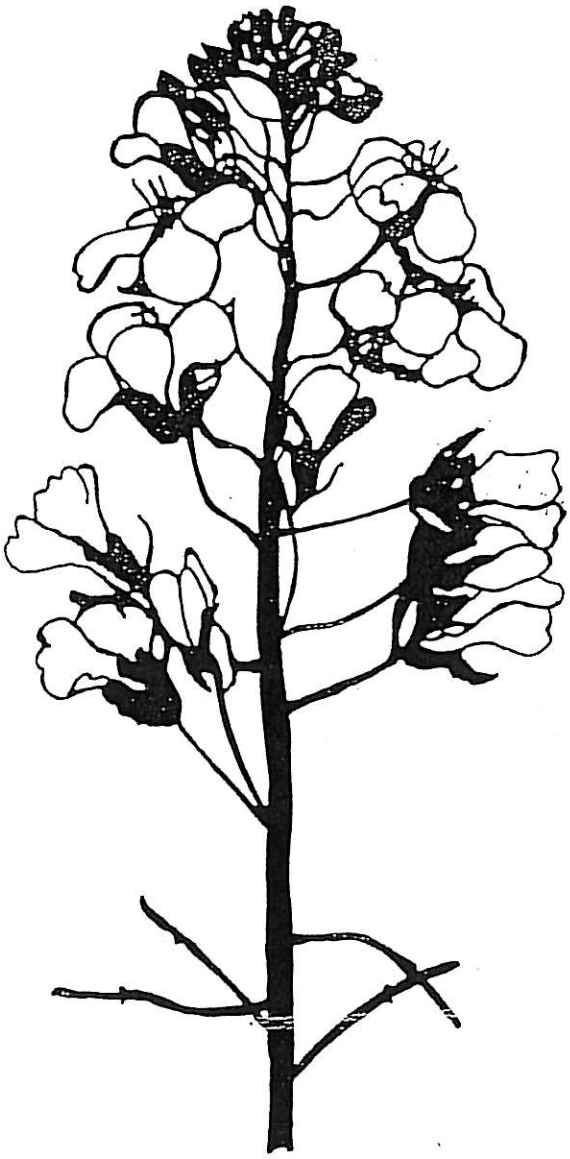
CRUCIFERAE NEWSLETTER



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Study of flavonoid patterns in some species of *Erucastrum* (*Brassicinae*)

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Introduction

The genus *Erucastrum* Presl, include about 20 species mainly distributed in the western Mediterranean region, though also some one (*E. strigosum*) is found in S. Africa. Although the genus has no economic importance, however it is one of the nearest wild relatives of the economically important genus *Brassica*. Therefore, *Erucastrum* constitutes a potential genetic resource for use in plant breeding programs.

Present work, continuation of a previous paper (Sánchez-Yélamo, 2000), represents the comparative study of the chromatographic patterns of *Erucastrum* species.

Materials and Methods

Plant material, cultivated in the greenhouse, was obtained from seeds that had been collected from their natural habitats and stored under long-term preservation conditions (Gómez-Campo, 1990) at the germplasm bank of the Departamento de Biología Vegetal, Escuela Técnica Superior de Ingenieros Agrónomos de Madrid (Table 1).
Botanical nomenclature is based on Greuter *et al.* (1986).

Table 1. Plant material

TAXON	Gametic number
E. varium (Eva)	7
E. virgatum (Evi)	7
E. strigosum (Est)	8
E. littoreum subsp. *glabrum* (Elg)	8 (2x)
E. littoreum subsp. *littoreum* (Ell)	16 (4x)
E. littoreum subsp. *brachycarpum* (Elb)	24 (6x)
E. elatum (Eel)	15

Phenolic compounds were extracted, from leaves, and isolated by two-dimensional paper chromatography (2D-PC) following Sánchez-Yélamo (1994, 2000). Compounds were identified using standard procedures by one-dimensional paper chromatography (1D PC) and thin layer chromatography (TLC) in comparison with authentic markers (Markham, 1982, Harborne, 1988,1989). The identification of sugars was carried out by TLC on pretreated Silica gel plates following Hansen (1975). With data of presence/absence of spots, a data matrix was elaborated and treated by NTSYS computer programs. The similarity matrix was employed to construct the dendrogram.

Results and discussion

A total of 18 flavonoids were isolated from the foliar extracts of the taxa surveyed. The compounds were glycosides derivatives of kaempferol and quercetin. Table 2 indicate the identified molecules which chromatographic characteristics and UV spectral data were reported in the mentioned previous paper (see Sánchez-Yélamo, 2000). Figure 1 represent the composite chromatograms (2D PC) of taxa showing the mobilities in the two chromatographic solvent used. The distribution of flavonoids glycosides of all taxa studied are also indicated in Table 2.

Each taxon shows a characteristic chromatographic pattern and some differences are detected among them, even the most related species as are the euploid series of *E. littoreum* subsp. *glabrum* (n=8), *E. littoreum* subsp. *littoreum* (n=16) and *E. littoreum* subsp. *brachycarpum* (n= 24). *E. elatum* was the richest of the group, showing twelve molecules. All taxa of *Erucastrum* share only the compound no 12 (kaempferol 3-galactoside-7 rhamnoside) and the specie-specific molecules were the glycosides 1, 2, 6, 11 and 23; the rest of compounds are shared by two or more taxa. The resulting similarity values among taxa can be interpreted as their relationships are very close, including *E. strigosum* that is a taxon with a remote distribution (S. Africa) from the others that are mediterranean.

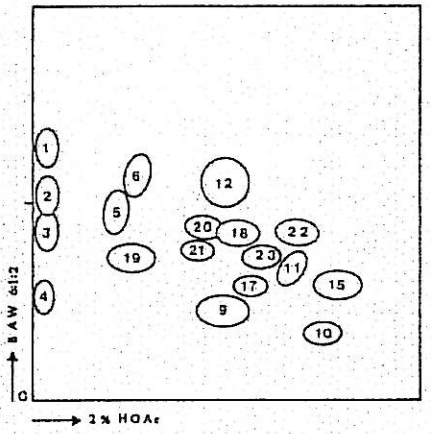

Fig. 1 Composite chromatogram of all studied.taxa.
BAW=BuOH-HOAc-H_2 O

Table 2. Flavonoids identified in studied taxa (*)

	Compound	Eva	Evi	Est	Elg	Ell	Elb	Eel
1	K 7-Gal	+	-	-	-	-	-	-
2	Q 7-Gal	+	-	-	-	-	-	-
3	K 7-Glc	-	+	+	+	+	+	+
4	Q 7-Glc	-	+	+	+	+	+	+
5	Q 3-Glc	-	+	+	+	+	+	+
6	K 3-Glc	-	+	-	-	-	-	-
9	Q 3-Digal	-	-	+	+	-	-	+
10	K Trigly	-	-	+	-	-	-	+
11	K 3-Digal	-	-	-	-	-	-	+
12	K 3-Gal-7-Rha	+	+	+	+	+	+	+
15	K Trigly (Rha+Gal)	-	-	+	+	+	+	+
17	K 7-Gal-3-Digal	-	+	-	-	-	+	-
18	Q 3-Glc-7-Rha	+	+	-	-	+	+	+
19	Q 3-Diglc	+	+	-	+	-	-	+
20	K (3,7)(Rha+Gal)	-	+	-	+	-	-	-
21	Q (3,7)(Rha+Gal)	-	+	+	-	-	-	+
22	K Trigly (Rha+Glc)	-	+	+	-	-	-	+
23	Q Trigly (Rha+Glc)	-	-	-	-	-	-	+

K= kaempferol; Q= quercetin; Glc= glucose; Gal= galactose
Rha= rhamnose; -gly: glycoside;
(+) presence (-) absence. (* For Abbreviations see Table 1)

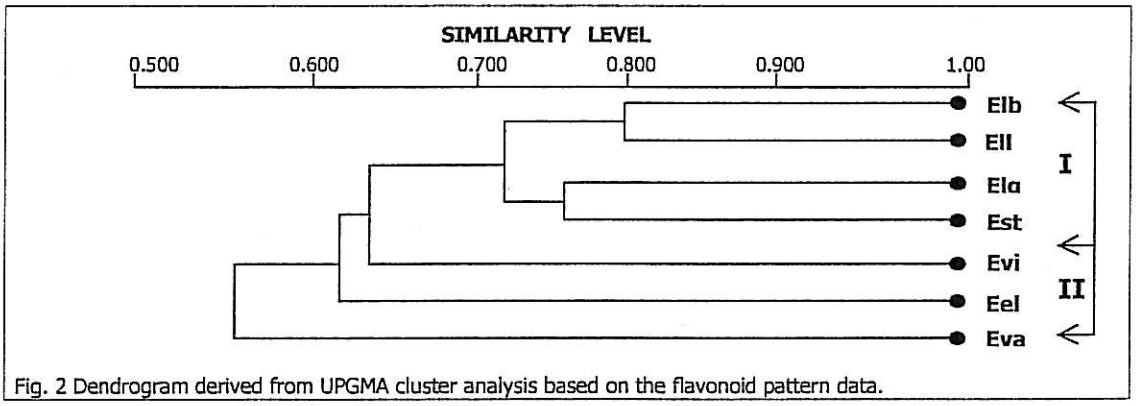

Fig. 2 Dendrogram derived from UPGMA cluster analysis based on the flavonoid pattern data.

