of additive gene action in determinations of these traits. It is advisable that

selection pressure applied on these desired direction coupled be useful. High heritability in combination of moderate genetic advance for oleic acid and linoleic acid revealed non-additive genetic effect in except of these traits. Low genetic advanced for those traits inspite of high heritability is due to environmental influence. Similarly results were observed by Ahuja *et al.* (1989), Simgel *et al.* (1991), Tang and Lie (1991) and Singh *et al.* (2000).

**Table 1: Mean of square, mean  $\pm$  SE, range, phenotypic (PCV) and genotypic coefficient of variation, broad sense heritability and genetic advanced for quality parameters of Indian mustard (*Brassica juncea*).**

Characters	Mean square	Range	Mean $\pm$ SE	PCV	GCV	Heritability	Genetic advance
Oil content	7.777**	35.94-44.89	40.41 $\pm$ 0.88	4.35	5.32	66.80	2.97
Protein content	12.332**	33.26-42.44	37.21 $\pm$ 0.88	6.24	7.08	77.76	4.22
Fiber content	8.325**	9.70-15.34	11.99 $\pm$ 0.38	16.71	17.30	93.27	3.99
Glucosinolate	1535.34**	29.62-176.55	115.25 $\pm$ 2.20	23.97	24.12	98.75	56.54
Palmitic acid	2.851**	2.23-6.99	4.64 $\pm$ 0.44	23.87	27.43	75.12	1.92
Stearic acid	0.492**	0.01-2.69	1.11 $\pm$ 0.10	43.77	45.74	91.56	0.96
Oleic acid	355.103**	7.92-60.43	26.21 $\pm$ 1.12	50.66	51.02	98.60	27.16
Linoleic acid	238.183**	10.73-43.10	27.59 $\pm$ 1.21	39.32	39.80	97.55	22.07
Linolenic acid	36.221**	8.37-26.29	15.28 $\pm$ 0.80	27.36	28.34	93.18	8.31
Ecosinoic acid	22.331**	0.01-12.31	4.10 $\pm$ 0.32	91.36	92.04	98.53	7.76
Erucic acid	989.131**	0.01-53.33	21.07 $\pm$ 0.84	105.4	105.6	99.71	45.71

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## Correlation in fatty acids in Indian mustard (*Brassica juncea*)

A.K. Choudhary, S.P. Singh, Kamendra Singh, Anshuman Singh and D.P. Pant

Lowest saturated fatty acid content has mustard oil among all cooking oils and two essential fatty acids linolenic and linoleic acids which are neither synthesized by the human nor can be sourced externally except mustard oil and vegetable oil. Erucic acid & eicosenoic acids were found high in Indian mustard as undesirable fatty acids. Desirable fatty acid composition of mustard varieties will be developed for edible or industrial purpose. Relationship of fatty acid are required to decide the breeding strategies. The study was design to under stands to factors in desirable direction.

### Material and methods:

According to objective, a study was conducted with genotypes of Indian mustard (*Brassica juncea*). The experiment was conducted with two replications during 2000-2001 at crop research station, Pantnagar. Fatty acid analysis of harvested seed was carried out in oil quality laboratory in the department of genetic and plant breeding. Fatty acids were determined by gas chromatograph using capillary column after converting fatty acid into their respective esters by Graig and Murty (1958). Correlation coefficients were estimated according to method given by Searle (1961).

### Results and discussion

Oleic acid was significantly positively associated with linoleic & linolenic acids whereas significantly negatively related with eicosenoic and erucic acids. Linoleic acid was significantly and positively associated with other essential fatty acid i.e linolenic acid whereas both significantly and negatively related with eicosenoic acid and erucic acid. Linolenic acid was significantly positively correlated with oleic, palmitic and linoleic acids. Eicosenoic acid was significantly and positively correlated with erucic acid and negatively with other fatty acids. Erucic acid is significantly and negatively correlated with all fatty acids i.e palmitic, stearic, oleic, linoleic and linolenic except eicosenoic acid. Palmitic acid was significantly positively correlated with linoleic acid while significantly negatively associated with linolenic, eicosenoic, erucic acids. Stearic acid was significantly positively correlated with oleic and linoleic acid while significantly negatively correlated with erucic acid. Similar results correlation among the fatty acids were reported by Ahuja *et al.* (1989), Singh *et al.* (1991) ad Singh *et al.* (2001). Correlation among the fatty acids indicated that reduction in erucic acid resulted in an increase in other fatty acids

**Table : Correlation among fatty acids in Indian Mustard (*Brassica juncea*).**

Characters		Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Eicosenoic acid (20:1)	Erucic acid (22:1)
Palmitic acid (16:0)	P	1.000	0.046	0.257	0.416**	0.454**	-0.224.	-0.408**
	G	1.000	-0.052	0.310	0.421**	0.549**	-0.262	-0.464**
Stearic acid (18:0)	P		1.000	0.458**	0.303	-0.032	-0.218	-0.360*
	G		1.000	0.472	0.339*	-0.026	-0.249	-0.373*
Oleic acid (18:1)	P			1.000	0.733**	0.407*	-0.709**	-0.898**
	G			1.000	0.758**	0.433**	-0.720**	-0.905**
Linoleic acid (18:2)	P				1.000	0.687**	-0.822**	-0.938**
	G				1.000	0.728**	-0.835**	-0.938**
Linolenic acid (20:3)	P					1.000	-0.509**	-0.672**
	G					1.000	-0.522**	-0.696**
Eicosenoic acid 20:1)	P						1.000	0.825**
	G						1.000	0.835**
Erucic acid (22:1)	P							1.000
	G							1.000

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**PRQ-9701 Low Erucic acid and yellow seeded strain of Indian Mustard  
(*Brassica juncea*) (L.), Czern and Coss**

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Brassica oilseeds normally contain high levels of erucic acid. Cultivars with little or no erucic acid was first identified in *Brassica napus* L. Later on, low erucic acid lines were identified in *B. rapa* and more recently in *B. juncea*. The consumers of western countries are more conscious about, nutritional aspects, hence breeder produced cultivars with low erucic acid (Sauer and Kumar, 1983). The first low erucic acid rapeseed (LEAR) cultivars developed were **Orlo** in *B.napus*, **Sapn** in *B.rapa* (Downey *et al.*, 1975) and **Zem-1** in *B.juncea* (Kirk and Oram, 1981). Although, low erucic acid genes are also available in *B.juncea* but only few commercial cultivars of *B.juncea* are low in erucic acid (Love *et al.*, 1990a).

The research efforts made at Pantnagar University related in the development of low erucic acid *B.juncea* mustard strain PRQ-9701. It is a transgressive segregant from a cross between *B.juncea* genotypes, **RC -781** (high erucic acid, black seeded) and **Zem-1** (Australian low erucic acid and yellow seeded cultivar). The low erucic strain **PRQ-9701** is at par with national check, **Kranti** in respect of yield and almost all the physio/morphological traits except fatty acid profile (Table ).

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**Table 1: Important characteristics of PRQ 9701 and its parents.**

Characters	Cultivars			
	PRQ-9701	Krantī (NC)	Parents	
			RC-781	Zem 1
<b>A. Physio/morphological traits</b>				
Days to flowering initiation (nos.)	42	46	62	58
Days to 75% maturity (nos.)	57	67	97	86
Day to maturity (nos.)	135	134	152	148
Plant height (cm)	178.5	187.9	221.3	230.5
Length of main shoot (cm)	54.5	72.2	25.5	36.7
Primary branches (nos.)	7	5	10	4
Secondary branches (nos.)	8	6	11	5
Siliquae on main shoot (nos.)	41	42	28	31
Length of siliquae (cm)	4.5	5.2	4.8	3.5
Number of seed/ siliquae	14	14	13	11
Seed colour	Yellow	Black	Black	Yellow
1000 seed weight (g)	3.62	3.69	2.55	2.85
Oil content (%)	41.80	40.00	39.85	40.25
<b>B. Quality traits</b>				
Fatty acid Composition				
Palmitic acid	4.91	4.83	2.28	4.47
Stearic acid	1.85	0.80	1.14	Trace
Oleic acid	39.89	8.93	10.89	30.61
Linoleic acid	32.45	14.49	14.86	31.39
Linolenic acid	19.68	13.79	13.47	26.84
Ecosinoic acid	1.21	7.44	7.84	7.27
Erucic acid	Trace	49.50	50.37	0.40
Protein content (%)	38.38	34.57	35.07	38.85
Fiber content (%)	9.94	15.34	13.38	9.38

# Production of extracellular enzymes by *A. brassicae* under different cultural conditions

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## Introduction:

Alternaria Blight (AB) caused by *Alternaria brassicae* (Berk.) Sacc. is a severe menace in sustaining higher yields of rape and mustard in many parts of the world and in addition also leads to remarkable losses in oil content (Kolte and Singh, 1997). Extracellular enzymes play an important role in the invasion of a plant by a pathogen. They help in breaking down the cell walls and macerating the plant tissue (Sharma et al., 2001). Pectinases and celulases are among the most important invading extracellular enzymes. In the present study, the effect of cultural conditions on growth related changes in extracellular enzymes of *A. brassicae* have been investigated.

## Materials and Methods:

*Alternaria brassicae* obtained from Department of Plant Pathology, PAU was grown in 250ml flasks containing 80ml Czapek's medium and Duggar's medium separately, at different pHs; 5.5 and 7.5 and different temperatures; 25°C and 30°C. The fungus was harvested at 3,5,7,9,11 and 13 days after inoculation and biomass was estimated. The culture filtrates were used for the estimation of cellulase and pectinases. Cellulase activity was estimated by the method of Jones et al., 1972. Pectinases [polygalacturonase (PG) and pectin methyl esterase (PME)] were estimated by the methods of Miller, 1959 and Dawson, 1962, respectively.

## Results and Discussion:

Production of pectinases and cellulase as a function of growth is shown in Table 1. It is evident that the pectinase activity increased as the fungus continued to grow and was maximum at 9<sup>th</sup> day of growth in both the media and declined thereafter. Soni and Bhatia, 1981 have also reported increase in pectinase activity with growth in case of *Fusarium oxysporum*. The PG activity was higher in Czapek's medium than in Duggar's medium (25°C, pH 5.5), whereas in case of PME the activity was almost same in both the media. When the pectinase activity was compared in relation to pH and temperature conditions, maximum activity was observed at 25°C and pH 5.5. The cellulase activity also showed similar trend but it was much less in comparison to PG activity. Whatever activity of pectinase or cellulase is observed in the culture filterates must be due to the constitutive pectinase and cellulase of the fungus because the carbon sources used in the media were sucrose in Czapek's medium and glucose in Duggar's medium. The pectinase activity reported by Soni and Bhatia (1981) was mainly inducible pectinase since they had used pectin as the sole source of carbon in the medium. The constitutive enzymes reported here are not repressed by the presence of glucose or sucrose in the medium (Trachet and Fevre, 1996). These constitutive enzymes provide the pathogen with the inherent ability to utilize the nutrients from host and then trigger inducible enzyme synthesis. Maximum growth of the fungus (as evidenced by biomass measurements in Table 1) was also observed in Czapek's medium (25°C, pH 5.5) indicating these to be the good cultural conditions favouring the growth of *A. brassicae* and further showing that this fungus prefers sucrose as carbon source for its growth rather than glucose which is utilized virtually by most of the cultivable fungi.

**Table 1: Growth and production of pectinases (PG and PME) and cellulase by *Alternaria brassicae* under different cultural conditions**

Growth period (days)	Mycelium dry wt. (g/dl)				Pectinases								Cellulase units/ml of culture filtrate <sup>c</sup>			
					Polygalacturonase units/ml culture filtrate <sup>a</sup>				Pectin methyl esterase units/ml culture filtrate <sup>b</sup>							
	Czapek's medium		Duggar's medium		Czapek's medium		Duggar's medium		Czapek's medium		Duggar's medium		Czapek's medium		Duggar's medium	
	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5	25°C pH 5.5	30°C pH 7.5
3	0.2	0.1	0.1	0.09	0.010	N.D.	0.007	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	3.7	2.6	0.6	0.5	0.067	N.D.	0.012	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
7	3.9	2.8	1.1	0.6	0.512	0.050	0.191	0.012	0.025	N.D.	0.025	N.D.	0.015	N.D.	N.D.	N.D.
9	4.1	2.9	1.9	0.9	1.154	0.381	0.976	0.249	0.056	0.035	0.056	0.035	0.158	0.103	0.033	N.D.
11	3.8	3.2	1.6	0.7	0.726	0.198	0.333	0.126	0.035	0.010	0.046	0.012	0.087	0.058	0.021	N.D.
13	3.5	2.3	1.4	0.4	0.559	0.167	0.214	0.083	0.035	0.010	0.035	0.010	0.062	0.043	N.D.	N.D.