The dendrogram shows two groups of samples: group I constituted by the n=8 taxa (*E. littoreum* subsp. *glabrum* and *E. strigosum*) and by the polyploids of the first. The group II is formed by the rest of the taxa. On the basis of present results, and considering that polyploids could be a evolutive consequence in the speciation process, *E. littoreum* subsp. *glabrum* and *E. strigosum* migth be primitive taxa, being *E. varium* the more evolved one of the studied group.

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Variability & relationship in quality parameters of Brassica and related species

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The emphasis is being laid upon improvement in seed size, colour, fiber content, oil content and quality of oilcake, because these character are directly associated with nutritional and market value of produce and product. In developing varieties with low fiber, high protein, high oil yield and yellow seed, bold seeds, the information about characters are of prime importance in deciding breeding strategies. Therefore, present study was conducted to observe relationships between different quality attributes.

Materials and methods:

Twenty one genotypes of Brassica and related species viz. *B.campestris* var toria (6), and var. yellow sarson (2); *B.juncea* (7), *B.napus* (2) *B.carinata*, *B.nigra*, Sinapis alba and *Eruca sativa* (one each) were grown in two replication in randomized block design at Crop Research Centre, Pantnagar, India, during rabi 1998-1999. Harvested seeds were analyzed for 1000-seed weight by using numerical seed counter, for oil content by Newport analyzer -4000 (NMR), for protein content by Micro kjeldhal, and for fiber content by AOAC (1984). Data recorded for above quality parameters were subjected to correlation analysis following Searle (1961). Variablity parameter were calculated as suggested by Burton and Davane (1953) genetic advance under selection were estimated Lush (1949).

Results and Discussion:

Significant variation was observed for all parameter except 1000 seed weight (Table 1). Widest range was recorded for glucosinolates (19.95-166.05 μ mole/g fat free meal followed by oil (20.78-44.095) and protein (33.55-44.70%) content. Genotypic coefficient of variation were almost equal to phenotypic coefficient of variation all characters, suggesting that environment had no major influence on them. Highest (PCV as well asGCV) were recorded for glucosinolates followed by oil content and test weight. The estimates of genetic advance for glucosinolate suggested that the selection pressure applied in desired direction may lead to improvement. Similar results were found by Ahuja et al (1989) Singh et al., (1991) and Baetzel et al., (2000).

Table 1 : Variability, range, phenolyhic (PCV) and genotypic (GCV) coefficients of variation, and genetic advance for quality parameters of Brassica and related species.

Character	Mean square	Mean ± SE	Range	PCV	GCV	Genetic advance
1000-seed weight (g)	0.428	3.539±0.062	2.39-4.48	13.14	13.02	0.94
oilcontent (%)	57.126**	39.021±0.161	20.78-44.09	13.70	13.69	11.0
Protein content (%)	16.432**	39.083±0.214	33.55-44.70	7.34	7.32	5.88
fiber content (%)	5.504**	13.073±0.110	10.88-19.05	12.70	12.68	63.41
glucosinolate content (μmole/g)	1991.819**	119.145±0.42	19.95-166.05	26.49	29.40	65.00

** Significant at 1% level.

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Correlation analysis is presented in (Table-2). 1000 seed weight had no significant relationship with other quality parameters. It was observed that oil content is negatively associated with fiber, protein and glucosinolate content. These results revealed that the increase in oil content will result in the reduce protein, fiber and glucosinolates content in meal. Protein was significantly and negatively associated with oil (-.0409). Fiber was significantly and negatively correlated with oil (-0.724) and positively associated with glucosinolates. It indicates that reduction in fiber also reduces glucosinolates. Thus these two character may be improved simultaneously These findings were in accordance with those reported earlier by HU (1988), Singh et al., (1991) and Baetzel, et.al.(2000)

Table 2: Association among quality traits in Brassica and related species.

Character		1000-seed weight (g)	Oil content (%)	Protein content (%)	Fiber Content (%)	Glucosinolates content (µmol/g)
1000-seed weight (g)	P	1.000	0.149	0.174	0.122	-0.071
	G	1.000	0.149	0.174	0.123	-0.071
Oil content(%)	P		1.000	-0.408**	-0.722**	-0.314*
	G		1.000	-0.409**	-0.724**	-0.314*
Protein content (%)	P			1.000	0.017	0.000
	G			1.000	0.018	0.000
Fiber content (%)	P				1.000	0.255*
	G				1.000	0.255*
Glucosinolates content (µmol/g	P					1.000
	G					1.000

*** Significant at 5% level, ** Significant at 1% level.**

Oilcontent was negatively correlated with protein, fiber and glucosinolates suggesting that reduction in protein, fiber and glucosinolates would be increase by optimising the oil content. Results of this study revealed that the development of varieties with high oil content low fiber and protein.

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Correlation among fatty acids of Brassica and related species

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Improvement in the Nutritional quality of rapeseed-mustard oil, is important because it is only source of two essential fatty acids (linoleic and linolenic) for human body. Its use as oil for human is restricted due to high amount of erucic acid in oil. In the development of varieties with low erucic acid, the information about associations between different fatty acids is of prime importance in deciding breeding strategies. Therefore, present study was conducted to elucidate relationship between different fatty acids.

Materials and methods:

Twenty one genotypes belonging to *B.Campestris var toria* (6) and yellow sarson (2), *B.juncea* (7), *B.napus* (2), *B.carinate*, *B.nigra*, *Sinapis alba* and *Eruca sativa*. (One each) were planted in two replications in randomized block design at Crop Research Centre, Pantnagar, INDIA, during rabi 1998-1999. Harvested seeds of 21 genotypes of different Brassica and related species were subjected to fatty acid determination by gas liquid chromatograph (GLC) after converting different fatty acid into their methylesters (Luddy et al. 1968) in the oilseed quality laboratory in the department. Data recorded for seven fatty acids subjected to correlation analysis (Searle 1961).

Results and Discussion:

Results of correlation analysis are presented in Table-1. It was observed that palmitic acid was significantly and positive correlated with oleic, linoleic, linolenic, and ecosinoic acid where as negatively correlated with erucic acid. Stearic acid was positively associated with oleic acid however negatively correlated with ecosinoic and erucic acid. Oleic acid was positively correlated with linoleic acid while negatively correlated with ecosinoic acid and erucic acid; Association of linoleic acid and linolenic acid was found positive. Erucic acid was negatively associated with all fatty acid except ecosinoic acid. These finding were in accordance with those reported earlier by Ahuja et al., (1989) Singh et al (1991), Hu, ZL (1988), Tang, Z. J. And Liu, R.J (1991) Erucic acid was negatively correlated with all the other fatty acids suggesting that reduction of erucic acid would be possible by incrasing other fatty acids viz. Palmitic, stearic, oleic, linoleic and linolenic. Breeder should apply selection pressure for low erucic acid in the breeding programme.

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Table 1: Association among fatty acids in Brassica and related species.

Character		Palmatic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Ecosinoic acid	Erucic acid
Palmitic acid	P	1.000	0.057	0.426**	0.565**	0.476**	0.284*	-0.452**
	G	1.000	0.057	0.427**	0.566**	0.477**	0.285*	-0.452**
Stearic acid	P		1.000	0.672**	0.153	-0.096	-0.413**	-0.741**
	G		1.000	0.671**	0.153	-0.095	-0.413**	-0.740**
Oleic acid	P			1.000	0.313**	0.062	-0.339**	-0.876**
	G			1.000	0.314**	-0.062	-0.340**	-0.876**
Linoleic acid	P				1.000	0.351**	-0.140	-0.461**
	G				1.000	0.352**	-0.140	-0.461**
Linolenic acid	P					1.000	-0.084	-0.238
	G					1.000	-0.084	-0.238
Ecosinoic acid	P						1.000	0.374**
	G						1.000	0.374**
Erucic acid	P							1.000
	G							1.000

Reference

Ahuja, K.L., Botta, S.K., Raheja, R.K., Labana, K.S., and Gupta, M.L. (1989). Plant Foods for Human Nutrition. 39: 155-160.

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ELECTRONIC GUIDE TO WILD GERMPLASM OF BRASSICA AND ALLIED CROPS (TRIBE BRASSICEAE, BRASSICACEAE) 2ND EDITION

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Highlights of the Guide:

This free electronic publication provides an update to the 1993-94 "Guide to the wild germplasm of *Brassica* and allied crops (Tribe Brassiceae, family Cruciferae) Parts I to V". Economically, the genera *Brassica, Crambe, Eruca, Raphanus* and *Sinapis* display enormous diversity and are used as a source of oil, vegetables, mustard condiments, and fodder. Weed and wild relatives in the tribe represent a potential source of genes, e.g. resistance to disease and insect pests and tolerance of cold, salt and drought conditions. Other species have potential value as industrial oils (*Crambe, Eruca*), value-added products, or as host systems for molecular farming. An understanding of the genetic potential of wild relatives in the Tribe Brassiceae is critical for the establishment of long-term breeding programs of these crops.

Edition 2 of the Guide has been expanded to include taxonomic, geographical and ecological data on an additional 18 species, providing information on a total of 235 species in 49 genera of the Tribe Brassiceae. The section on chromosome numbers has been expanded to include information on all original citations for both wild and crop species, and includes 380 references with data on over 1400 chromosome reports. The section on inter-specific and inter-generic hybridization includes data from 240 original references, with the addition of 144 new references. The information provided in this guide is intended to be useful in providing direction for future genebank needs for these crops and for assisting biotechnologists and plant breeders in the utilization of these genetic resources in their research programs. It is also an extremely valuable resource for regulators concerned with the possibility of gene flow between transgenic cruciferous crops and wild relatives not only in Canada but on a global basis.