- a            μmoles of reducing sugar produced/min/ml  
b            μeqs of methoxyl groups liberated/min/ml  
c            μmoles of reducing sugar produced/min/ml

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## Effect of salicylic acid spray on sugar metabolites in *B. Juncea* leaves infected with *A. brassicae*

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### Introduction:

Indian Mustard (*Brassicae juncea*) is one of the most economically important oil seed crops. It can play a vital role in imparting self-dependence in the oil sector. However, its yield as well as oil content is drastically affected by a fungal disease *Alternaria blight* caused by *A. Brassicae*. Elicitor, Salicylic Acid (S.A.) spray has been reported to induce protection against plant pathogens (Raskin, 1992) by changing biochemical constituents of the plant. The present investigation describes the effect of salicylic acid on percent severity, sugar and starch levels in leaves of *B. Juncea*.

### Materials and Methods:

Indian mustard (*Brassica juncea* L: Zern and Coss Var. RLM 1359) was raised following standard package and practices. The field was divided into A,B,C. and D. plots. Each plot was sprayed with specific concentration of salicylic acid (10,25 and 50  $\mu\text{g/ml}$ ) respectively. The control plot was sprayed with water. Top five leaves were collected at different time intervals (24,48,96,144 and 192 h) after spray and brought to laboratory under cold conditions. Percentage infection and severity in leaves was calculated as per scale suggested by Conn et al (1990). Total sugars, reducing sugars and starch were estimated by the method of Dubois et al (1956) and Nelson (1944) respectively from leaves dried at 70°C.

### Results and Discussions:

The data on percentage infection and severity is shown in Table 1. The infection was minimum at 50  $\mu\text{g/ml}$  S.A. spray and increased only till 48 hours. The decrease in disease symptoms due to S.A. spray have also been reported in tobacco against TMV by White (1979). The S.A. spray at 25  $\mu\text{g/ml}$  and 50 $\mu\text{g/ml}$  resulted in increased sugar content with maximum level observed at 96hrs. as compared to infected control. The increase in sugar content could be due to restriction of the fungal growth and thereby less utilization of the plant sugars by the pathogen, as evidenced by the percent infection and severity (Table 1). Sugars play important role towards disease resistance by maintaining healthy flora of saprophytic microbes that help to restrict the pathogens (Juniper and Jeffrae, 1983). The decrease in starch content followed the same pattern at all the three concentration of S.A. sprayed. The decrease in starch content (which must be due to hydrolysis of starch) could be responsible for the increasing sugar content.

Table 1: Percentage infection and severity in leaves of *Brassica Juncea* after spray with salicylic acid solution

HAS	Control (Water)	Conc. of S. A.		
		10µg/ml	25µg/ml	50µg/ml
<b>% Infection</b>				
24	12	14	14	13
48	18	19	18	16
96	32	32	29	16
144	44	48	29	16
192	59	61	29	16
CD (P<0.05) Sampling = 1.18 Treatment = 2.12				
<b>% severity</b>				
24	8	10	11	11
48	16	15	14	14
96	24	21	19	14
144	48	46	19	14
192	64	62	19	14
CD (P< 0.05) Sampling = 2.27 Treatment = 2.04				
HAS : Hours after spray.				

Table 2: Biochemical constituents in S.A. sprayed Indian Mustard Leaves.

HAS	Control (Water)	Conc. of S.A.		
		10µg/ml	25 µg/ml	50µg/ml
<b>TOTAL Sugars</b>				
24	108.3±1.26	112.4±2.32	107.8±1.21	104.3±2.11
48	101.7±1.49	106.3±1.62	112.3±2.02	109.6±3.78
96	96.2±1.12	98.2±2.31	109.4±1.32	114.7±1.39
144	89.0±1.32	97.4±2.64	101.3±2.61	111.0±2.61
192	87.2±0.98	92.3±1.91	97.4±0.91	106.2±0.84
CD (P< 0.05) Sampling = 1.32 Treatment = 0.12				
<b>REDUCING SUGARS</b>				
24	63.4±2.18	61.2±0.73	68.2±2.12	58.9±1.72
48	60.2±1.19	58.3±1.62	65.4±1.18	60.2±2.13
96	53.1±1.27	52.2±3.12	60.3±2.06	61.3±2.22
144	52.7±1.23	50.7±2.18	55.2±2.11	59.4±2.14
192	50.3±1.19	50.3±1.19	52.8±1.96	58.3±2.92
CD (P< 0.05) Sampling = 1.47 Treatment = 0.32				
<b>STARCH CONTENT</b>				
24	59.8±0.93	54.3±0.73	53.2±0.97	54.1±0.99
48	59.6±0.98	52.1±0.92	52.9±1.03	53.6±1.12
96	52.3±1.12	52.0±0.86	48.3±1.08	51.2±1.07
144	51.2±0.94	49.6±0.86	46.2±0.96	48.4±0.86
192	51.0±0.83	48.3±0.89	45.9±0.82	48.2±0.89
CD (P< 0.05) Sampling = 1.18 Treatment = 0.73				

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## Evaluation of Cabbage Genotypes for Thrips Damage

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### Introduction:

Onion thrips (*Thrips tabaci*) are a major pest of the cabbage industry in New York State and the northeast, largely due to the ineffectiveness of chemical control. Thrips damage can occur in the outer third of a cabbage head making them unmarketable due to bronzing of the leaves and damage up to 22 leaves inside the cabbage head (North and Shelton, 1986). Cabbage varieties are available that show tolerance to thrips but this is limited and insufficient. The current study screened 50 head forming cabbage (*Brassica oleracea* var. *capitata*) accessions previously identified with low thrips damage from the 345 accessions available from the USDA-ARS (Griffiths and Shelton, 2003). Accessions were screened with 24 thrips tolerant and susceptible commercial cultivars in replicated trials in summer 2004

### Materials and Methods:

A total of 50 cabbage accessions from the US collection were planted in 4 replicated plots with 10 plants per plot during the 2003 field season in New York State. Twelve cultivars that have previously been associated with thrips tolerance (Azurro, Blue Thunder, Bobcat, Fresco, Galaxy, Huron, King Cole, Little Rock, Lynx, Rona, Transam, Vitaro) and 12 susceptible controls (Atlantis, Autoro, Bartolo, Bronco, Genessee, Marvelon, Morris, Octoking, Ramada, Rinda, Storage #4, Super Red 80) were included in the trial. Seeds were sown in April and transplanted to the on April 30<sup>th</sup> 2003 (one month earlier than regular trials to encourage thrips damage).

Inoculation was achieved through natural infestation of thrips which was high during the 2003 season. Pest management limited chemicals disruptive to thrips populations, and plants were allowed to grow to maturity and form heads for evaluation. Cabbage heads of 3 plants from each of the four reps were evaluated using a rating scale that ranged from 1 – 5 for thrips damage (where 1= no thrips damage, and 5 = extreme damage) during August 2003. The genotypes were also evaluated for the depth of damage within the head, their market class and their head density. Means groupings were calculated according to Duncan's means separation at  $p \leq 0.05$ .

## Results:

The most resistant accessions were PI 235043, PI 343529, PI 246097, G30754, PI 246060, PI 246103, PI 246046, G30429, PI 235044, PI 246079, G30739 and PI 343636. The red cabbage cultivars were the most resistant commercial types in this trial [Rona (1<sup>st</sup>), Vitaro (3<sup>rd</sup>), Azurro (4<sup>th</sup>) and Autoro (5<sup>th</sup>)] with 4 green cultivars performing well [Transam (6<sup>th</sup>), Galaxy (8<sup>th</sup>), Bobcat (14<sup>th</sup>) and Blue Thunder (16<sup>th</sup>)].

Genotypes were sorted relative to market class and head density (Table 1 and Table 2). Savoy cabbages had the least damage, followed by red and flat dutch types based on mean severity ratings. Head density was also important, with looser heads suffering less damage from thrips. It was not possible to separate head density as a significant factor between commercial varieties, as all varieties used had high head density. The lower damage attributable to market type and head density in the accessions should be considered when being used for improvement of cabbage genotypes for thrips tolerance.

**Table 1:** Thrips damage based on market class.

Type	No. Tested	Mean Rating	Mean Depth
Green	379	2.79 a <sup>z</sup>	4.80 a
Red	81	1.89 bc	2.94 b
Savoy	43	1.74 c	1.47 c
Flat Dutch	126	2.13 b	2.79 b
Cone	19	2.74 a	4.26 a
Mixed	56	2.84 a	4.59 a

<sup>z</sup> Means separation according to Duncan's multiple range test ( $p \leq 0.05$ )

**Table 2:** Thrips damage based on head density.

Head Density	No. Tested	Mean Rating	Mean Depth
Low	38	2.11 b	2.34 b
Medium	179	1.93 b	2.56 b
High	487	2.75 a	4.65 a

<sup>z</sup> Means separation according to Duncan's multiple range test ( $p \leq 0.05$ )

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Griffiths, P.D. and Shelton, A. (2003). Evaluation of cabbage accessions for thrips damage. *Cruciferae Nesletter* 25.

North, R. and Shelton, A. (1986). Colonization and intra-plant distribution of *Thrips tabaci* (Thysanoptera: Thripidae) on cabbage. *J. Econ. Entomol.* 79: p219-223.

## Evaluation of Cabbage Accessions for Thrips Damage

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### Introduction:

Onion thrips (*Thrips tabaci*) are a major pest of the cabbage industry in New York State and the northeast, largely due to the ineffectiveness of chemical control. Thrips damage can occur in the outer third of a cabbage head making them unmarketable, as they can cause bronzing of the leaves and bumps damage up to 22 leaves inside the cabbage head (North and Shelton, 1986). Cabbage varieties are available that show tolerance to thrips but this is limited and insufficient. The current study screened the 345 available cabbage (*Brassica oleracea* var. *capitata*) accessions stored at the USDA-ARS germplasm repository for thrips damage during summer 2002.

### Materials and Methods:

A total of 345 cabbage accessions (those available for distribution) from the US collection were planted in 2 replicated plots with 10 plants per plot during the 2002 field season in New York State. Six varieties that have previously been associated with thrips tolerance ('Transam', 'Bobcat', 'Fresco', 'Vitaro', 'Rona' and 'Huron') and 6 susceptible controls ('Rinda', 'Ramada', 'Genesee', 'Storage #4', 'Bartolo' and 'Saratoga') were included in the trial. Seeds were sown in April and transplanted to the field in early May (one month earlier than regular trials to encourage thrips damage).

Inoculation was achieved through natural infestation of thrips which was extremely high during the 2002 season. Pest management limited chemicals disruptive to thrips populations, and plants were allowed to grow to maturity and form heads for evaluation. A total of 178 accessions of the 345 planted were evaluated as the remainder did not form suitable heads for accurate scoring. Cabbage heads of 3 plants from each of the two reps were evaluated using a rating scale that ranged from 1 – 5 (where 1 = no thrips damage, and 5 = extreme damage several layers inside the head) during September. The heads were sliced in half, and thrips damage was recorded based on extent and depth within the head.

## Results:

Accessions that formed loose heads or low head solidity showed lower levels of thrips damage in this trial. As this might influence selections for breeding purposes, only those selections that formed solid heads were considered for selection and future crossing. The ten accessions that exhibited the lowest levels of thrips damage are listed in Table 1.

<u>LINE</u>	<u>TYPE</u>	<u>SIZE</u>	<u>MATURITY</u>	<u>SOLIDITY</u>	<u>MEAN</u>	<u>Ivp</u>	<u>Ivno</u>	<u>Ivs</u>	<u>Genus</u>	<u>Species</u>
T295	G/R	M	M	H	2.00	PI	357373	78OI	<i>Brassica</i>	<i>oleracea</i>
T162	R/G	M	M	H	2.17	PI	246103	80OI	<i>Brassica</i>	<i>oleracea</i>
T214	R	M	M	H	2.17	PI	275004	79CI	<i>Brassica</i>	<i>oleracea</i>
T128	GR	M	E	L(R),H(G)	2.25	PI	246050	80OI	<i>Brassica</i>	<i>oleracea</i>
T163	R/G	M	M	H	2.25	PI	246108	80CI	<i>Brassica</i>	<i>oleracea</i>
T099	G	M	M	M/H	2.50	PI	225861	76OI	<i>Brassica</i>	<i>oleracea</i>
T177	R/G	S	M	H	2.50	PI	255561	79CI	<i>Brassica</i>	<i>oleracea</i>
T239	G	L	M	H	2.50	PI	302984	80OI	<i>Brassica</i>	<i>oleracea</i>
T269	G	L	M	H	2.50	PI	343572	78OI	<i>Brassica</i>	<i>oleracea</i>
T126	R	S	M	H	2.58	PI	246046	78CI	<i>Brassica</i>	<i>oleracea</i>

**Table 1: Cabbage accessions with thrips tolerance and medium-high head solidity (G=Green, R=Red, E= Early, S=Small, M=Medium, L=Large, L=Low, H=High).**

No accessions were free of thrips damage during this trial, and the varieties categorized as 'tolerant' failed to score higher than 3.0. However, the tolerant varieties did show lower levels of damage than the susceptible controls. The accessions exhibiting low levels of thrips damage provide potential sources for increasing host plant resistance to thrips damage in commercial cabbage varieties.

## References:

North, R. and Shelton, A. (1986). Colonization and intra-plant distribution of *Thrips tabaci* (Thysanoptera: Thripidae) on cabbage. *J. Econ. Entomol.* 79: p219-223.



# Performance of newly developed strains in *Brassica juncea* and their reaction to mustard aphid (*L. erysimi*)

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Amongst Brassicas, *Brassica juncea* is an important crop of Indian sub-continent. The development of new varieties is viewed as a possible way out for stepping up the average yield level of this crop and it is largely grown under the rainfed, biotic and abiotic stresses (Rai, 1997). An exciting and challenging task before the oilseed breeder is to develop the cultivars with built-in genetic resistance to devastating insect-pests like mustard aphid and *Alternaria* leaf blight disease in Indian sub-continent. Therefore, the present study was undertaken to develop high yielding varieties with built-in genetic resistance to mustard aphid (*L. erysimi*).

## MATERIALS AND METHODS

The material consisting of thirteen strains developed through intraspecific hybridization (Table 1). All these strains were grown in a randomized block design with three replications during rabi 2001-2002 at SKUAST Research Farm in the division of plant breeding and genetics, R.S. Pura, Jammu. The data was recorded for seed yield and screening for aphid resistance parameters (Bakhetia & Sandhu, 1973 and Pathak, 1961).

## RESULTS AND DISCUSSION

Amongst thirteen strains developed through interspecific hybridization, RSPR-69 recorded highest seed yield of 16.33 q/ha followed by RSPRO-03 (13.133 q/ha). Although, the strain RSPRO-03 ranked second yet it showed minimum plant infestation and aphid population per plant both at flower initiation stage (FIS) and full bloom stage (FBS) (Table-1). The other strains which showed the lowest plant infestation and aphid population/plant are low yielders. The strain RSPR-03 has been nominated in initial varietal trial (IVT) testing in All India Co-ordinated Project on rape seed-mustard during rabi 2002-2003 because of its inbuilt genetic resistance and high yield performance in station trials.

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Table 1. Pedigree of the strains developed through intraspecific hybridization and their screening against *Lipaphis erysimi*.

S.No.	Name of the strain	Pedigree	Plant infestation *		Aphid pop./*	
			(%)		Plant	
			FIS	FBS	FIS	FBS
1.	RSPR - 68	PSR 10 x Varuna	33.33	80.00	48.26	194.30
2.	DS - 13	Sel. from exotic material	33.33	46.67	27.67	83.00
3.	RSPR - 65	BIO - 424 x Varuna	26.67	66.67	20.20	70.67
4.	RSPR - 71	R.L - 1359 x RH - 30	13.33	86.67	9.60	72.53
5.	RSPR - 67	Pusa bold x Kranti	20.00	80.00	9.30	20.900
6.	RSPR - 69	RLM - 198 x Varuna	13.33	54.33	5.10	30.80
7.	RSPR - 72	BIO - 424 x R.L - 1359	33.33	46.67	16.47	77.87
8.	RSPR - 66	Pusa bahar x R.L - 1359	26.67	73.33	17.50	86.53
9.	RSPR - 70	Varuna x R.L - 1359	13.33	66.67	5.87	73.73
10.	RSPR - 03	Kranti x Pusa bold	26.67	46.67	19.47	22.13
11.	RSPR - 74	Pusa barani x Kranti	33.33	66.67	41.47	78.07
12.	RSPR - 75	R.H - 30 x Varuna	20.00	80.00	12.47	80.00
13.	Pusa bold	Check	33.33	66.67	3.87	47.60

\*: Mean based on 15 plants

FIS = Flower initiation stage

FBS = Full bloom stage

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## Cabbage Butterfly, *Pieris brassicae* L.- An Upcoming Menace for Brassica Oilseed Crops in Northern India

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The cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) is so far known as serious pest of cabbage, cauliflower, knol-khol and other cruciferous plants, is now emerging as serious pest of *Brassica* oilseeds in eastern Uttar Pradesh and other parts of India. This has threatened the cultivation of these crops as this pest caused severe damage to the crop during *rabi* 2001-02. The occurrence of this insect was observed in quite large area causing severe damage to *Brassica campestris* var. yellow sarson in the month of February 2002. Later on this insect shifted to late maturing *B. carinata* in the month of March 2002. In case of severe damage, plants got completely defoliated by caterpillars and became devoid of siliquae giving plants "bamboo-broom" look. Thus the occurrence of cabbage butterfly in *Brassica* oilseed crops has emerged as serious threat and hence deserves concerted research efforts.

Earlier, cabbage butterfly was found attacking rapeseed-mustard, especially *Brassica napus* late in the season (Bakhetia and Sekhon, 1989). The activity of this insect was mostly confined on cabbage, cauliflower and knol-khol during October-February (Sachan and Gangwar, 1990). This insect was also observed damaging cabbage crop during March-April in *tarai* region (Chaudhari et al, 2001). In recent past there has been tremendous change in cropping patterns in this area that has probably caused a shift in the host range of this insect including *Brassica* oilseed within its reach. The change in cropping pattern has extended the sowing of rapeseed-mustard from as early as late August to early December. Thus, cropping span has considerably extended in time and space. The insect which preferred hilly areas for its breeding, has now established in plains due to change in climatic factors and also on account of extensive cultivation of cabbage and cauliflower in this area. All these factors taken together appear to be responsible for spreading of this insect on such a large scale.

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Evaluation of Indian mustard (*Brassica juncea* (L) Czern & Coss)  
crosses and segregating generations against downy mildew  
(*Peronospora parasitica*)

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Downy mildew caused by *Peronospora parasitica*(Pers.) ex Fr. is an important disease of Indian mustard. Completely reliable information on losses in seed yield by downy mildew is not available because it occurs almost invariably with white rust. However, combined infection with both pathogens on *B.juncea* may cause 37-47 per cent and 17-32 per cent reduction in siliquae formation and seed production in India (Bains and Jhooty, 1979)

Symptoms of this disease appear on all above ground parts but usually on leaves and inflorescence. Usually few days after sowing, small translucent light-green lesions first appear on cotyledonary or first few true leaves in seedling stage. Such lesions later enlarge and develop into grayish-white, necrotic patches on the leaf, bearing downy growth of fungus (conidia and conidiospore) on its under surface. In severe incidence the affected leaf dries up and shrivels. The downy mildew symptoms are also found associated with white rust (*Albugo candida* (Lev.) Kunz.) symptoms on leaves. Usually the downy growth of *P.parasitica* appears in or around the white rust pustules but when the downy lesions appears first on the leaves, the appearance of white pustules in or around the downy mildew lesions is seldom seen.

Resistant varieties are most feasible, cheapest and environment friendly means for exploitation of full potential of improved varieties and ultimately stabilizing and increasing production of rapeseed-mustard. Therefore, efforts are underway to develop downy mildew resistant varieties of Indian mustard. Identified resistant sources EC 399299, EC 399313 and EC 399296 were crossed with improved Indian mustard cultivars / strains and different segregating generations derived from these crosses were screened at seedling stage. The seedlings were grown in plastic pots and inoculated at cotyledonary stage (7 days after sowing) with downy mildew spore suspension ( $2.5 \times 10^4$  conidia/ml) as per method described by Williams (1985). After inoculation pots were covered with plastic sheets to allow humidity to reach almost up to 100%. The pots were incubated for 3 days for the development of disease.

Interaction phenotypes were scored 7 days after inoculation on scale 0 to 9 (Williams, 1985) and resistant genotypes with score 0 to 1 were selected and transferred to bigger pots. These plants were bagged with butter paper bags before flower initiation to ensure genetic purity of seeds of each plant. The details of plant screened and selected are given below.

Generations	Grown and Screened	Selected Individual Plants
F <sub>1</sub> crosses	22	50(21)*
F <sub>2</sub> population	13	27(11)
F <sub>3</sub> progenies	17	38(15)
F <sub>6</sub> progenies	47	78(31)
Individual Plant Selection	67	171

\* The figures in parentheses indicate number of crosses/populations/progenies.

These selected plants will be further screened and evaluated for important physio-morphological characters including yield and oil content. The plants selected from F<sub>6</sub> generation will be evaluated in replicated trials for preliminary yield evaluation.

**Acknowledgements:**

Thanks are due to Dr.N.I.Nashaat, Project Leader, "Indo-UK Collaborative Project on Oilseeds for Disease and Drought Resistance", IACR, Rothamsted, Harpenden (UK) for encouragement.

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## Evaluation of mustard germplasm against *Albugo candida*

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White rust caused by biotrophic oomycete pathogen *Albugo candida* (Pers. Ex Hook) Kunze is an important disease of Indian mustard (*Brassica juncea* (L) Czern Coss) and *B.rapa*. The pathogen can infect all above ground parts of plant producing characteristic white blisters (sori). Severe infection culminates in systemic "staghead" infection of the inflorescence (often in association with *Peronospora parasitica*), which is main cause of yield loss in susceptible cultivars (Awasthi *et al.* 1997; Bisht *et al.* 1994; Petrie 1988). Local infection of leaves and cotyledon result in the appearance of raised pustules (1-2 mm in diameter) on the abaxial (lower) surface, which later coalesce to form blisters. The adaxial (upper) surface, corresponding to the pustule on lower surface, is tan yellow and prevalence of disease is easily recognized from upper surface of the affected leaves. After the complete development of the pustule, it ruptures and releases a chalky dust of spores. When plants are infected through stem or at flowering stage, it causes extensive distortion, hypertrophy, hyperplasia and sterility resulting in a staghead (Verma & Petrie, 1980; Goyal *et al.* 1996).

Resistant varieties are the cheapest and environment friendly means for the exploitation of full potential of the improved varieties and ultimately stabilizing and increasing the production of rapeseed-mustard. Therefore, efforts are underway to develop white rust resistant varieties of Indian mustard. Identified resistant sources Domo, Cutlass, PWR 9538, PWR 9541 and YRT-3 were crossed with improved Indian-mustard cultivars/strains and different segregating generations derived from these crosses were screened at seedling stage. The seedlings were grown in plastic pots and drop inoculated at cotyledonary stage ( 7 days after sowing ) with the inoculum (concentration of zoospores  $8 \times 10^4$  ml) as per method described by Sachan *et al.*(2000). After inoculation pots were covered with plastic sheet to allow the relative humidity to increase to almost 100%. The pots were incubated 13-16 days for the development of disease.

Interaction phenotypes were scored after incubation on scale 0 to 9 and resistant plants with score 0 to 1 were selected and transferred to bigger pots. These plants were bagged with butter paper bags before flower initiation to ensure the genetic purity of seeds of each plant. The details of plants screened and selected are given below.

Generation	Grown and screened	Selected Individual plants
F <sub>2</sub> population	24	15 (11)*
F <sub>3</sub> progenies	78	42 (31)
F <sub>5</sub> progenies	29	14 (12)
F <sub>6</sub> progenies	34	25 (18)

\* Numbers in parenthesis indicates number of crosses/ populations/ progenies .

These selected plants will be further screened and evaluated for important physiomorphological characters including yield and oil content. The plants selected from F<sub>5</sub> and F<sub>6</sub> will be evaluated in replicated trial for preliminary yield evaluation.

**Acknowledgements:** Thanks are due to Dr.N.I.Nashaat, Project Leader, "Indo-U.K.collaborative project on oilseeds for disease and drought resistance", IACR, Rothamsted, Harpenden,(U.K.) for encouragement.