ELECTRONIC ADDRESS: http://res2.agr.ca/ecorc/cwmt/tech.htm

Edition 2 of the Guide has been divided into nine separate PDF files which can be downloaded and printed separately using an Acrobat reader version 4

1. Introduction
2. Taxonomic Checklist
3. References to Parts I and V
4. Chromosome Numbers
5. References to Part II
6. Interspecific and Intergeneric Hybridization Data
7. References to Part III
8. Wild Species as Sources of Agronomic Traits
9. References to Part IV
10. Geographical, Life History and Ecological Data
11. References to Parts I and V

Examples of the contents of each of Parts I to V are shown below:

PART I: TAXONOMIC CHECKLIST

ACC	SYNONYM OF	TAXON
Y		***Ammosperma cinerea*** (Desf.) Hook. f.; Benth. & Hook. f., Gen. Pl. 1: 82. (1862).
Y		***Ammosperma variabile*** Nègre & Le Houér.; Bull. Soc. Bot. France 106:149.(1959).
N	*Vella aspera*	*Boleum asperum* (Pers.) Desv.; J. Bot. Agric. 3: 163. (1815).
N	*Hirschfeldia incana*	*Brassica adpressa* Boiss.; Voy. Bot. Midi Espagne 2: 38. (1839).

PART II: CHROMOSOME NUMBERS

TAXON [Name Reported as]	n	2n	REFERENCE
Brassica balearica Pers.	-	18	Manton (1932)
Brassica balearica	-	32	Dahlgren et al. (1971)
Brassica balearica	16	-	Salmeen (1979)
Brassica balearica	18	36	Cardona & Contandriopoulos (1980)
Brassica balearica	18	-	Snogerup & Persson (1983)
Brassica balearica	16	32	Cardona (1991)

PART III. INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION DATA

TAXON	PARENTAL COMB. (♀ x ♂)	CROSS TYPE	REFERENCE
Arabidopsis thaliana [n= 5]	*Brassica napus* + *A. thaliana*	PROT	Forsberg et al. (1994)
Brassica barrelieri [n= 10]	*B. barrelieri* x *Brassica fruticulosa*	SEXL	Takahata & Hinata (1983)

PART IV: WILD CRUCIFER SPECIES AS SOURCES OF AGRONOMIC TRAITS

1. **North American Germplasm**
2. **Morphological Characters**
3. **Genome Arrangement**
4. **Chemical Traits**
5. **Photosynthesis**
6. **Breeding System/Cytoplasmic Male Sterility**
7. **Edaphic and Aquatic Adaptation**
8. **Cold Tolerance**
9. **Drought Tolerance**
10. **Herbicide Resistance**
11. **Disease Resistance**
12. **Insect Resistance**
13. **Nematode Resistance**
14. **Crop Diversification**

PART V. GEOGRAPHICAL, LIFE HISTORY AND ECOLOGICAL DATA

Species:	***Ammosperma cinerea*** **(Desf.) Hook. f.**
Life/Form:	annual
Ecology:	desert plains and hills; dry steppes, pastures and stream beds, damp sandy places
Geography:	Africa: nc & c Algeria, n Libya, s Tunisia
Phytogeo:	Saharo-Sindian

MOLECULAR CHARACTERIZATION OF GENETIC RELATIONSHIPS IN *BRASSICA RAPA* BASED ON RAPD MARKERS

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Introduction

Brassica rapa (n = 10, Genome AA) is a very polymorphic crop species domesticated in both Europe and Asia for edible oil, industrial oil, vegetable, and fodder use. Such varied use and corresponding morphological variability has led to a high number of infraspecific taxa (subspecies, varieties and cultivar groups). The last current authoritative treatment is by Hanelt in Schultze-Motel (1986) [Update 2000]. Hybridization studies by Olsson in 1954 showed that *B. rapa*, *B. campestris* and Asian n=10 taxa *B. chinensis*, *B. dichotoma*, *B. narinosa*, *B. nipposinica*, *B. pekinensis*, and *B. trilocularis* were fully interfertile and latter names were recombined as subsp. of *B. campestris*. Hanelt (1986) recombined these names as subspecies of *B. rapa* [7-8 subsp.], defined on basis of morphology, use and area of cultivation. Subspecific rank of n =10 cultivated species: *B. dubiosa*, *B. perviridis*, *B. purpuraria* and *B. ruvo*, *B. septiceps* is still not resolved. The objective of our study was to use RAPDs (random amplified polymorphic DNA) markers to test the validity and genetic relatedness of the infraspecific categories in *B. rapa*, and in particular to test the affliation of three previously unassigned species within the *B. rapa* genome.

Materials and Methods

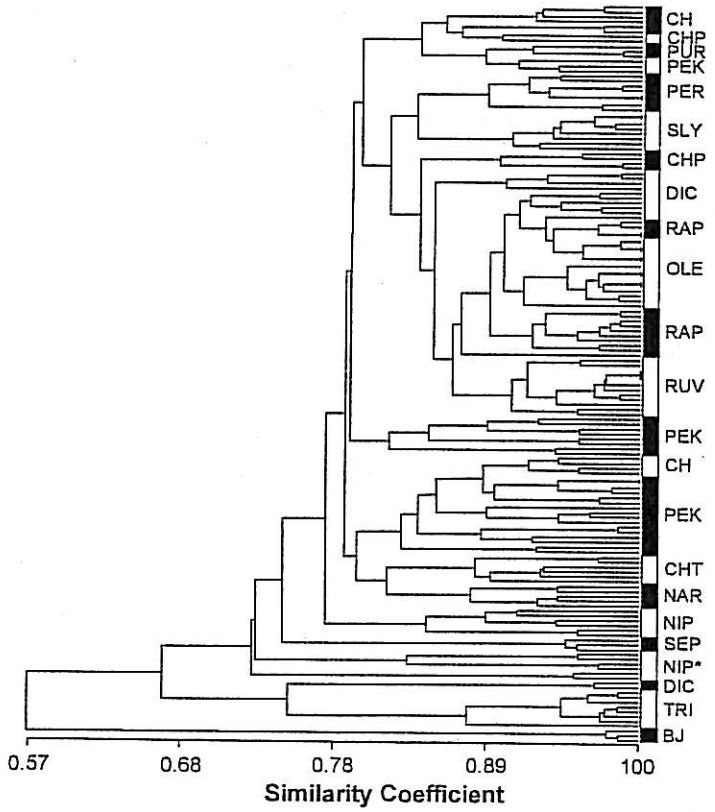
Taxonomic framework - 68 accessions of the *Brassica rapa* cytodeme were included [see Fig. 1 footnote]. *B. juncea* was included as the outgroup. DNA extraction - DNA was extracted from 2 individuals per accession. Frozen tissue (ca. 0.1 g) were ground, suspended in 400 μL extraction buffer (200mM Tris-HCl pH 7.5, 250mM NaCl, 25mM EDTA, 0.5% SDS), and centrifuged. The pellet was dried and resuspended in 100 μL Tris EDTA buffer. Random amplified polymorphic DNAs (RAPD) - Seven 10-oligomer primers from the Univ. British Columbia (Nos. 134, 153, 174, 181, 184, 185, 304) were used. Amplification reactions contained 3.8μL H_2O, 2.5 μL of 10 X PCR buffer + Mg, 4.0μL of 1.25mM dNTP, 1μL of 10pmol primers, 11.5μL of genomic DNA [1 μL of DNA to 10.5 of deionized water], 0.2μL of Taq DNA polymerase at 5 units/μL. The PCR reactions were 45 cycles (94°C for 15 sec, 32°C for 30 sec and 72°C for 2 min), followed by 72°C (10 min). The PCR products were run on a 1.2% agarose gel in TBE running buffer at 80 volts for 4-5 hr. Gels were stained with 25 μL of ethidium bromide and photographed under UV light. Data analysis - Presence/ absence of 70 polymorphic RAPD markers were scored. Pairwise genetic similarity measures among accessions were obtained with NTSYSpc, version 2.00, option SIMQUAL and SM coefficient and dendrograms generated using the UPGMA clustering procedure, option SAHN.

Results and Discussion (Fig.1)

Eight of the taxa formed single clusters: Narinosa, Perviridis, Purpuraria, Septiceps, Ruvo, Sylvestris, Taitsai, and Trilocularis. The accessions did not separate clearly into European and Asian groups. European subcluster included Oleifera, Rapa, Ruvo as expected, and 4 of 5 accessions of Dichotoma. Wild accessions Sylvestris formed a separate cluster from Oleifera, and did not support its treatment as a synonym of ssp. oleifera. Rapa and Oleifera did not form separate groups, instead clustered closely together supporting the close relationship suggested by several researchers. Asian oilseed, Dichotoma was split between two groups, supporting both a European and Asian derivation. Most accessions clustered with the European Oleifera/Rapa group and one accession grouped with Trilocularis, supporting derivation of Trilocularis from Dichotoma. Asian vegetable groups showed the greatest variability. Chinensis [sensu lato] was the most variable(s) with accessions in several clusters, which is consistent with morphological diversity described for the group. Parachinensis was divided among two clusters. One accession clustered with Chinensis accessions and two others

formed a separate cluster. Narinosa and Taitsai, both morphologically similar, formed a separate cluster from Chinensis. Pekinensis was divided within three clusters suggesting multiple origins. Two of which also contained accessions of Chinensis. There was no evidence to support hybrid origin with Rapa and Chinensis. Nipposinica separated in three independent clusters. Nipposinica is a leaf vegetable cultivated in Japan and China, although var. utilis [NIP 4-5], is grown for oil seed production in China. RAPDs data supports a separate origin of this Asian oil type [marked with asterisk in Fig. 1]. Perviridis formed aseparate cluster with Sylvestris and data did not support its proposed affiliation with Nipposinica. Pururaria, a Chinese vegetable with limited cultivation, grouped with two accessions of Chinensis, Parachinensis and PEK2 accession, partially supporting suggestion of its affiliation with Chinensis. Septiceps, cultivated in the eastern USA as a vegetable, and a proposed affiliate of Rapa, formed a distinct group and its genetic relationship to European or Asian groups could not be resolved.