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**Evaluation of Indian mustard (*Brassica juncea* (L) Czern & Coss) breeding lines against *Alternaria* blight**

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*Alternaria* blight caused by *Alternaria brassicae* (Berk), Sacc is an important disease of Indian mustard. The symptoms of the disease are characterized by formation of dark brown coloured spots on leaves, stems and siliquae. Lower leaves show the symptoms first, with the appearance of black points which later on enlarge to develop into prominent cocentric rings or lesions of various sizes (Kolte, 1985). As the disease progresses, the lower leaves defoliate and spots subsequently appear on stem and siliquae. The losses in yield due to *Alternaria* blight at different locations in northern part of India ranged from 10 to 70 per cent (Anonymous, 1980, 1981; Kolte *et al.* 1987). Incorporation of resistance/tolerance for *Alternaria* blight in improved cultivars may be the most acceptable and feasible eco-friendly means for exploitation of full genetic potential of these varieties. Although completely resistant sources for *Alternaria* blight are not available, some tolerant genotypes such as PAB 9331, PAB 9332, PAB 9337, PAB 9511, PAB 9534, PAB 9836, PAB 9841, PHR-1, PHR-2, ABR-15, ABR-17, RC-781 and Ornamental rai are available and hence these were crossed with some improved Indian mustard cultivars/strains. Different segregating generations derived from these crosses were screened under field conditions by growing spreader-cum-infecter rows of yellow sarson variety YST-151 after every five rows of test lines and spray of *Alternaria* spore suspension ( $10^4$ - $10^5$  spores/ml) at first true leaf stage. *Alternaria* spore suspension was also sprayed at pod stage. Plants showing infection more than 10% were rouged out. The resistant/tolerant plants having 0-1 rating score (ranging from 1 to 10% leaf area covered with small pin-headed spots on the leaves and pin-headed spots on pods) were tagged at maturity stage. These tagged plants were harvested individually. The details of plants screened and selected are given below:-

Generations	Grown and Selected	Selected Individual Plants
F <sub>1</sub> crosses	29	17(11)*
F <sub>2</sub> population	31	32(11)
F <sub>3</sub> progenies	130	179(63)
F <sub>4</sub> progenies	35	79(14)
F <sub>5</sub> progenies	26	84(18)
F <sub>6</sub> progenies	7	25(6)
Individual plant selection	20	25(6)

\*Figures in parentheses indicate number of crosses/populations/progenies.

These selected plants will be further screened and evaluated for important physio-morphological characters including yield and oil content. The plants selected from F<sub>5</sub> and F<sub>6</sub> generations will be evaluated in replicated yield trial for primary yield evaluation.

**Acknowledgement:** Thanks are due to Dr.N.I.Nashaat, Project leader, "Indo-UK collaborative project on oilseeds for transfer of disease and drought-resistance". IACR, Rothamsted, Harpenden(UK) for encouragement.

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# THE DETERMINATION OF SUITABLE KOHLRABI (*Brassica oleraceae* var. *gongylodes* L.) CULTIVARS FOR AUTUMN GROWING PERIOD IN TEKİRDAĞ

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## INTRODUCTION

Kohlrabi is cultivated commonly in America and Europe countries such as Germany, France, Holland and Italy. It is a fast growing cool season vegetable crop, and its enlarged stem and fresh leaves are eaten raw in salads or can be cooked like turnip (Splittstoesser, 1990; Liebster, 1991). According to vegetable production records, there is no kohlrabi production in Turkey, while other members of Cruciferae family such as cabbage, cauliflower, and radish are grown in almost all parts of the country (Anonymous, 2000; Vural et al., 2000). On the other hand, broccoli and Brussels sprouts which are members of Cruciferae has become more popular in recent years. Whereas kohlrabi can be one of the alternative crops for vegetable growers due to having short growing season and its export possibility. Also, kohlrabi can be a good choice for protected cultivation because it can grow without any additional heating during winter months. So, the present study was aimed to investigate this possibility and to determine the suitable kohlrabi varieties.

## MATERIAL and METHOD

Study was conducted at the experimental field of the Faculty of Agriculture, University of Trakya (40°59' E, 27°29' E and altitude 4 m) during the autumn season of 2001. According to long-term climatic data, the mean annual temperature, precipitation and relative humidity are 13.8 °C, 575 mm and 76% respectively. The soil was a clay-loam at 7.4 pH with 1.7% organic matter, 20.1 ppm P<sub>2</sub>O<sub>5</sub> and 230.3 ppm K<sub>2</sub>O. Constant rates of 120 kg N ha<sup>-1</sup> and 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was applied to the plots for nitrogen and phosphor needs of the plants prior to planting. The seed of nine kohlrabi varieties (Neckar, Lahn, Lippe, Express Forcer, Delikateß Blauer, Erko, Qickstar, Rapidstar, and White Danube) were sown into plug-trays filled with commercial peat-like mixes on August 16<sup>th</sup>. The seedlings which have three true leaves were transplanted in the plots on September 12<sup>th</sup>. Plants were spaced 0.30 m within the rows and 0.35 m between rows. Sowing and planting dates were determined by taking account of meteorological data and requirement of kohlrabi.

The plants were harvested ~2 months after planting. Following parameters were measured or evaluated for each cultivar: Leaves number (bigger than 2 cm), leaves weight (g), tuber weight (g), tuber diameter (mm) and total yield [(kg. ha<sup>-1</sup>) with tuber and leaves].

The experiment was established according to randomized block design with three replications. Data were subjected to analysis of variance.

## RESULTS and DISCUSSION

According to results, leaves weight varied from 63.3 to 334.9 g, leaf number from 12.2 to 24.5, tuber weight from 90.5 to 356.6 g, tuber diameter from 44.8 to 88.4 mm and yield from 45 571 to 23 428 kg ha<sup>-1</sup> depending on cultivars (Table 1). These results are in agreement with those of Mehwald (1976) in which the average tuber weight and leaves weight of nine kohlrabi cultivars were 330-475 g and 130-330 g respectively, with those of Sritharan et al. (1992) in which the measured tuber diameter was between 3.47 and 7.01 cm and plants had average leaf number of 7.9-17.7 in different CO<sub>2</sub> concentration, and with those



of Glukhov (1973) who obtained 23 t ha<sup>-1</sup> yield in kohlrabi. It is seen that kohlrabi can be successfully grown under Trakya conditions and acceptable yield can be taken.

The differences among cultivars with respect to yield, and some tuber and leaf properties were statistically important. Yield of kohlrabi is generally evaluated as the result of tuber size, and Krug (1991) stated that tuber diameter must be more than 40 mm for fresh market. When the results dealing with tuber weight and diameter were taken into consideration, it was seen that Rapidstar gave higher values than other cultivars. This finding is consistent with the reports of Dunbar (1998) who determined that the Rapidstar was the best among fourteen kohlrabi cultivars. Erko which gave the highest leaves weight can be used by different goal, for instance as fodder. Delikateß Blauer that had the lowest tuber weight and diameter after than Erko was markedly lower in yield.

Table 1. Leaves weight and number, tuber weight and diameter, and yield in nine kohlrabi cultivars.

	Leaves weight (g)	Leaves number	Tuber weight (g)	Tuber diameter (mm)	Yield (kg ha <sup>-1</sup> )
Rapidstar	128.9	24.5	356.6	88.4	44 571
Lippe	77.7	14.2	317.2	81.4	32 619
E. Forcer	63.3	15.3	308.9	81.7	37 333
Neckar	100.5	14.2	306.6	84.7	33 761
W. Danube	189.9	25.2	282.7	80.4	41 476
Lahn	79.4	14.5	271.1	77.2	34 333
Quickstar	69.9	16.5	209.4	71.4	26 857
D. Blauer	117.7	12.2	113.9	53.6	23 428
Erko	334.9	17.1	90.5	44.8	45 571
LSD (0.05)	88.5	3.6	95.5	11.2	9 667

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## Effect of irrigation levels on brown sarson

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### INTRODUCTION

Mostly the germination of brown sarson gets impaired due to lack of pre-sowing irrigation and improper germination affects the proper establishment of plant stand due to decrease in temperature with the advancement in winter season. The frequent drought also induce the stress at flowering and siliqua formation stages and thereby reduces the seed yield. Hence water management at critical crop growth stages was initiated to over come the problems of moisture stress.

### MATERIALS AND METHODS

A field experiment consisting of eight irrigation schedules as per Table 1 was tried during 1999-2000 and 2000-01 in R.B.D. design on silty clay loam soils of Shalimar campus of SKUAST-K. The soil with medium N, P & K status had soil moisture storage of 24.2 and 11.3cm in 90 cm soil layer depth at field capacity and permanent wilting point, respectively. Variety 'KS-101' (*Gulchien*) of brown sarson (*Brassica campestris*) was sown in the first fortnight of October in rows 30cm apart using seed rate of 7.5kg ha<sup>-1</sup> during both the years. Intra row spacing of 10 cm was maintained by thinning the crop 30 days after sowing. A basal dose consisting of 30+30+20+10 kg N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and S ha<sup>-1</sup> was applied before sowing. Remaining N was top dressed at flower and siliqua initiation stages. Crop received a rainfall of 258.1 and 101.1 mm during respective years against normal rainfall of 595.6 mm. Crop was harvested at physiological maturity stage.

### RESULTS AND DISCUSSION

The data in Table 1, indicated that application of irrigation as pre-sowing at flowering and siliqua initiation stages recorded significantly highest seed yield than rest of the treatments during both the years. Pre-sowing irrigation may have favoured uniform and timely germination and thereby resulted in better establishment before the decline in the temperature with the advancement in winter. Further due to deficit of 56.7 and 83.0 per cent in precipitation over normal during the respective years of

cropping seasons, the crop may have responded to irrigation at critical stages and thereby increased seed yield than other treatments. Application of 3 irrigations (pre-sowing, at flowering and at siliqua initiation stages) improved water expense efficiency and benefit cost ratio during both the years. The results are in accordance with those of Yadav *et al.* (1995) and Bhalerao (2001). Treatment involving no pre-sowing irrigation gave very less returns.

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Table 1. Influence of various irrigation schedules on seed yield, water expense, water expense efficiency and economics of brown sarson (mean of 2 years).

Treatment	Seed yield (q ha <sup>-1</sup> )	Water expense efficiency (kg ha <sup>-1</sup> mm <sup>-1</sup> )	Net returns (Rs. ha <sup>-1</sup> )	B:C
T <sub>1</sub>	10.16	3.14	4700	0.78
T <sub>2</sub>	4.29	1.37	-1580	-0.27
T <sub>3</sub>	12.01	2.99	5960	0.98
T <sub>4</sub>	6.50	1.79	540	0.09
T <sub>5</sub>	11.67	2.93	5615	0.93
T <sub>6</sub>	6.24	1.77	275	0.05
T <sub>7</sub>	13.05	2.94	6955	1.15
T <sub>8</sub>	7.42	1.88	1370	0.23
CD 5%	0.58	-	-	-

T<sub>1</sub> = pre-sowing irrigation; T<sub>2</sub> = without pre-sowing irrigation; T<sub>3</sub> = T<sub>1</sub>+ irrigation at flower initiation; T<sub>4</sub> = T<sub>2</sub> + irrigation at flower initiation; T<sub>5</sub> = T<sub>1</sub> + irrigation at siliqua initiation; T<sub>6</sub> = T<sub>2</sub> + irrigation at siliqua initiation; T<sub>7</sub> = T<sub>1</sub>+ irrigation at flower and siliqua initiation; T<sub>8</sub> = T<sub>2</sub> + irrigation at flower and siliqua initiation

# Evapotranspiration of mustard (*Brassica juncea* L.) in relation to weather under rainfed conditions

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## Introduction

Efficient water management is the most important factor that affects the yield crop to an appreciable extent. Information on evapotranspiration rates and water requirement of crop at different crop growth stages helps in development of water management practices in watershed areas.

## Materials and Methods

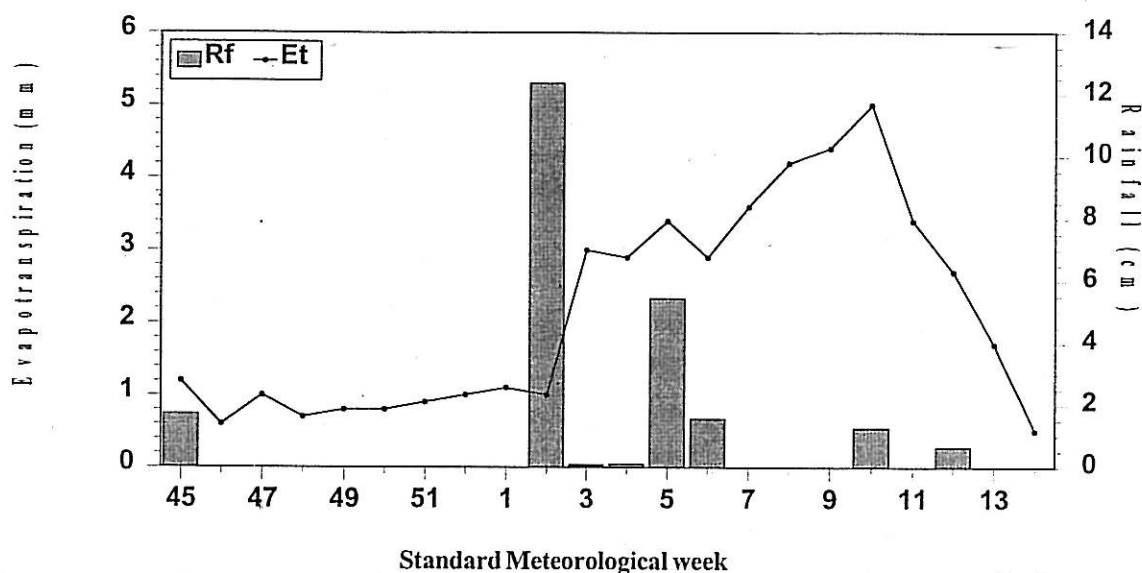
An experiment was conducted during during *rabi* 1999-2000 at Dryland Research Station SKUAST-J, Dhiansar (32°-39' N, 74°-58' E and 332 meters above mean sea level). The mustard cv Pusa Bahar was sown in gravimetric lysimeters and adjacent field under rainfed condition with standard agronomic practices. The daily evapotranspiration (Et) rates of mustard crop from sowing to harvest were measured with the help of lysimeter and weather data for corresponding period were used from Agromet observatory at Dhiansar.