Fig. 1. Dendrogram based on UPGMA analysis



EUROPEAN - OILSEEDS: **OLE**: Oleifera, Polish rape; **VEGETABLES**: **RAP**: Rapa, Rapifera, turnip; **RUV**: Ruvo, broccoletto; **SEP**: Septiceps, seven-top turnip. **ASIAN -OILSEEDS**: **DIC**: Dichotoma, brown sarson; **TRI**: Trilocularis, yellow sarson; **VEGETABLES**: **CH**: Chinensis, Pak Choi; **CHP**: Parachinensis; **CHT**: Taitsai, Rosularis; **NAR**: Narinosa, broad-beaked mustard; **PUR**: Purpuraria; **NIP**: Nipposinica, Mizuna; **PER**: Perviridis, Spinach mustard; **PEK**: Pekinensis, Chinese cabbage. **WILD - SLY**: Sylvestris.

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GENETIC DIVERSITY IN *BRASSICA CARINATA*, *B. JUNCEA* AND *B. NIGRA* BASED ON MOLECULAR AFLP MARKERS

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Introduction

Cultivated species *Brassica carinata* ($n = 17$, BBCC) and *B. juncea* ($n = 18$, AABB) are amphidiploids derived from diploid taxa, *B. rapa* ($n = 10$, AA), *B. nigra* ($n = 8$, genome BB), and *B. oleracea* ($n = 9$, genome CC). *Brassica carinata* is primarily an oilseed crop believed to have originated in the Ethiopian plateau. There is little differentiation into various crop types and genetic levels are predicted to be low. *Brassica juncea*, Indian or brown mustard is grown in North America and Europe for condiment use, on the Indian subcontinent for seed oil and the Far East as a vegetable. The species is very variable, and includes oleiferous, semi-oleiferous, rapiferous and leafy types. Genetic diversity is expected to be higher than in *B. carinata*, and multiple origins likely. The objectives of the study were two-fold: 1) to develop AFLP [amplified fragment length polymorphism] methodology for *Brassica* crop species, including screening of primer pair combinations to select primers with a high relative multiplex ratio and comparison of manual and automated (LICOR) techniques; and 2) to evaluate the utility of AFLPs for estimating levels of genetic diversity among *Brassica* crop species, particularly *B. carinata*, *B. juncea*, and *B. nigra*.

Materials and methods

Plant material - Plant material from 75, 28 and 7 accessions of *Brassica carinata* , *B. juncea* , and *B. nigra* were included. DNA extraction and AFLP analysis - AFLP method is described extensively by Vos et al. (1995). DNA was extracted from frozen leaf material according to a modified CTAB procedure (Doyle and Doyle 1987). 500 ng of genomic DNA was digested with *EcoR*I and *Mse* I restriction enzymes. Adapters of known sequences were ligated to the ends of restricted fragments using T4 DNA ligase (Promega). A preamplification PCR was carried out using *EcoR* I and *Mse* I universal primers each containing an additional selective nucleotide. PCR products were used as template for the second round of amplification using *EcoR* I and *Mse* I selective primers containing 2 to 3 selective nucleotides. *EcoR* I selective primer were labelled with either 33P or IRD-700 for autoradiography and infra-red detection on LI-COR machine, respectively. Both manual (radiolabelled) and automated (LI-COR) AFLP methods were efficient for Brassica genotyping. The IRD-based automated AFLP method was found to be faster and eliminated the need for radioactive material, but showed lower resolution for high molecular weight bands compared to the manual method. Data acquisition and diversity estimation - Autoradiographs and IRD-based gel images were scored based on the presence or absence of bands at polymorphic loci. The data matrix was used to generate a genetic similarity matrix with SIMQUAL routine based on DICE similarity coefficient from the NTSYS-pc statistical package (Rohlf, 1990). Clustering was performed using subroutine SAHN based on the genetic similarity matrix with the UPGMA clustering method. A taxonomic identification key [not shown] was constructed to key out *B. carinata* accessions using DELTA program (Dallwitz et al., 1993).

Results and discussion

An average of 100 loci were assayed simultaneously with each primer pair and DNA markers falling in the range of 50 bp to 500 bp were considered for analysis. A subset of 7 primer pairs, was selected from an initial screening of 30 primer pair combinations. A total of 121 AFLP polymorphic markers were obtained using 7 primer pairs, with an average of 17 polymorphic markers observed per primer pair (Table 1). UPGMA analyses of the data indicated three distinct clusters at the phenon line of 0.75 on the genetic similarity scale corresponding to the three taxa [Fig. 1]. Two main subclusters were evident for

B. juncea, primarily separating vegetable versus seed/oil use. No major subclustering was detected for *B. carinata* or *B. nigra*. Mean within-species genetic diversity was 0.22, 0.21, and 0.14 for *B. juncea*, *B. nigra*, and *B. carinata*, respectively (Fig. 2). Measures of genetic diversity for *B. juncea* and *B. nigra* should be considered preliminary, and are most likely underestimates of diversity due to smaller accession number sampled. An AFLP-based taxonomic key based on 17 markers was sufficient to distinguish each of the 75 accessions of *B. carinata*.

Fig. 1. Dendrogram based on UPGMA analysis.

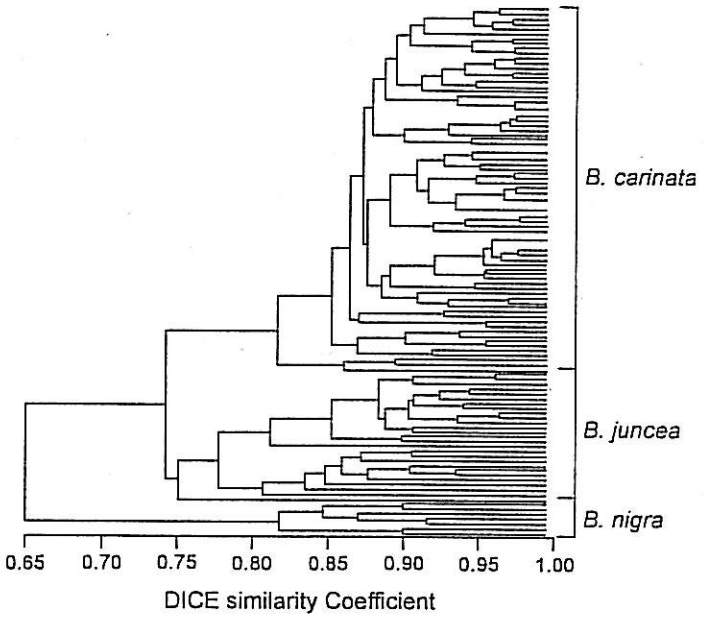
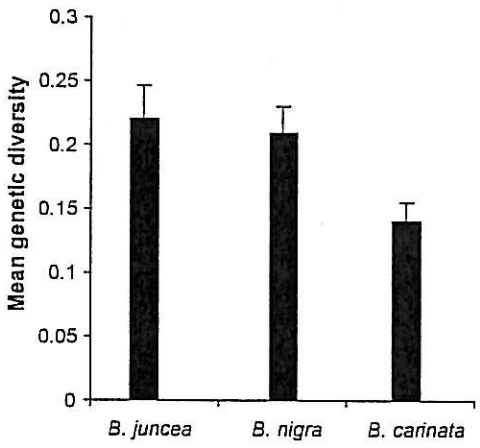


TABLE 1. AFLP primer pairs, number of loci assayed and number of polymorphic markers

Primer pairs	No. loci	No. polymorphic markers
EAAC/MCAG	81	17
EAAC/MCAA	125	25
EAAC/MCAT	76	14
*EAG/MCTA	152	24
EAGG/MCTA	105	15
EAGG/MCAC	98	19
*EAG/MCAA	42	7
Total: 7	679	121

* *EcoR* I primer with two selective nucleotides

Fig. 2. Comparison of mean genetic diversity level among three species of *Brassica*



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Intergeneric hybridization between *Brassica juncea* and *Erucasturum virgatum* and the meiotic behavior of F_1 hybrids

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Wild species of cultivated relatives are often variable source of important genes in crop improvement. It is also important to study the phylogenetic relationship of the genomes between cultivated plants and the wild relatives. Many intergeneric and interspecific hybrids showed the high bivalent chromosome associations in the first meiotic division of the F_1 hybrids (Harberd and McArther, 1980; Inomata 1997). Genetic recombination might occur between cultivated brassica and the wild relatives, and the possibility of gene transfer to crop brassica might be possible. This study produced intergeneric hybirds between *Brassica juncea* and *Erucastrum virgatum*, and showed the behavior of meiotic chromosomes in the F_1 hybrid. The crossability of the progenies was further investigated.

The materials used in the experiment were *Brassica juncea* ($2n$=36) and *Erucastrum virgatum* ($2n$=14), which seed was provided by Gómez-Campo. When emasculated flowers of *B. juncea* bloomed, the conventional cross with the pollen of *E. virgatum* was made. Ovary culture of the F_1 hybrid was carried out according to the previous paper (Inomata, 1990). Somatic chromosomes and chromosome associations in the first meiotic division were checked by using the method of Inomata (1994).

Ninety-three ovaries were cultured in the Murashige and Skoog's medium (1962) with 300mg/*l* of casein hydrolysate. Seventy-two embryos which were late torpedo- and walking stick-shaped were picked up in 36 days after ovary culture and moreover, the embryo culture was carried out in the half of Murashige and Skoog's medium with 300 mg/*l* of casein hydrolysate. Two plants grow up and they matured in the field. Other one hybrid was obtained by leaving the pollinated flowers as a control. It seems that this hybrid is important to the standpoint of evolution among the tribe *Brassiceae*. Three F_1 hybrids showed 25 somatic chromosomes that were dihaploid. The leaves of the F_1 hybrids were intermediate in morphology between the parents. Table 1 shows the results for pollen fertility and chromosome associations in the first meiotic division. No pollen fertility was observed. The type of chromosome associations in the first meiotic division of the three F_1 hybrids resembled each other. The mode in the first meiotic division was $4_{II}+17_{I}$. The chromosome associations showed $(0\text{-}1)_{III}+(1\text{-}8)_{II}+(9\text{-}23)_{I}$. High homology of chromosomes existed between the parents. Table 2 shows the results for different types of division in microspore mother cells of the F_1 hybrids. The range was from monad to hexad. The mode was dyad. Most microspore division was not uniform in triad, pentad and hexad. Though about 40% of pollen tetrad were observed, there was no pollen fertility. Self-polination and

open pollination of the F_1 hybrids were made in 242 and 1917 flowers, respectively, and no seeds were obtained. No seeds were also obtained from 483 flowers, which were backcrossed with *B. juncea*. If the diplodization of these hybrids was made by colchicine, the seeds of the progenies might be produced.

Acknowledgment

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

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Table 1. Pollen fertility and chromosome associations in the first meiotic division of the F_1 hybrids of *Brassica juncea* x *Erucastrum virgatum*

Plant number	Chromosome number in root tip	Pollen fertility (%)[a]	Number of PMCs observed	Mean chromosome associations per cell at metaphase I (range in parenthesis)		
				III	II	I
1	25	0	34	0 (-)	3.4 (1~7)	17.5 (11~23)
2	25	0	33	0 (-)	3.8 (1~6)	16.9 (13~23)
3	25	0	33	1 (0~1)	5.5 (3~8)	13.8 (9~19)
Total or range		0	100	0.01 (0~1)	4.3 (1~8)	16.2 (9~23)

[a]: 500 pollen grains were counted.

Table 2. Different types of division in microspore mother cells of the F_1hybrids in *Brassica juncea* x *Erucastrum virgatum*

Plant number	Different types of division in microspore mother cell						
	Monad	Dyad	Triad	Tetrad	Pentad	Hexad	Total
1	74	106	24	95	5	0	304
2	2	188	6	174	2	0	375
3	4	240	12	161	0	1	418
Total	80	534	42	430	7	1	1094

Original

A Study on Genome Separation in Rapeseed

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Abstract

In the meiotic division of pollen mother cells (PMCs) of rapeseed (*Brassica napus* cv oro) the following chromosome behaviors, such as the separation and asynchronous division of two groups of chromosomes were observed. It is suggested that the above two groups of chromosomes are related respectively to "a" and "c" genomes .The study on genome separation and related chromorome behaviors of rapeseed may have some importance for the phylogenetic research and breeding work of this crop.

Key words: *Brassica napus*, meiotic division, asynchronous division, genome separation

Studies on chromosomes are important for the phylogenetic research and breeding work of plants. In rapeseed Guo (1979) described genome segregation in some artificial octoploid plants of rapeseed (*B. napus* cv shengli). In order to know whether similar chromosome behaviors are present in the natural rapeseed, the following experiments were carried out.

Materials and methods

A natural rapeseed cultivar oro (*Brassica napus* cv oro, n=38=20+18, aacc) was used in the experiment. Rapeseed seeds were gathered from the selfed plants cultivated in the experimental plots of Sichuan University. In the preparation of chromosome slides Carnoy's fluid (ethyl alcohol 3+ acetic acid 1) was used for fixation and carbol fuchsin solution was used for stainning.

Results

Cytological observations were made on meiotic divisions of some PCMs of rapeseed (*B. napus* cv oro). At early diplotene chromosomes were congregated into two groups in some cells (Fig.l). Later the asynchronous division of two groups of chromosomes was observed at diplotene and early diakinesis (Fig.2), where a group of 9 (sometimes 7) chromosomes condensed and became more visible a little earlier. Later two groups of chromosomes were observed , one with 9 (sometimes 7) chromosomes, the other with 10 (sometimes 12) ones. In this case the genome separation was observed.

Discussion

1. U (1935) suggested that rapeseed (*B. napus*, 2n=38, aacc) was derived from a natural cross between *B. campestris* (2n=20, aa) and *B. oleracea* (2n=18, cc), with 19 (10+9, ac) chromosomes in its gametes. Later Guo (1979) discribed genome segregation in some artificial octoploid plants of rapeseed (*B. napus* cv shengli). Now in our experiment the separation of two groups of chromosomes (mostly 10+9) was observed at diakinesis of some PMCs of the material rapeseed (*B. napus* cv oro, 2n=38=20+18, aacc). It is reasonably suggested that the above two groups of chromosomes are related respectively to "a" and "c" genomes.

2. The phenomenon of asynchronous division and separation of genomes is somewhat common in plants. Li et al. (1947) observed asynchronous division of genomes in hybrid plants of *Triticum timopheevi* × *Aegilops bicornis*. Recently Yin et al. (1998) described this similar phenomenon in hybrid plants of *B. alboglabra* x *Orychophragmus violaceus*. Schwarzacher et al. (1992) reported chromosome separation in

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hybrid plants of *Hordeum vulgare* x *H. bulbosum*. Later, Li et al. (1993) and Wu et al. (1996) observed respectively genome separation in hybrid plants of *Brassica* species x *O. violaceus*. It is suggested that the phenomena of asynchronous division and separation of genomes are somewhat common in hybrid plants and the study of genome separation might have some importance for the phylogenetic research and crop breeding of rapeseed.

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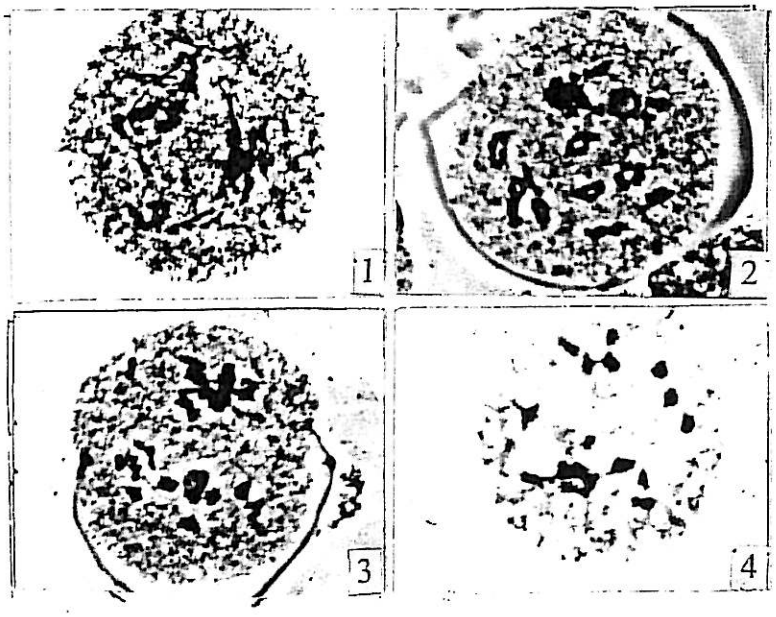

Plate: Phases of the meiotic division of some PCMs in rapeseed

Fig.1 Two groups of chromosomes at early diplotene

Fig2 Nine chromosomes appeared earlier at diplotene

Fig.3 Genome separation (9+10) at diakinesis

Fig.4 Genome separation (7+12) at diakinesis

Studies on Cytology of Visible Chromosome Formation under the Light Microscope during Cell Cycle in Rapeseed

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INTRODUCTION

In the present paper we report the feature on cytology of visible chromosome formation in rapeseed. These studies are important for cell cycle, micronucleus formation and mechanism of chromosomal banding.

MATERIALS AND METHODS

Material offering

There are cultivates Xianglongyou 2. Xiangyou 11, Xiangyou 13, Xiangyou 14 of *B. napus* from Hunan Agricultural University Changsha, China.

Chromosome preparation

Seeds were germinated on wet fiter in a petri dish at 25℃. Germinating seeds with roots 1.0—1.5 cm long were immersed in 0.002 M 8-hydroxyquinoline solution for 30 min at 25℃, then thoroughly washed in distilled water. The good root tips were excised and immersed them in 0.075 M KCl solution for 30 min at 25℃, and then macerated in 2.5% of mixture enzymatic solution of cellulase and pectinase for 3—4 h at 25℃.