## Results and Discussion

The data so obtained are presented in the Table.1 and weekly evapotranspiration during crop growth are depicted in Fig no1. The result reveals that the total evapotranspiration was

**Table:1 Growth and yield of mustard (Cv.Pusa Bahar) in Rabi 1999-2000.**

<u>S.No.</u>	<u>Observation</u>	<u>Lysimeter</u>	<u>Field</u>
1	Date of sowing	08.11.1999	08.11.1999
2.	Date of 50% flowering	04.01.2000	03.01.2000
3.	Date of harvesting	06.04.2000	06.04.2000
4.	Duration in days	151	151
5.	Plant height(cm)	149.2	110.2
6.	No. of pods plant <sup>-1</sup>	163	146
7.	Seed Yield (q ha <sup>-1</sup> )	7.81	6.33
8.	Stalk Yield (q ha <sup>-1</sup> )	25.9	20.2
9.	Consumptive use	325.4	325.4
	of moisture (mm)		
10.	Moisture use efficiency	2.40	1.95
	(Kg ha <sup>-1</sup> mm <sup>-1</sup> )		
11.	Rainfall during crop growth period.	214.5	214.5
12.	No. of rainy day.	12	12



**Fig.1 Evapotranspiration (mm) and Rainfall (cm) during crop growth (1999-2000)**

325.4 mm against the rainfall of 214.5 mm received during crop growing period. The weekly average of evapotranspiration was low around 1mm / day upto 2<sup>nd</sup> Standard Meteorological Week (SMW) and thereafter it increases sharply and reached maximum to 5.0 mm/day at 10<sup>th</sup> SMW. The sharp increase in Et after flowering of mustard crop was because of sufficient availability of moisture in soil due to heavy rainfall of 123 mm and 54 mm received during 2<sup>nd</sup> and 5<sup>th</sup> SMW, respectively. The moisture use efficiency was calculated 2.40 kg ha<sup>-1</sup> mm<sup>-1</sup>. The correlation between weekly evapotranspiration and maximum temperature (T max), minimum temperature (T min) relative humidity morning (RH1) and relative humidity evening (RH2) of corresponding week were computed and presented in Table 2. It can be seen that no weather parameter is significantly correlated with Et. This may be due to that the evapotranspiration under rainfed condition is mainly governed by plant characteristic leaf area index, root density as well as soil moisture.

**Table:-2 Coorelation coffiecient between Et and weather parameters**

	ET	T.max	T.min	RH-1	RH-2
ET	1	-0.226	-0.195	-0.229	0.087
T.max		1	0.802**	-0.778**	-0.717**
T.min			1	-0.728**	-0.208
RH-1				1	0.503*
RH-2					1

\*\* Highly significant

## Planting ratio for hybrid seed production in *Brassica rapa* L.

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**ABSTRACT:** Using YSMS-8163, a GMS line of yellow *sarson* as seed parent and NDYS-921 as pollen parent (10 female: 2 male), planting ratio was investigated. Results revealed that 4 female: 2 male row ratios are most appropriate for hybrid seed production based on GMS line in yellow *sarson*.

Hybrid breeding is being extensively explored and pursued in *Brassica* oilseeds. In yellow *sarson*, (*Brassica rapa* var yellow *rapa*), development of male sterile lines [1,2] has led to increased efforts in this direction. In this endeavour, various aspects that define and determine the success of hybrid breeding programme need to be investigated. Production of hybrid seed by cross-pollination is the most important factor affecting the success of hybrid breeding programme. Success depends upon maximizing the seed setting on female line using various production techniques. Among various factors that influence the seed and siliquae setting on seed parent lines, female: male row ratio is the most important. Using YSMS-8163, as seed parent line, row ratio was studied and results on the same are reported herein.

### MATERIAL AND METHODS

YSMS-8163 was grown with NDYS-921 (as pollen parent) in 10 female: 2 male row ratio. The experiment was laid out in three replications in an isolated block. Fertile sibs in GMS line were rouged out at the time of flower initiation. Row-wise observations were recorded on 10 randomly selected plants in each row for seed yield and its key components viz. number of siliquae per plant and number of seeds per seeds.

**Row Arrangements:** In 10 female: 2 male row ratio plantings, the Row-1 and Row-10 are equivalent as both are adjacent to the pollen parent rows. Similarly Row-2 and Row-9 are equivalent as both are at equidistance, placed next only the adjacent rows. Based on the same premise Row-3 may be treated as equivalent to Row-8, Row-4 to Row-7 and Row-5 to Row-6. Based on this consideration five row arrangements viz. Row Arrangements-I (RA-I), RA-II, RA-III, RA-IV and RA-V were established.

The mean observations for 5 Row Arrangements were subjected to analysis of variance for RBD as per standard procedure.

### RESULTS AND DISCUSSION

The analysis of variance for the design of experiment showed highly significant differences due to row arrangements for all the three characters studied viz. seed yield per plant, number of seeds per siliqua and number of siliquae per plant. The observed means for different characters and their relative performance in different row arrangements as percentage of RA-I has been presented in Table-1.

The results showed significantly higher seed yield per plant in RA-I, which was statistically at par with RA-II. Both these row arrangements (RA-I and RA-II) were significantly superior to other row arrangements.

In terms of seed yield realized in different Row arrangements as percentage of RA-I, RA-II exhibited 97.98% seed yield, which was followed by 85.20% in RA-III, and the lowest was in RA-V (69.97%). However, the result of siliquae set exhibited

significant differences between RA-I and RA-II. The later was statistically at par with RA-III and both were statistically superior than RA-IV and RA- V. Similar trend was observed for number of seeds per siliqua in respect of which RA-I and RA-II were at par and statistically superior than other row arrangements. Considering of the result of these characters together, the row arrangement II appears to have distinct superiority over other row arrangements. Based on this, a row ratio of 4 female: 2 male may be considered appropriate for hybrid seed production in yellow *sarson* X yellow *sarson* hybrid combinations. As such the findings of the present investigation are in agreement with earlier report (1). In *Brassica napus*, the results indicated the superiority of 4 female: 2 male row ratio over 2 female: 1 male and 3 female: 1 male. (6) Investigated a series of row ratio in *Brassica napus* and reported that 3: 1 and 4: 1 row ratios produced a higher yield of hybrid seed.

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**Table 1:** Effect of pollen parent rows-distance on seed set, siliquae set and seed yield in female parent (ms lines) rows in different row arrangements.

Row arrangements	Row numbers	No. of siliquae/Plant/row		No of seeds/siliqua/plant		Seed yield/plant/row	
		Mean	% of Adjacent row	Mean	% of Adjacent row	Mean	% of Adjacent row
RA - I	1 & 10	78.75	100	28.48	100	4.46	100
RA - II	2 & 9	72.43	91.98	23.30	99.23	4.37	97.98
RA - III	3 & 8	71.23	90.46	22.79	97.06	3.80	85.20
RA - IV	4 & 7	68.89	87.48	22.28	94.89	3.73	88.63
RA - V	5 & 6	64.17	81.49	20.35	86.67	3.08	68.97
General mean		71.09		22.44		3.88	
C. D. at 5%		4.30		1.26		0.49	



Character association and path analysis under dryland condition in  
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### Introduction

Indian mustard (*Brassica juncea* L.) is the major oilseed crop of India. The successful cultivation of Indian mustard is hampered in dry land areas mainly due to the lack of suitable cultivars which can withstand long dry spells. For development of drought tolerant varieties availability of divergent form of drought tolerant germplasm is imperative. The present study was therefore, planned to study genetic variability, character association and path analysis in diverse genotypes of Indian mustard to form coherent breeding programme.

### Materials and Methods

The present study was conducted at Dryland Research Station, Dhiansar, Jammu during *rabi* 2001-02. Twenty diverse genotypes of Indian mustard were grown in randomized block design with three replications. Recommended package of practices were followed to raise the crop. Observations were recorded for the yield and yield attributing characters from ten competitive plants. Components of variability and heritability were estimated according to Johnson *et al* (1955). The correlation and path analysis were carried using methods of Al-Jibouri *et al* (1958) and Dewey and Lu (1959) respectively.

**Table 1** Estimates of genetic parameters of variability for ten different characters of twenty Mustard genotypes.

Components	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches plant <sup>-1</sup>	Secondary branches Plant <sup>-1</sup>	Siliquae plant <sup>-1</sup>	Seeds siliquae	Siliquae length (cm)	1000seed weight (g)	Seed yield plant <sup>-1</sup> (g)
Mean	50.07	138.80	151.68	5.79	11.46	254.08	14.56	5.09	4.23	21.83
Range	30-62	109-150	86-190	4.27-7.13	7.13-14.47	154-324	11.33-18.07	4.08-5.83	2.87-5.80	11-30
PCV	16.83	07.88	17.20	14.63	18.20	18.23	17.11	10.20	19.93	22.09
GCV	16.13	7.83	16.51	7.97	15.06	16.87	14.83	7.33	19.27	21.20
Heritability(%)	98.8	98.8	92.1	29.7	68.4	85.6	75.1	51.6	93.5	92.1
GA as % of mean	34.3	16.0	32.7	8.9	25.6	32.2	26.5	10.9	38.4	41.9

### Results and Discussion

Estimates of phenotypic coefficient of variance were slightly higher than the corresponding genotypic coefficient of variance (Table 1) for all the traits indicating that environmental component had lesser influence on the expression of these characters. Genotypic coefficient of variance was high for seed yield plant<sup>-1</sup> and 1000 seed weight. High heritability in broad sence was recorded for all characters except primary branches plant<sup>-1</sup>, siliquae length, secondary branches plant<sup>-1</sup> and seeds siliquae<sup>-1</sup>. High estimates of heritability for seed yield and yield related attributes revealed reliability in phenotypic selection. Genetic advanced as percentage of mean was high for seed yield plant<sup>-1</sup>, 1000 seed weight, days to 50% flowering, plant height and siliquae plant<sup>-1</sup>. Similar results were also reported by Singh and Chaudhary (1988) and Reddy *et. al.*, (1994). High heritability and high genetic advance for seed yield plant<sup>-1</sup> and 1000 seed weight revealed predominance of additive gene action and thus reliability for direct selection.

The results presented in table 2 indicated seed yield exhibited significant positive correlation with 1000 seed weight, seed siliquae<sup>-1</sup>, siliquae plant<sup>-1</sup>, length of siliqua and primary branches plant<sup>-1</sup>. Singh and Chaudhary (1988) and Goyal and Kumar (1995) have also reported similar results. Path coefficient analysis at genotypic level revealed that siliquae plant<sup>-1</sup> had high positive direct effect. Seed siliqua<sup>-1</sup>, primary branches<sup>-1</sup> and 1000 seed weight having high correlation with seed yield had maximum indirect effect via siliquae plant<sup>-1</sup>. These results are in conformity with Singh and Chaudhary (1988). Hence, in the present set of material it was indicated clearly that selection of longer and more siliquae number with more primary branches plant<sup>-1</sup> would promote higher yield.

**Table 2** Path coefficient analysis showing direct and indirect effect of nine characters on seed yield.

Characters	1000 seeds weight	Seeds siliqua <sup>-1</sup>	Siliquae plant <sup>-1</sup>	Length of siliqua (cm)	Secondary branches plant <sup>-1</sup>	Primary branches plant <sup>-1</sup>	Plant height (cm)	Days to 50% flowering	Days to maturity	Genotypic correlation with seed yield
1000 seed weight	.2114	.0936	.3869	.0387	-.0439	-.0219	-.0083	.0254	-.0074	.6746**
Seeds siliqua <sup>-1</sup>	.0724	.2734	.4410	.0001	.0445	-.1002	.0006	.0073	-.0036	.7355**
Siliquae plant <sup>-1</sup>	.1262	.1860	.6482	.0340	.0499	-.1167	-.0099	.0318	-.0107	.9388**
Length of siliqua	.1117	.0003	.3008	.0732	.0131	-.1291	-.0205	-.0026	.0151	.3619**
Secondary branches										
Plant <sup>-1</sup>	-.0475	.0623	.1657	.0049	.1951	-.1514	-.0338	.0830	-.0248	.2535
Primary branches										
Plant <sup>-1</sup>	.0242	.1433	.3959	.0494	.1546	-.1911	-.0475	.0821	-.0192	.5918**
Plant height	.0273	-.0025	.0995	.0234	.1029	-.1416	-.0641	.1129	-.0369	.1207
Days to 50% flowering	.0288	.0107	.1110	-.0010	.0871	-.0844	-.0389	.1859	-.0449	.2542
Days to maturity	.0226	.0142	.1004	-.0161	.0702	-.0533	-.0343	.1211	-.0689	.1558

Residual effect = 0.0561

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# **IN VITRO REGENERATION SYSTEM IN CRAMBE VIA PROTOPLAST CULTURE**

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## **INTRODUCTION**

The genus *Crambe* belongs to the Cruciferae and comprises annual species. As a potential renewable oil source with a high content of erucic acid (58–62 %) *Crambe* has been objected to agronomical as well as technological investigations. Products and derivatives of high-erucic acid oil may be applied in a wide range such as detergents, cosmetics, coatings and pharmaceuticals.