The macerated roots were rinsed with distilled water 2—3 time and then immersed it in the distilled water for 5-10 min. Pour out the distilled water, add 4—5 ml fresh prepared methanol: acetic acid (3:1) to the material. Put on 3-4 root tips on the glass slides, smashed with forceps, and add 2 drops fixation solution on the slide, then bake it on the alcohol burner. The preparations were stained in 1:9 (v/v). Giemsa solution for 20 min, followed by rinsing with distilled water, when the slides became completely dry and mounted in damar balsam.

MAIN RESULTS

1. Chromatin morphological characteristic analysis during interphase stage of cell cycle in *B. napus*.

The most generally accepted chromatin was chromosomal different movement state. The chromatin state appear in interphase nucleus on B. napus were observed. The chromosomes are not visible and the nucleus has different size granular appearance. Their average number in per nucleus were observed that is Xianglonyou 2 varies from 30±4, Xiangyou 11 varies from 31±3, Xiangyou 13 varies from 30±4 and Xiangyou 14 varies 29±3 respectively. These granules are composed of heterochromatin and are evenly distributed. They were referred to as similar globular pre-chromosome type. These granules do not undergo despiralization and decondensation at the end of each cell division. Instead they remain tightly coiled at a time when the rest of the chromosomes are in a relatively uncoiled condition.

2. The morphological variable on chromosome formation with progressing prophase in *B. napus*

There are a lot of darker staining granules with different shape and size. The darker staining granules joined by chromonematal fibrils. These fibrils between granules are strained to straighten. So that these granules become spindle shape or quite long. It has been shown the threadlike

Table 1 The number variation on heteromatin granular in *B.napus*.

Variely	No.of cells	No. variation on heterochromatin granular ($X \pm S.E$)
Xianglongyou 2	1000	30±4
Xiangyou 11	1000	31±3
Xiangyou 13	1000	30±4
Xiangyou 14	1000	29±3

chromosome formations are closely correlated with that of drake staining granules. One side or two sides of granules may de seen fine thread, these fine thread commence shorter thinner and lightly stained. then increase longer and thicker to become visible threadlike chromosomes in the nucleus. These granules were disappear with progressing prophase and at the some time the threadlike chromosome formation. At the end of chromosome can be seen coiling is lax. It shows the chromosome is composed of two chromatids. The chromosomes were stained with Giemsa and showed dark segment and light segment along the entire length of chromosomes.

3. The variable law on the chromosomal morphology during mitotic prophase in *B. napus*

Throughout prophase each chromatin develops coils and so becomes shorter, thicker and more readily visible by increased spiralization. The chromatids are seen to be twisted around each other in relational coil. The coils interlock in a manner that the chromatics cannot be separated without unwinding the coil. This kind of twisting is also called plectonemic coiling in mitosis. At the end of prophase, when the chromosomes attain their maximum contraction, the nuclear membrane breaks down and disappears. There is no longer much relational coiling present, and consequently, the chromatids are no longer twisted about each other but lie side by side. The number of chromosome of cell can be counted as $2n$=38 in *B. napus*.

DISCUSSION

The result is shown:(1)when chromosomes were induced to produce aberration during cell cycle plants, from chromatin to form threadlike chromosome are most easily produced aberration phase. Because of the chromosome are fine thread, which is broken easily, or produces some chemical changes in the cell, which in turn cause anomalies when the chromosomes replicate. So in general, dividing cells are much more sensitive to induction factor than non-dividing cells. But due to exist repair function during chromosome replication and may be have some an effect on aberration frequency of chromosomes and chromatic. (2) When induction aberration, the nucronuclei were formed by association between heterochromatin granules. (3) These granules may have relation to the number of chromosomes and C-banding. (4) In a lot of hypothesis of chromosome ultrastructure, the solenoid hypothesis (Finch, 1975)can explain chromosome ultrastructure more than other one.

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EVALUATION OF PLANT REGENERATION AND SOMACLONAL VARIATION IN *BRASSICA JUNCEA* L. (COSS AND CZERN)

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INTRODUCTION

The application of gene transfer techniques / creation of variability for crop improvement requires an efficient method by which plants can be regenerated from single cells, tissues or organs via organogenesis or embroyogenesis. Many *in vitro* techniques have been used to complement conventional plant breeding in different crops. Somaclonal variation is one of them which takes advantage of naturally occurring genetic variation in *in vitro* regenerated plants and provides value addition in already accepted and adopted varieties for commercial use. The technique has been used to generate and release agriculturally useful variation in different crops (Evans *et al,* 1984; Katiyar and Chopra, 1995; Duncan, 1997). We describe here evaluation of plant regeneration in four different cultivars released by Punjab Agriculture University, Ludhiana for commercial cultivation and somacolonal variation in the most regenerative cultivar RLM 198.

MATERIAL AND METHODS

Seeds of four cultivars of *Brassica juncea* namely RLM198, RLM 514, RLM 619, RL1359 were obtained from oilseed section, Department of plant breeding , PAU, Ludhiana. Seeds were germinated on HMS containing half major salts of MS (Murashige and Skoog, 1962), 2% sucrose, pH 5.8. Seed cultures were kept in dark for 3 days followed by light/dark regime of 16/8 hours at 25±1°C in incubation room. Cotyledon and hypocotyl explants were excised from 5-7 days old seedlings and cultured on MS supplemented with BAP (2mg/l). Cultures were scored for percent shoot regeneration every week for one month. Regenerated shoots were transferred to HMS for rooting. Plantlets were hardened by placing on cotton plugs supported in test tubes containing tap water for 15 days. Hardened plants were transferred to field soil in small pots covered with transparent coffee cups for one week. Data for survival, growth and development was recorded. Comparative studies were made for seed and seed progeny characteristics of selfed seeds obtained from regenerated plants and check variety RLM198.

RESULTS AND DISCUSSION

The plant regeneration ability varied with explant and cultivar genotypes. (Table 1) as also documented in *Brassica* spp. by Murata and Orton (1987), Jain *et al* (1988), Sharma *et al* (1990). Based on high regenerative ability, RLM198 was selected for further studies. The data for plant regeneration and different stages of growth and development are shown in Table 2. Selfed seeds were obtained from 4 of cotyledon (C1,C2,C3,C4) and 1 of hypocotyl (H) origin. Heritable somacolonal variation was scored for (1) 1000 seed weight (2) speed and percent *in vivo* and *in vitro* seed germination and (3) in vitro seedling height. Data for these parameters is recorded in Tables 3-5. Variation for 1000 seed weight and plant height was also noted by Jain *et al* (1987, 1989).

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Table 1. Pattern of shoot regeneration in *Brassica juncea* cultivars.

Cultivar	% Regeneration in Cotyledon	% Regeneration in Hypocotyl	Time taken for shoot initiation response (days)
RLM 198	89	20	10
RLM514	44	0	15
RLM619	40	0	15
RL1359	36	0	15

Table 2. Shoot regeneration, different stages of growth and development in RLM198

Explant type	Numbers					
	Shoots regenerated	Shoots rooted	Plantlets hardened	Plantlets survived in pots	Plants flowered	Plants set seeds
Cotyledon	561	271	243	61	18	4
Hypocotyl	142	85	66	5	5	1

Table 3. 1000 seed weight

Plant type	C1	C2	C3	C4	H	Control
Mean 1000 seed weight (g)	1.952	1.782	2.168	0.981	1.81	3.172

Table 4.Patteren of seed germination

Percent *In vivo* seed germination					Percent *In vitro* seed germination							
Plant type	12 hours	24 hours	48 hours		Day1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 15
C1	1.7	36.2	64.6		0	14.3	20.4	26.5	26.5	30.6	38.7	83.7
C2	42.3	75.0	92.3		0	26.3	31.5	34.2	34.2	44.7	52.6	81.5
C3	26.1	47.8	65.2		0	23.5	38.2	38.2	38.2	41.1	41.1	85.3
C4	--	--	--		0	2.3	2.3	2.3	2.3	2.3	2.3	2.3
H	6.2	21.2	52.5		0	0	0	0	0	10.0	10.0	40.0
Control	0	0	50.3		0	20.4	67.3	79.6	79.6	81.6	83.7	87.8

Table 5. Frequency distribution data for *in vitro* seedling height after 3 weeks

	Percent plants lying between range		
Plant type	0-20 (mm)	20-40(mm)	>40 (mm)
C1	42.1	42.1	15.8
C2	38.1	47.6	14.3
C3	18.75	75.0	6.25
Control	7.3	7.3	85.4

CONCLUSION

Somaclonal variation is low cost, labour intensive, easy to use technology which can be effectively utilized for creating and evaluating useful variability for value addition of cultivars.

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Microspore mutagenesis in *Brassica napus*

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Introduction:

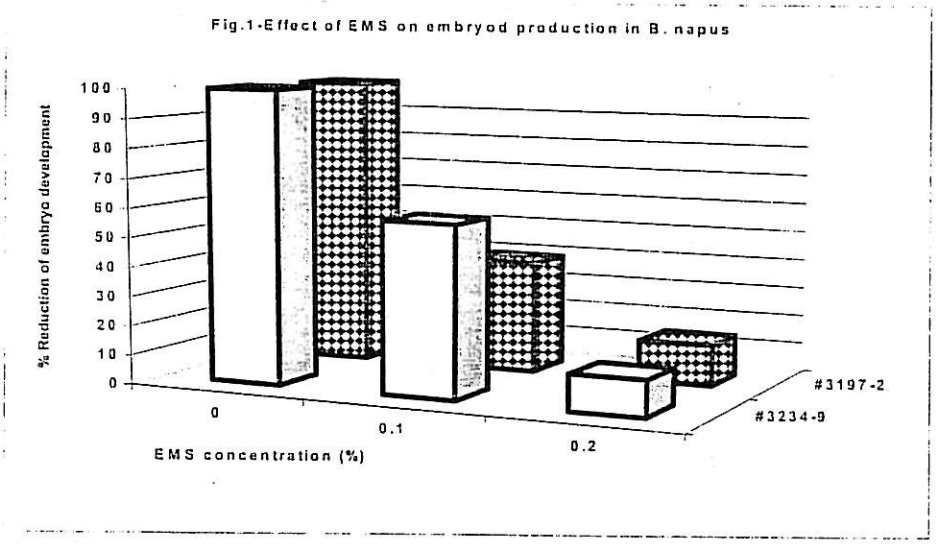
Muatagenesis is an established tool for increasing genetic variability in crop plants. Since the recovery of recessive mutants through classical breeding methods is difficult in higher plants because of diploid nature of sporophyte tissue, mutagenesis of haploids seems very attractive alternative. Microspore mutagenesis coupled with in vitro selection can provide an extremely powerful system for modifying and selection of traits in crop plants, because this culture system exhibits rapid embryo development and allows easy manipulation of extremely large population of individuals with minimum labor or cost. Without difficulty, several times per week, hundred million microspores can be isolated, and mutagenized. So in view of above objectives, for the development of doubled haploid mutant plants microspore mutagenesis of *B. napus* has been started.

Materials and Methods:

Microspores of *Brassica napus* lines # 3197-2 and #3234-9 provided by Dr. Gerhard Rakow AAFC, Saskatoon, Canada, were isolated following the microspore culture protocols of Ferrie *et al* (1995). The isolated microspores were incubated at room temperature for 1.5 hours in 0.1 and 0.2 % EMS added NLN media. After EMS treatment the mutagenized microspores were washed properly and cultured on NLN induction media. Embryos were counted three weeks after induction. They were then placed on a shaker in a tissue culture room (22 $^{\circ}$ C, 16 hr light). Embryos remained on the shaker until green. The embryos were then plated on petri plates containing B5 media. Plantlets were developed after three weeks. The normal plantlets were transferred to solid B5 media and then to soil.

Results and Discussions:

A sharp decrease in the embryogenesis in less embryogenic line # 3234-9 as well as in efficient embryogenic line # 3197-2 was observed. In case of line # 3197-2, 0.1 % EMS concentration reduced the production of embryoids up to 60 % while 0.2 % EMS reduced the embryo production up to 90 %. Same results were noted in case of line # 3234-9, however, 0.2% EMS treatment resulted in more drastic reduction of development (Fig.-1). In brief both EMS treatments decreased the embryo production in both lines. These results are in agreement with the results



reported by Swanson *et al* (1989) and Ahmad *et al* (1991). Nevertheless, the present study revealed that low concentration of EMS (0.1%) enhanced cell division and growth of embryoids. The high concentration of 0.2%, reduced cells division as well as slowed down the development and growth of the embryoids as compared to untreated embryoids (control).

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INFLUENCE OF LEAF EXTRACTS ON CULTIVATED TURNIP IV. FERTILIZATION VALUE

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ABSTRACT

The lower doses of aqueore leaf extract of neem (*Azadirachta indica* A. Juss.) have enhancing effect on number of ovules/pistil, number of seeds/siliqua and fertilization value of turnip (*Brassica rapa* L.), but the higher doses are fatal. The carrot weed (Parthenium hysterophorus L.) extract exhibits a deleterious effect on these traits. Only higher doses of periwinkle (*Catharanthus roseus* Don.) extract are stimulating on number of ovules/pistil, but the extract has a retarding effect on the rest two fertility attributes.

INTRODUCTION

The commonly used chemical mutagens are not only expensive but also injurious to health and environment. Therefore, search for safe and effective mutagens of biological origin can be an important approach. There are a few reports on turnip in this context (1-5). Seed set is a character of paramount importance to plant breeders. Number of ovules present in the ovary may be regarded as an important component of fertility for they develop into seeds after successful fertilization. In fact, seed set depends upon the ability of these ovules to get fertilized with pollen grains. Present work was undertaken in order to know the effect of aqueous leaf extract of periwinkle, carrot weed and neem on number of ovules/pistil (MNO), number of seeds/siliqua (MNS) and Fertilization value (FV) of turnip. With the help of this, it is possible to evaluate the number of ovules capable of forming seeds.

MATERIALS AND METHODS

The mother solution was prepared by grinding of 250g green leaves and from that the solutions of different concentrations (20%, 40%, 60%, 80% & 100%) were made by adding required amount of distilled water separately. The seeds of 'Rose Red' variety of turnip were treated in different concentrations of the leaf extracts for 24 hours. Then they were sown in the field immediately at Hazaribag to raise M1 plants along with control. M2 plants were raised from the seeds obtained from M1 by selling. Pistils were pressed in between two microscopic slides after putting a drop of KI solution to count the number of ovules. The silique were dissected with the help of a blade and a pair of needle to count the number of seeds. The ratio of MNS to that of MNO X 100 gave the percentage of FV. All results are presented in Table 1.

RESULTS AND DISCUSSION

A marked effect of the extracts was noted on MNO. Periwinkle extract, from 20-60% concentrations, had no effect on it, but an enhancing effect was observed at the higher doses in M1 with a further improvement in M2. M1 plants under 80% treatment had maximum number of ovules in their pistils. Carrot weed extract exhibited a gradual decrease in MNO from lower to higher doses in M1 with a considerable recovery in M2 at all doses but not to the extent of control. Overall, there was an enhancing effect of neem extract on MNO at all the doses in M1 which further improved in M2. The plants under 40% treatment had maximum number of ovnles in their pistils. Periwinkle as well as carrot weed extracts demonstrated deleterious effect on MNS. A gradual decline from lower to higher doses was seen in M1, with a slight improvement in M2 but not to the level of control. There was an enhancing effect of neem extract at all the doses in M1 (except at 100%). A further improvement occurred at all the doses, except 100%. A retarding effect of periwinkle and carrot weed extract was seen on FV in M1. Noticeable recovery took place in M2, but not to the extent of control. Neem extract had an enhancing effect on FV at all the doses (except 100%) in M1, but surprisingly with decline in M2.

Seed set is a complex character. It is the ultimate product of interactions among a number of quantitative traits which are known to be controlled by different sets of polygenes. A great many alkaloids have been isolated, purified and determined chemically from periwinkle, and two most active among them are Vinblastine and Vincristine (6). They have antitumour effect. They are known to interfere with some metabolic reactions related to DNA and RNA synthesis. Carrot weed yields a non-alkaloidal, non-glycosidic substance called parthenin (7), along with some soluble inhibitors like caffeie acid, P-coumaric acid, Anedie acid, Vanillic acid and P-hydroxy benzoic acid (8-9). The plant materials inhibit seed germination, growth and yield of wheat (9), cowpea (10), turnip (11) and ragi (8). Present results also indicate the deleterious effect of carrot weed on all the three attributes. Burning of the whole plant along with its seeds in the only effective method of its eradication. The plant should not be used as green manure by farmers even by mistake as it

exhibits allelopathic activity (8, 12). Neem elaborates a vast array of biologically active and chemically diverse constituents. Leaf extract of neem has nimbin, nimbenene, 6 - desacetabimbinene, nimbadiol, nimbolide and querection (13). Appreciable amount of protein, minerals, carotenes and trace elements (except Zinc) are also present (14). N, P and C balances are positive. Obviously, all or a few of these, are directly or indirectly related with the enhancing effect of the extract on the fertility traits of turnip. However, a further biochemical investigation is required in this direction.

Table 1. Effect of botanical extracts on number of ovules / pistil, no. of seeds / siliqua and fertilization value of turnip.