*In vitro* culture of *Crambe* should enhance breeding efficacy in this genus and constitutes a prerequisite for the use of *Crambe* genotypes as genetic sources *via* somatic hybridization or genetic transformation. The present paper reports on plant regeneration from protoplasts isolated from mesophyll tissue of *in vitro* leaves.

## **MATERIAL AND METHODS**

Seeds from different lines of *C. abyssinica* and *C. hispanica* were used as starting material for the establishment of *in vitro* cultures. Seeds of *C. hispanica* accession '13/81' was provided by Institute of Horticultural Crops, Quedlinburg and '15/84' by Gene Bank, Gatersleben. Seeds of *C. abyssinica* accession '665/1' was obtained from Institute of Agricultural Crops, Groß Lüsewitz, cv. 'BelAnn' from Research Centre for Agriculture and Fishery, Biestow and cv. 'Carmen' from CEBECO, Celle.

For protoplast isolation leaves were excised from 3-4 week-old seedlings and cut into pieces in a solution containing 0.3 M mannitol and 0.05 M CaCl<sub>2</sub>. After about 16 h incubation in solution of cellulase 'Onozuka R10' and macerozyme 'R10' at 24°C protoplasts were isolated as described by Gerdemann-Knörck (1995) with minor modifications. Protoplast solutions were cultured with different liquid culture media: MI (Li & Kohlenbach 1982), Nitsch (Nitsch & Nitsch 1969) and Gamborg B5 (Gamborg *et al.* 1968) at a final density of  $4 \times 10^5 \text{ ml}^{-1}$ . One week after isolation, the protoplasts were diluted with fresh liquid medium. When the microcalli reached a size of 1-2 mm they were transferred onto different solid media: N2 (Sacristan *et al.* 1989, modified) or N3 containing MS medium (Murashige & Skoog 1962) supplemented with 1.0 % sucrose, 2.0 % mannitol, 250 mg/l casein hydrolysate, 0.5 mg/l naphthaleneacetic acid (NAA), 0.5 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l thidiazuron (TDZ). Each medium was changed every three weeks.

## **RESULTS AND DISCUSSION**

Identification of a suitable osmoticum influencing the yield of viable protoplasts was tried with two different concentrations of mannitol (0.4 and 0.6 M) and with 0.4 M sucrose. The best yields of viable protoplasts were obtained at a concentration of 0.6 M mannitol. Protoplasts with higher viability and in some cases with better yields were obtained at a concentration of 0.2 % cellulase, 0.04 % macerozyme and 0.6 M mannitol.

After 2-4 days cell division was observed and small colonies were formed two weeks after culture initiation. The MI medium was effective for cell division and colony formation with all genotypes except of '665/1'. The combination of 0.5 mg/l 2,4-D, 0.5 mg/l NAA and 0.5 mg/l BAP was the most effective for microcallus regeneration. In all tested liquid media microcallus regeneration could not be obtained with genotype '665/1'.

On the solid media N2 and N3 shoots were produced after 3-4 weeks in dependence on the genotype. The genotypes also were found to differ in percent regeneration and mean number of shoots per callus. Genotypes of *C. hispanica* showed a higher regeneration response than genotypes of *C. abyssinica*. N2 and N3 media had a positive effect on the regeneration response of *C. hispanica* '13/81' and '15/84' and *C. abyssinica* 'BelAnn' but not for 'Carmen'. The frequency of shoot regeneration was between 2.5 % and 5.7 % (table 1).

Table 1: Shoot regeneration of *Crambe* genotypes

Genotype	Calli (n)	Calli with shoot regeneration	Number of shoots
'13/81'	3835	220	285
'15/84'	3324	98	120
'BelAnn'	80	2	2

When transferring microcalli from suspension culture to solid shoot proliferating media use of agarose as a solidifying component was more favourable as compared to agar which often resulted in the release of brown, possibly polyphenolic exudates. This observation is also reported by Robertson *et al.* (1988) and Sakhno and Skarzhinskaya (1990).

The regenerated shoots were elongated on MS basal medium without hormones before transfer to the greenhouse.

The ability to culture and to regenerate plants from isolated protoplasts in *Crambe* is a prerequisite for biotechnological approaches and will facilitate application of somatic cell manipulation technique to the improvement of this crop genus.

In tests with protoplasts of *Crambe* for fusion experiments with *Brassica napus* we observed that cell wall regeneration took place. Depending on the combination of genotypes regeneration rates up to 19 % after the fusion treatment were observed. These fusion and regeneration frequencies were approximately the same as those obtained in hybridization experiments between related species (Fahleson *et al.* 1994).

From the results can be concluded that MI medium supplemented with 2,4-D, NAA and BAP at 0.5 mg/l each is suitable for protoplast division and colony formation in *Crambe*. When protoplast-derived colonies were transferred onto N2 and N3 medium plantlets could be regenerated.

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#### Acknowledgements

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# PERFORMANCE OF CAULIFLOWER (*Brassica oleracea* var. *botrytis*) SELECTIONS IN THE TEMPERATE CLIMATE OF INDIA

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## ABSTRACT

Fourteen advance generation selections of late cauliflower were assessed for their performance for yield and other horticultural traits during 2001-2002 at Baragraon Farm of IARI, Regional station, Katrain (Kullu Valley) HP, India situated at 1560 m.a.s.l. Kt-2 gave the highest yield of 36.36 kg/ plot (9 m<sup>2</sup>) followed by Kt-16 (32.76 kg/plot). Kt-17 was earliest in maturity besides minimum number of outer leaves. Both the high yielding selections produced good quality curds. Kt-2 is resistant to black rot and tolerant to sclerotinia rot too.

## INTRODUCTION

Cauliflower is one of the important vegetable grown in tropical, subtropical and temperate climate of India. However, suitable varieties are selected on the basis of their maturity period and the prevalent climate because cauliflower is temperature sensitive. Snowball group of cauliflower is most suitable for the main season crop (September–April) for the temperate regions. Curds are available when there is lien period of its production in the tropical and subtropical regions of India. Hence it brings more returns to the growers. Therefore, superior advance generation selections of late cauliflower developed at IARI, Regional Station, Katrain were evaluated for their performance.

## MATERIALS AND METHODS

Fourteen selections of late cauliflower were tested against standard check Pusa Snowball-1 during rabi season (i.e. September-April). All these selections were planted in a randomised block design with three replications at Indian Agricultural Research Institute, Regional Station, Katrain (Kullu Valley) H.P. during 2001-2002. Each plot measured 9 m<sup>2</sup> and plants were spaced 45 cm apart within and between the rows. Seeds were sown in nursery beds in first week of September 2001 and transplanted in first week of October. All the recommended cultural practices were used to raise a good crop. Data were recorded for yield and other horticultural and quality traits.

## RESULTS AND DISCUSSION

There were significant differences among variety, selections for all the six traits. Mean performance of the five high yielding selections are presented in Table 1. A glance over the values revealed that Kt-17 was earliest which took 132.66 days from transplanting to curd formation. The minimum numbers of outer leaves were recorded in Sel. 44 (14.1) followed by Kt-17 (15.2) while leaf size index was lowest in Kt-17 (450.7 cm<sup>2</sup>). Curd size index, gross weight/curd and yield/plot was maximum for selection Kt-2 (36.36 kg) and Kt-16 (32.76kg). Kt-2 is resistant to black rot and tolerant to Sclerotinia rot. Hence both these selections are the promising and can prove most suitable for temperate regions of India or in other countries of the world having similar climate. Thakur and Singh (2001) found Selection SWI-1 the highest yielder and minimum number of outer leaves out of five entries assessed for summer production.

Kt-17 has been observed to possess earliness besides lower number of outer leaves/plant and can be utilized in further breeding programme. These selections will be tested in other climatic regions of India under All India Coordinate Research Project on Vegetable Crops.

## REFERENCES

Thakur, P.C. and Veerpal Singh 2001. Assessment of cauliflower (*Brassica oleracea* var. *botrytis*) varieties for summer production in temperate climate. *Cruciferae Newsletter* 23: 85-86.

**Table 1. Mean performance of top five cauliflower selections for various quantitative characters.**

Characters	Rank					CD at P=0.05
	I	II	III	IV	V	
Days to 50% curd formation	Kt-17 132.66	RSK-1301 133.66	Kt-6 147.00	Kt-15 152.00	Kt-18 153.00	5.83
No. of outer leaves	Sel-44 14.1	Kt-17 15.2	Kt-15 15.6	Kt-6 15.67	Kt-18 15.7	2.30
Leaf size index (cm <sup>2</sup> )	Kt-17 450.7	Sel-22 682.1	Sel-44 686.1	Kt-8 769.7	RSK-1301 808.0	184.60
Curd size index (cm <sup>2</sup> )	Kt-2 213.2	Kt-16 196.0	Sel-22 191.2	Kt-4 185.3	Kt-19 185.0	43.84
Gross wt./curd (kg)	Kt-16 2.02	Kt-2 2.01	Kt-4 1.71	K-9 1.52	Kt-20 1.48	0.41
Yield (kg/plot/ 9 m <sup>2</sup> )	Kt-2 36.36	K-16 32.76	Kt-20 32.04	Kt-6 31.68	Sel-22 28.80	5.76



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## Evaluation, characterisation and regeneration of *Brassica oleracea* germplasm collections in the EU GENRES CT99 109-112 project 'Brassica, including *B. carinata*'

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### Introduction

In the GENRES CT99 109-112 project "Brassica Collections for Broadening Agricultural Use, including Characterising and Utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop" the *B. oleracea* subgroup was formed to conduct the tasks as defined in the Technical Annex the project. One major task was the evaluation of collections for important agricultural properties. As the number of *B. oleracea* accessions in the European collections was far too large (approx. 10 000) to be evaluated, a core collection was established representing the available diversity. The properties to be evaluated were decided by the partners before the start of the project. Another purpose of the project was to make material of European collections more accessible. Therefore part of the material of partner's collections were characterised and regenerated in the project. Priority was given to material included in the core collection.

### Core collection

A core collection of 396 accessions of *B. oleracea* and related species was established with help of all partners of the subgroup using the *B. oleracea* core of the EU AIR3 CT920463 *Brassica* project as starting point. The aim was to represent the broadest variation of *B. oleracea* germplasm possible, using the European Brassica database (Bras-EDB). At the start of the project the partners agreed on the number of accessions per crop type and country of origin, considering also the importance of the crop. Next, all partners holding *B. oleracea* collections identified a fixed number of accessions per crop type from their collection for inclusion in the core. The chosen accessions had to meet the necessary requirements for the evaluations regarding seed quality and quantity. Wild *Brassica* species (n=9) related to *B. oleracea* were generously donated by Dr. Cesar Gomez-Campo from the 'Universidad Politecnica de Madrid', Spain.

During field tests for resistance and characterisation activities of the *B. oleracea* core, part of the unknown types of the core could be identified. It also became clear that some core accessions were misclassified. The accessions not belonging to *B. oleracea* will be discarded from the core. Accessions selected for the core will be tagged in the Bras-EDB.

### Characterization and regeneration

A total of 495 *B. oleracea* accessions have been characterised. For the characterisation a minimum descriptor list was used, which was defined by the partners and based on the minimum descriptors as defined by the ECP/GR Brassica Working group. In addition some partners used other descriptors.

Regeneration protocols were discussed in the first year of the project. During the whole project approx. 240 accessions of *B. oleracea* were regenerated.

### Evaluation

The complete core of 396 accessions was evaluated for resistance to the three pathogen's: *Plasmiodiophora brassicae*, *Xanthomonas campestris* pv *campestris* and *Mycosphaerella*

*brassicicola*. Part of the core was evaluated for salt tolerance. The evaluation tests were conducted according to protocols, which were defined in the first year of the project.

#### ***Plasmodiophora brassicae* (Clubroot)**

The core collection was evaluated against two isolates of clubroot (MS6 and K 92-16). The screening tests were successfully completed and disease indexes for the two isolates varied between 18 -100% and 44 -100% respectively. The five most resistant *B. oleracea*'s were accessions of kohlrabi, tronchuda, Brussels Sprouts and two kale's.

#### ***Xanthomonas campestris* pv *campestris* and**

For *Xanthomonas* no resistant accessions were found, but especially in accessions of cauliflower, broccoli and kale (including also Chinese kale) resistant plants were found. Only in five accessions the percentage resistant plants was more than 50%: These were a kale and four cauliflower accessions. Tests of progenies of selfed plants of part of the resistant material confirmed the resistance. The resistance found was of a high level, but not complete.

#### ***Mycosphaerella brassicicola***

The *Mycosphaerella* screening tests in the field detected a few accessions of which all plants showed a high level of resistance. Especially in red cabbage, but also in broccoli, accessions with many resistant plants have been found. The most resistant accessions, of which all tested plants showed resistance, were five red cabbages. The Chinese kale accessions could not be evaluated because they were flowering so early that infection had not yet taken place.

#### **Salt tolerance**

A total of 100 accessions were screened for salt tolerance in three different tests. The material screened included mainly the cauliflower (67) and broccoli (30) accessions of the *B. oleracea* core collection. The salt doses in these tests were 0, 2 and 4 g/l. In the first two tests the reduction for plant and curd weight was recorded. For the last test only the plant weight reduction could be measured.

The mean reduction in curd weight induced by the highest salt concentration was for both cauliflower and broccoli around 40%. Broccoli showed some more tolerance than cauliflower. For both cauliflower and broccoli 8 accessions were found with less than 20% curd weight reduction at the highest salt concentration.

#### **Conclusions**

The *B. oleracea* subgroup regenerated and characterised a substantial number of *B. oleracea* accessions. The complete core collection of 396 accessions was evaluated for resistance to *Xanthomonas* and *Mycosphaerella* and for two isolates of clubroot. Part of the core (100 accessions, mainly cauliflower and broccoli) was screened for salt tolerance. The evaluations gave some interesting results, which can be further exploited in breeding programmes.

All the results will become available on the website of the Bras-EDB:

<http://www.cqn.wur.nl/pgr/collections/brasedb/>

#### **Acknowledgement**

The work of this GENRES CT99 109-112 project has been supported by the European Commission in the framework of Council Regulation (EC) No 1467/94.

## SURVEY OF SEED STORAGE COMPONENTS IN ETHIOPIAN MUSTARD (*Brassica carinata* A. Braun)

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### INTRODUCTION

Ethiopian mustard (*Brassica carinata* A. Braun) is an important crop within its natural environment (Ethiopia) and is increasingly being looked on as an additional oilseed crop with high potential for the dry areas of southern Europe, where its drought tolerance and pest and disease resistance may make it suitable as an alternative oilseed within the crop rotation. In addition, it contains many agronomic traits that would be beneficial if they could be incorporated within other *Brassica* crops grown in more temperate areas of Europe (Fereres *et al.* 1983; Gugel *et al.* 1990).

In the framework of the European project “*Brassica* collections for Broadening Agricultural Use”, including “Characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop” (Contract no: RESGEN CT99 109-112), the *Brassica carinata* subgroup was made up of the following partners:

- P15-Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain (*sub-coordinator*) (IAS), and
- P16- John Innes Centre, Norwich, United Kingdom (JIC).

One of the objectives of that subgroup was to evaluate multiple seed storage components of *B. carinata* in accessions grown at 2 different environments through the years 2000, 2001, 2002 and 2003. These analyses were performed over seed samples from 4440 individual plants by Near Infrared Spectroscopy (NIRS). This technique has been successfully used for decades in the agricultural field for the prediction of quality parameters in *Brassica* (Panford *et al.* 1988; Font *et al.* 2002a; Font *et al.* 2002b; Font *et al.* 2003). The objective of this paper is to present the main results obtained in the evaluation of 222 accessions of *Brassica carinata* after three years of field trials in two localities Norwich (UK) and Córdoba (Spain).

### MATERIAL AND METHOD

Ten plants from 222 accessions of *B. carinata*, chosen at random from the collections at IAS (Córdoba, Spain) and Centre for Genetic Resources (Wageningen, The Netherlands), were grown in field plots both at Norwich, UK (from March to September 2000, 2001 and 2002) and at Córdoba, Spain (from November 2000 to June 2003). Plants of each accession were grown in two randomised blocks of 5 individuals. All the plants were grown in self-pollinating conditions. All the accessions were evaluated for the following seed storage components (SSC): crude protein (CP), oil, total glucosinolate content (GSL), acid detergent fiber (ADF), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and erucic acid (C22:1). The determination of the SSC was carried out by Near Infrared Spectroscopy (NIRS).

To perform NIRS calibration for SSC, 3g samples of intact seed from 4440 plants (222 accessions x 10 plants/accession x 2 localities), were scanned in an NIR spectrophotometer (NIRSystems model 6500, Foss-NIRSystems, Inc., Silver Spring, MD, USA), acquiring their spectra at 2 nm. intervals over a wavelength range from 400 to 2500 nm. On the basis of their spectral features, between 100 and 200 samples were selected as representative subsets of the whole spectral variability contained in the entire set, for performing NIR calibrations. Reference analytical values for the selected samples were obtained for CP by Kjeldahl; for oil by Nuclear Magnetic Resonance (NMR) and Soxhlet; for GSL by High Performance Liquid Chromatography (HPLC); for ADF following the method proposed by Goering and Van Soest (1970) and Gas-Liquid Chromatography for the fatty acid composition of the oil. Using the program GLOBAL v. 1.50 (WINISI II, Infrasoft International, LLC, Port Matilda, PA, USA), different mathematical treatments (0,0,1,1 (derivative, gap, first smooth, second smooth);



1,4,4,1; 2,5,5,2) were used to correlate spectral and chemical data. The equations for each SSC were then validated on an external validation set, selecting those equations with the highest prediction abilities on the basis of the coefficient of determination ( $r^2$ ) and standard error of prediction (SEP). The selected equations, were then used to predict the SSC of the different accessions of *B. carinata* from both localities from 2000 to 2003.

## RESULTS AND DISCUSSION

All the NIRS equations selected as having the highest prediction abilities (Table 1), showed good precision (ADF, C18:2; C18:3) or excellent precision (CP, oil, GSL, C18:1; C22:1) in the prediction, on the basis of their respective  $r^2$  values (Shenk and Westerhaus 1996).

From the application of the equations shown in Table 1 to the 4440 individual plants collected at both environments (IAS and JIC) from 2000 to 2003, a total of 35520 prediction data for SSC were generated, which are summarized in Table 2.

**Table 1.** Calibration and validation statistics for the different seed storage components studied.

<u>seed storage component</u>	<u>n</u>	<u>range</u>	<u>mean</u>	<u>sd</u>	<u>SEP</u>	<u><math>r^2</math></u>
CP (% dw)	100	16.80-37.80	24.21	4.66	<b>0.51</b>	<b>0.99</b>
oil (% dw)	100	25.6-54.6	43.13	5.4	<b>1.35</b>	<b>0.94</b>
ADF (% dw)	100	5.33-16.31	11.00	2.18	<b>0.83</b>	<b>0.81</b>
GSL ( $\mu\text{mol/g dw}$ )	150	56.53-221.10	117.62	31.65	<b>8.5</b>	<b>0.93</b>
C18:1 (% oil)	200	4.53-65.75	14.38	10.51	<b>3.77</b>	<b>0.90</b>
C18:2 (% oil)	200	7.15-33.46	17.17	4.67	<b>2.04</b>	<b>0.81</b>
C18:3 (% oil)	200	3.11-21.18	12.28	2.99	<b>1.17</b>	<b>0.85</b>
C22:1 (% oil)	200	9.0-60.5	38.26	12.25	<b>2.81</b>	<b>0.95</b>

n= number of samples used in the calibration; sd= standard deviation; SEP= standard error of performance;  $r^2$ = coefficient of determination.

**Table 2.** Seed storage components of *B. carinata* at IAS and JIC for the years from 2000 to 2003.

		<u>1st year</u>		<u>2nd year</u>		<u>3rd year</u>	
		<u>IAS</u>	<u>JIC</u>	<u>IAS</u>	<u>JIC</u>	<u>IAS</u>	<u>JIC</u>
<u>CP (% dw)</u>	mean	22.7	25.7	22.7	23.0	24.7	28.8
	sd	3.3	2.8	3.0	2.8	3.2	4.2
<u>OIL (% dw)</u>	mean	44.7	33.3	45.0	43.1	45.3	40.3
	sd	3.8	4.1	3.3	2.8	4.3	3.6
<u>ADF (% dw)</u>	mean	13.8	9.6	12.9	10.8	12.0	9.0
	sd	2.2	1.7	1.2	0.9	2.4	1.3
<u>GSL (<math>\mu\text{mol/g dw}</math>)</u>	mean	108.8	105.2	107.5	86.7	111.2	116.2
	sd	18.6	19.1	18.6	16.9	25.7	21.4
<u>C18:1 (% oil)</u>	mean	9.8	8.9	7.9	11.6	11.7	10.2
	sd	2.7	1.4	3.2	3.7	4.5	3.8
<u>C18:2 (% oil)</u>	mean	15.8	17.4	15.9	17.9	19.1	23.4
	sd	1.9	1.4	2.0	2.0	2.9	3.6
<u>C18:3 (% oil)</u>	mean	12.9	13.2	12.6	13.0	9.9	13.6
	sd	1.6	1.6	1.3	1.5	2.4	2.3
<u>C22:1 (% oil)</u>	mean	42.9	38.4	44.6	32.0	44.7	37.7
	sd	3.4	2.6	3.1	5.4	6.9	8.2

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## **Brassica Collections for Broadening Agricultural Use: RESGEN CT99 109/112**

### **Brassica rapa Subgroup**

The *B. rapa* subgroup comprised 4 of the project partners:

- Genetic Resources Unit, Horticulture Research International, UK (Angela Pinnegar & Dave Astley)
- Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany (Klaus Dehmer, Marie-Luise Graichen & Evelin Willner)
- Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal (Eduardo Rosa, Alfredo Aires & Rosa Paula)
- Mision Biologica de Galicia, Pontevedra, Spain (Amando Ordas & Elena Cartea)

The main tasks of the *B. rapa* subgroup in the project covered: production and preparation of data for the Bras-EDB; the development of a project core collection for *B. rapa*; characterisation of the core; regeneration of the core and other priority accessions in partner collections; evaluation of the core for i) resistance to White blister (*Albugo candida*), b) glucosinolate content; safety duplication of material.

#### Documentation:

During the project the partners of the *B. rapa* sub-group transferred passport data sets for all relevant *Brassica* accessions to the Bras-EDB manager for incorporation in the European database. The Co-ordinator (CGN) distributed information outlining the standard formats for the presentation of characterisation and evaluation data in association with the Bras-EDB. The data from the characterisation trials of the *B. rapa* core collection produced by IPK, MBG HRIGRU have been forwarded to Noortje Bas in CGN. These data will be available on the Internet at the CGN web site.

#### B. rapa Core Collection

The *B. rapa* core collection of 100 accessions was established to provide the broadest representation of the available gene pool for use in the characterisation and evaluation programmes. The group took note of 4 recent review papers on the taxonomy of *B. rapa* in the development of the project *B. rapa* core collection (Hanelt 1986, Gladis 1989, Gladis & Hammer 1992 & Diederichsen 2001). Where possible the group used the taxonomic names of accessions utilised in the Bras-EDB. All the accessions proposed for the draft core collection were cytometry tested, thus providing an additional check on their taxonomic status. Not all the accessions in the *B. rapa* core collection are represented in the Bras-EDB. The *B. rapa* group see the development of the core collection as a major impact in providing a well characterised group of accessions that are available for further usage, and in particular, is relevant to the increasing interest in molecular characterisation as shown by various attendees at the project Symposium in Giessen, Germany (11<sup>th</sup> December 2003).

#### Characterisation of the Core

The members of the *B. rapa* sub-group developed 4 sets of minimum characterisation descriptors for use with different *B. rapa* crop types within the project, namely for tumip rape, root tumip, tumip tops/greens and Asian leaf/head types. Over the period of the project a total of 216 accessions of *B. rapa* were characterised by the sub-group partners including all the 100 accessions of the *B. rapa* core collection and additional accessions from the collections in HRIGRU, IPK Gatersleben and MBG Galicia. For most of the accessions characterised digital images were captured and stored. As reported above the *B. rapa* characterisation data will be made available on the Internet in association with the Bras-EDB.

#### Regeneration of Collections

A total of 210 Brassica accessions was regenerated by the *B. rapa* sub-group including 192 accessions of *B. rapa*, 13 of *B. oleracea* and 5 of *B. napus*. All the seed produced in the regeneration programmes have been stored under long-term conditions following international protocols. The group achieved a significant output for the regeneration of *B. rapa*, but this must be viewed in the context of the genepool defined in the Bras-EDB of >3000 accessions. It is important that national programme gene banks continue to collaborate in deciding which material is prioritised for regeneration in order to reduce duplication of effort and the waste of valuable resources. The seed produced of the core collection and other partner accessions

were used in the project characterisation and evaluation work, a majority of which are available for further utilisation depending upon the conditions of the original Material Transfer Agreements.

#### Pathology Screen with *Albugo candida*

The *B. rapa* core collection of 100 accessions was screened for reaction to the fungal disease White blister (*Albugo candida*) using 2 different isolates, namely 7V and Acem2. The results of the 2 pathology screens showed no obvious relationship between the reactions of the core to the 2 disease isolates. The results from the screen with isolate 7V showed a range of susceptible/resistance responses between and within accessions. Some accessions showed a resistant response with more than 80% of individuals with no pustules. In the second screen using isolate Acem2 a majority of the *B. rapa* core accessions were totally resistant to this *A. candida* isolate. However, 5 accessions exhibited plants that were susceptible to this *A. candida* isolate. The Acem2 isolate was collected from *Capsella bursa-pastoris* (L.) Medicus, Shepherd's Purse. This weed plant has long been suspected of acting as a winter host for the White blister fungus. The objective of using the Acem2 isolate was to score the reaction of the *B. rapa* core collection for susceptibility in order to assess the extent cross contamination from a common field crucifer weed. It is interesting to note that the majority of the *B. rapa* core accessions were completely resistant to the Acem2 isolate. However 5 accessions showed susceptible plants, thus some wild and weedy plants may act as an over-wintering source of the fungus.

#### Glucosinolates

The *B. rapa* core collection of 100 accessions was screened in the glucosinolate evaluation programme for 8 glucosinolate compounds, namely 2-Hydroxybut-3-enyl; 2-Propenyl; But-3-enyl; Pent-4-enyl; Indol-3-ylmethyl; 2-phenethyl; 4-Methoxyindol; 1-Methoxyindol-3-ylmethyl. Plants were grown in field trials and in parallel under controlled conditions in growth cabinets. This dual approach has provided significant data in relation to the glucosinolate composition relating to cultural and environmental factors and in relation to growth habit and plant growth stages. The analysis of the qualitative and quantitative composition of glucosinolates in the *B. rapa* core collection has provided an insight into the environmental impact on these compounds in relation to flavour, thus providing the possibility of flavour manipulation in the future. The material exhibited significant diversity in the quantitative characteristics in relation to growth stages (seedling, leaf, inflorescence and root).

Data from the *Albugo* screens were compared with data from the glucosinolate analyses to check any relationship between the compounds and the disease. The data revealed no significant interactions. However, it was recognised that further studies should screen the pathogen and glucosinolates on the same plant material for higher accuracy.

#### Safety duplication:

The system for the safety duplication of the *B. rapa* collections was agreed by the subgroup early, thus partners have developed black box bilateral agreements for their safety duplicates with other project partners or gene banks with suitable agreed standard storage conditions.

Note: The work of this GENRES CT99 109-112 project has been supported by the European Commission in the framework of Council Regulation (EC) No 1467/94 and by the partner national programmes.

## The European *Brassica napus* Core Collection – Characterisation, Evaluation and Establishment

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<sup>8</sup>Institute of Plant Pathology and Plant Protection, University of Göttingen, D-37077 Göttingen, Germany.

<sup>9</sup>IPK Branch Station Malchow, D-23999 Malchow/Poel, Germany

In the EU genres project project CT99 109-112 a subgroup was dedicated to *Brassica napus*. This species primarily represents oilseed rape (ssp. *oleifera*) with spring and winter types. Additionally, it comprises leafy fodder and green manure types (ssp. *napus*) of spring and winter forms. Swedes or rutabagas (ssp. *napobrassica*) are cultivated as a vegetable and for forage purposes and finally, a mixed group of “exotic” material primarily is used as vegetables comprising hakuran, kale types and couve nabica, where the inflorescence is used as vegetable.

By means of the ECP/GR Central *Brassica* Crop database (BrasEDB) a preliminary core collection of about 200 accessions was selected based on the above systematic frame and prioritising great diversity in country of origin. The BrasEDB contains a total of 3787 *Brassica napus* accessions.

This genetically diverse core collection was established to make a comprehensive screening of traits with agricultural interest among genebank accessions. A number of

pests and diseases infesting *Brassica* crops were given high priority:

Disease / Pest	
Clubroot	<i>Plasmodiophora brassicae</i>
Cabbage stem flea beetle	<i>Psylliodes chrysocephala</i>
Cabbaggestem weevil	<i>Ceutorhynchus pallidactylus</i>
Rape stem weevil	<i>Ceutorhynchus napi</i>
Field slugs	<i>Deroceras</i> spp.
Alternaria Sclerotinia Phoma	<i>Alternaria brassicae</i> , <i>Sclerotinia sclerotiorum</i> , <i>Phoma lingam</i> ( <i>Leptosphaeria maculans</i> )

Additionally, the seed quality was considered embracing parameters such as oil-, protein- and glucosinolate content as well as fatty acids composition of the seed oil.

Furthermore, it was decided to make a morphological characterisation of the core collection accessions to allow the phenotypic appearance and biology of plants to influence the final selection. The minimum descriptors scored for the characterisations were:

### Oilseed and Forage rape

Anthocyanin colouration of

- flower stem
- petiole and midvein

Leaf, number of lobes

Leaf colour

Flowering time

Petal colour

Vernalisation requirement/seasonality

### Swede

Root shape

Root colour of skin above ground

Root colour of skin below ground

Interior root colour

Leaf, number of lobes (optional)

### Kales

Plant growth habit

Leaf blade curling

Leaf colour





## The achievements of the EU GENRES CT99 109-112 project *Brassica*, including *B. carinata*

Loek J. M. van Soest, Ietje W. Boukema & Noor Bas

Centre for Genetic Resources, The Netherlands (CGN), Wageningen, The Netherlands

### Introduction

The GENRES CT99 109-112 project “**Brassica Collections for Broadening Agricultural Use, including Characterising and Utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop**” started 1 January 2000 and officially ended on 31 December 2003. The project was co-financed by the European Commission under the Council Regulation (EC) No 1467/94.

The activities were conducted by four subgroups, each dealing with a *Brassica* species:

- *B. oleracea* - Cole crops, e.g. kales, cabbages, cauliflower, broccoli, kohlrabi, couve tronchuda, Brussels sprouts
- *B. rapa* - turnip and turnip rapeseed
- *B. napus* - rapeseed or colza and swedes
- *B. carinata* - Abyssinian mustard

The major aims of the project were to conserve, document (computerise), characterise, evaluate and rationalise European collections of these *Brassica* species. The project is complementary to the activities co-ordinated in the framework of the ECP/GR (“European Cooperative Programme for Crop Genetic Resources Networks”) *Brassica* Working Group

Annex 1 lists the 16 partners of 8 European countries, which participated in the project. Ten partners are collection holders and conserve collections of *Brassica* species, three partners conduct research in *Brassica* species and three partners are private plant breeding firms with programmes in some of the *Brassica* species. One of the latter partners includes 7 breeding companies.

The results contribute to a better knowledge of the genetic resources of these *Brassica* species and improve the utilisation of the gene pools in Europe by plant breeders and growers. A mid-term assessment of the project was previously published (van Soest *et al.*, 2003).

### Major achievements of the project

The achievements according to the tasks and milestones as defined in the Technical Annex of the contract with the European Commission can be summarised in five major areas:

#### A. Database and core collections

##### European Brassica Database (Bras-EDB)

A new version of the Bras-EDB (Boukema *et al.*, 2003), including passport data of more than 19.000 accessions, has been placed on the Internet (<http://www.cgn.wur.nl/pgr/collections/brasedb/>). The characterisation and evaluation data obtained from this project will be linked to this database thus ensuring access to this information

##### Creation of core collections of the four *Brassica* species:

The four *Brassica* subgroups discussed the structure and size of the respective core collections and the groups developed their final core collections, on the basis of the research conducted in the project. The size of the four core collections is as follows:

<i>B. oleracea</i> : 396 accessions	<i>B. napus</i> : 150 accessions
<i>B. rapa</i> : 100 accessions	<i>B. carinata</i> : 73 accessions

#### B. Characterisation and regeneration

##### Characterisation of the *Brassica* species using minimum descriptors

The subgroups agreed upon sets of minimum descriptors for each of the four *Brassica* species. The cores and parts of the collections were characterised for these descriptors (Table 1). Part of the *B. carinata* collection was characterised using DNA finger printing.

##### Regeneration of collections with priority on accessions included in the core collections

The project regenerated nearly 800 accessions of all four *Brassica* species (Table 1).

Table 1. Overview of the completed characterisations and regenerations.

<i>Brassica</i> species	No of accessions characterised	No of accessions regenerated
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<i>B. oleracea</i>	480	290
<i>B. rapa</i>	215	195
<i>B. napus</i>	320	210
<i>B. carinata</i>	220	100
<b>Total</b>	<b>1235</b>	<b>795</b>

### C. Evaluation for different properties, priority on material included in the cores

More than 4000 samples were evaluated for a range of properties (Table 2). Results of these evaluation experiments will be linked to the Bras-EDB. The different evaluations resulted in the detection of accessions with interesting properties for further breeding (see papers of subgroup coordinators).

**Table 2** Overview of the evaluation conducted.

Property group	Number of properties	Brassica species
Pest and diseases	12	All four <i>Brassica</i> spp.
Quality	4	<i>B. napus</i> , <i>B. rapa</i> , <i>B. carinata</i>
Salt Tolerance	1	<i>B. oleracea</i>
Agronomic evaluation	2	<i>B. carinata</i>

### D. Rationalisation, safety duplication and recommendations for further collecting

#### Rationalisation of collections

The much-improved new version of the Bras-EDB allows tracing duplicates between the collections of the different *Brassica* species.

#### Safety duplication of the Brassica collections

The system of safety duplications of *Brassica* collections in Europe has been highly improved, compared to the situation before the start of the project. The majority of the genebanks have now safety duplicated their *Brassica* collections in other safe places.

#### Recommendations for collecting activities made

All subgroups have made recommendations for future collecting in order to fill gaps in the *Brassica* collections.

### E. Dissemination of information

The project co-ordinator submitted 5 progress reports to the European Commission. The results of the project were disseminated by means of a workshop, open for interested researchers and breeders in 2003. The Bras-EDB is on line searchable and downloadable from the Internet. Several project-partners published and presented papers dealing with the research conducted in the framework of the project.

### Acknowledgement

The work of this GENRES CT99 109-112 project has been supported by the European Commission in the framework of Council Regulation (EC) No 1467/94.

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### Annex I. Participants in the RESGEN CT99 109-112 project

(*Brassica* Collections for Broadening Agricultural Use, including Characterising and Utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop).

- P1 Centre for Genetic Resources, the Netherlands, Wageningen, The Netherlands.
- P2 Genetic Resources Unit, Horticulture Research International, Wellesbourne, UK.
- P3 Nordic Gene Bank, Alnarp, Sweden.
- P4 Institute National de la Recherche Agronomique, Rennes, France.
- P5 Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany.
- P6 National Agricultural Research Foundation, Agricultural Research Center of Makedonia and Thraki, Greek Gene Bank, Thessaloniki, Greece.
- P7 Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA) Università degli Studi di Catania, Italy
- P8 Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal.
- P9 Consejo Superior de Investigaciones Cientificas, Mision Biologica de Galicia, Pontevedra, Spain.
- P10 Federal Centre for Breeding Research on Cultivated Plants, Gene Bank, Braunschweig, Germany.
- P11 Norddeutsche Pflanzenzucht Hans Georg Lembke KG, Hohenlieth, Holtsee, Germany.
- P12 Deutsche Saatveredelung Lippstadt - Bremen GmbH, Lippstadt, Germany.
- P13 Universidad Politecnica Valencia, Valencia, Spain
- P14 Plantum NL, representing 7 breeding firms, Gouda, The Netherlands.
- P15 Consejo Superior de Investigaciones Cientificas, Instituto de Agricultura Sostenible, Cordoba, Spain.
- P16 John Innes Centre, Norwich, UK.



# EUCARPIA CRUCIFERAE NEWSLETTER Nr. 26

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Next Cruciferae Newsletter Nr 26 will be produced and edited at the beginning of year 2004. The editing group of Rennes will take charge but, as previously, it will be produced by direct photocopying the material you submit. Therefore, we should be grateful if you would, please, follow instructions below, from which depend the quality of your script.

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