Conc.	Gen.	No. of ovules / pistil (MNO)			No. of seeds / siliqua (MNS)			Fertilization Value (FV %)		
		Periwinkle	Carrot weed	Neem	Periwinkle	Carrot weed	Neem	Peri-winkle	Carrot weed	Neem
Control	M_1	23.4±0.14	32.1±0.06	16.2±0.22	17.3±0.17	25.1±0.05	12.3±0.24	73.9	78.2	75.9
	M_2	23.1±0.15	33.1±0.07	16.3±0.29	18.2±0.17	23.6±0.08	12.1±0.25	78.8	71.3	74.2
20%	M_1	23.7±0.15	30.2±0.07**	16.9±0.29*	16.9±0.15	16.2±0.06**	14.6±0.21**	71.3	53.6	86.4
	M_2	23.8±0.15	33.0±0.08	17.9±0.28**	17.2±0.16	18.3±0.05**	15.4±0.21**	72.3	55.4	86.0
40%	M_1	23.5±0.15	29.6±0.05**	18.4±0.18**	15.4±0.17**	13.3±0.06**	18.1±0.27**	65.5	44.9	98.4
	M_2	23.5±0.14	30.2±0.06**	19.4±0.27**	15.6±0.16**	18.7±0.06**	19.0±0.27**	66.4	61.9	97.9
60%	M_1	23.5±0.16	30.3±0.05**	16.9±0.30	13.9±0.15**	15.1±0.05**	16.7±0.25**	59.1	49.8	98.8
	M_2	23.5±0.16	32.2±0.09**	18.4±0.27**	14.1±0.16**	15.1±0.05**	17.8±0.21**	60.2	46.9	96.7
80%	M_1	26.4±0.17**	29.5±0.06**	16.6±0.19	15.5±0.16**	13.5±0.07**	13.4±0.23**	58.7	45.8	80.7
	M_2	27.6±0.17**	32.5±0.09**	16.9±0.25*	17.3±0.17**	21.7±0.06**	14.6±0.24**	62.7	66.8	86.4
100%	M_1	26.1±0.16**	28.2±0.04**	15.6±0.27	13.2±0.16**	05.5±0.08**	10.2±0.25**	50.6	19.5	65.4
	M_2	25.5±0.17**	32.0±0.07**	16.6±0.22	16.9±0.14**	12.4±0.06**	09.3±0.26**	66.3	38.8	56.0

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

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INFLUENCE OF LEAF EXTRACTS ON CULTIVATED TURNIP V. HETEROCHROMATIN

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ABSTRACT

Aqueous leaf extracts of neem (*Azadirachta indica* A. Jues) and carrot weed (*Parthenium hysterophorus* L.) have retarding effect on constitutive hetechromation of turnip (*Brassica rapa* L.). Lower doses of periwinkle (*Catharanthus roseus* Don.) extract are stimulating, while higher doses are deleterious. A further decline occurs in M2 under periwinkle and neem extract treatments, with no change in the distribution pattern. However, under carrot weed treatment it occurs only at lower doses, whereas at the higher doses a rapid recovery takes place with a noticeable change in the distribution pattern.

INTRODUCTION

Chromocenters are dark-staining heteropycnotic bodies found in the interphase nuclei in all plant groups. They have been attributed considerable role in taxonomy and evolution (1,2). Shape and size of chromocenters are quite variable due to differential DNA replication and endoploidy (3). They represent pericentric constitutive heterochromatin. Probably this is the only type of heterochromatin found in plant cells. They are rich in highly repetitive and satellite DNA, mitotically condensed throughout the interphase and detectable as C-bands in mitotic chromosomes after differential Giemsa staining and late replicating. Recently study of heterochromatin has aquired a special attention in view of its possible role in gene regulation, chromosome pairing and genetic recombination and evolution (2, 4 - 8). Turnip is a suitable material for genetic study of heterochromatin. Commonly used chemical mutagens are not only expensive but also injurious to health and environment. Therefore, search for safe and effective mutagens of biological origin can be an important approach (9 - 13). Present work has been undertaken in order to know the effect of aqueous leaf extracts of periwinkle, carrot weed and neem on heterochromation of turnip.

MATERIALS AND METHODS

'Rose Red' variety of turnip constituted the material for present study. Methods were the same as described earlier (13). All results are presented in Table 1.

RESULTS AND DISCUSSION

There was an enhancing effect of periwinkle extract on mean number of chromocentres/ nucleus (MNC) of turnip at lower doses, while the higher doses were retarding. On the whole, a gradual reduction in MNC was observed from lower to higher concentrations. In M2 a further decline occurred at all the doses, with no charge in the distribution pattern of the chromocenters within the nuclei. Carrot weed extract also caused a gradual decrease in MNC from lower to higher concentrations. Some recovery took place in M2 at higher doses, but a further decrease was noted at the lower doses. In contrast to periwinkle, distribution pattern of the chromocentres drastically changed. Neem extract too, had a deleterious effect on this nuclear phenotype. A gradual decrease in MNC from lower to higher doses was seen in M1, with a further decline in M2 at all the doses but with no change in the distribution pattern.

Number of chromocentres within the nuclei is characteristic for the varietal populations of radish (14) and turnip (15). In F1 hybrids MNC averaged mid way between their parents (14,16). Amount and distribution of chromocentres in radish (14) and turnip (16) are under the control of genotype and regulated polygenically. An increase in the amount of chromocenters adversely affects chiasm frequency and consequently, genetic recombination in radish (17). Air pollution causes an increase in the amount of heterochromation which may be utilized in the measurement of pollution level of different localities (18). The chromocenter counts can be exploited as quick, easier and reliable cytological method for determining the ploidy level (19). Different varietal populations of turnip react differently to different doses of gamma rays in turnip (20).

A great many alkaloids have been isolated, purified and determined chemically from periwinkle. Two most active among them are vinblastine and vincristine. They have antitumonr effects. They are known to interfere with some metabolic reactions related to DNA and RNA synthesis (21). Carrot weed yields a non-alkaloidal, non-glycosidic substance called parthenin (22), along with some soluble inhibitors like caffeic acid, P-coumaric acid, anelidic acid, vanillic acid and P-hydroxy benzoie acid (23-24). Parthenium extract inhibits seed germination, seedling survival, growth, photosynthetic activities, root weight, fertility traits and MNC of turnip (25). Leaf extract of neem has nimbin, nimbinene, 6 - desacatabimbinere, nimbadiol, nimbolide and querection

(26). Naturally, all or a few of these, are concerned with the inhibitory effects on heterochromatin of turnip. Reduction in the number of chromocentres, particularly at higher doses, is due to their fusion as a consequence of mutagenic effets of the extracts.

Table 1. Effect of botanical extracts on the chromocentres of turnip

Conc.	Gen.	Perwinkle Chromocentres / nucleus			Carrot weed Chromocentres / nucleus			Neem Chromocentres / nucleus		
		Mean ± SE	CV (%)	Range	Mean ± SE	CV (%)	Range	Mean ± SE	CV (%)	Range
Control	M_1	12.4 ± 0.18	14.4	7-16	10.7 ± 0.09	08.5	9-12	12.0 ± 0.27	11.2	10-14
	M_2	12.7 ± 0.17	13.3	7-16	10.6 ± 0.09	08.9	9-12	12.0 ± 0.27	11.2	10-14
20%	M_1	14.1 ± 0.18**	12.5	7-17	09.3 ± 0.12**	12.7	7-11	11.9 ± 0.20	08.6	10-13
	M_2	13. ± 0.18**	13.1	7-17	09.0 ± 0.80**	08.7	6-9	11.8 ± 0.23	09.7	10-13
40%	M_1	13.9 ± 0.18**	12.6	7-16	09.1 ± 0.13**	13.8	7-11	11.4 ± 0.15	06.5	9-12
	M_2	13.7 ± 0.18**	13.0	7-16	08.6 ± 0.13**	14.6	6-11	11.3 ± 0.16*	07.2	9-12
60%	M_1	13.9 ± 0.19**	13.8	7-17	08.9 ± 0.13**	14.6	7-11	11.3 ± 0.15	06.7	10-12
	M_2	09.8 ± 0.16**	16.4	7-17	08.5 ± 0.10**	11.9	6-10	11.0 ± 0.16**	07.3	10-12
80%	M_1	09.4 ± 0.16**	16.9	6-14	08.3 ± 0.11**	12.8	6-10	10.7 ± 0.13	05.9	9-12
	M_2	09.2 ± 0.18**	19.0	6-14	10.0 ± 0.11**	11.0	8-12	10.9 ± 0.19**	08.7	9-12
100%	M_1	08.7 ± 0.13**	15.4	6-13	06.5 ± 0.09**	13.8	5-8	10.3 ± 0.22**	10.9	10-12
	M_2	07.3 ± 0.12**	16.9	6-13	10.4 ± 0.14**	13.3	8-13	10.5 ± 0.27**	12.9	10-12

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

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High frequency production of doubled haploid plants of Chinese cabbage derived from microspore embryogenesis without colchicine treatment

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Introduction

Since haploid embryoids were first obtained from microspore culture of *Brassica napus* (Lichter, 1982), there has been a rapid progress in the application of this technique in genetic studies and breeding programs of *Brassica* crops (reviewed by Takahata, 1997). Desirable genetic recombinants resulting from microspore embryogenesis can be exploited by recovering haploid plants from microspore-derived embryos. The interesting recombinants can be used for the development of new varieties or as homozygous breeding lines. Because haploid plants are sterile, practical utilization in breeding programs relies on an efficient chromosome doubling technique to obtain fertile diploid plants. A low frequency of fertile diploid plants was obtained through androgenesis in *B. napus* (Zhao and Simmonds 1995). However, in Chinese cabbage, high frequency production of doubled haploid plants was observed in our previous work (Zhang et al. 1993) and present study.

Materials and Methods

Microspore culture was carried out in 45 Chinese cabbage varieties. 2-3 mm flower buds in length were collected and surface-sterilized in sodium hypochlorite (2% active chlorite) for 15 min and then rinsed three times with sterile distilled water. After the buds were macerated in a mortar containing B5 medium supplemented with 13% sucrose at pH 6.0 (B5-13), microspores were obtained by filtration through miracloth and then washed three times with B5-13 medium by centrifuging at 1000 rpm for 3 min. The microspores were suspended at a density of 1 x 10^5/ml in 1/2NLN-10 medium. Two ml of the microspore suspension was cultured in 60 x 15 mm plastic petri dish. The petri dishes were incubated at 32.5 ℃ for one day prior to maintenance at 25 ℃ in darkness. After 3 weeks of culture, cotyledonary embryos were transferred to 90 x 20 mm petri dishes containing B5 medium with 2% sucrose (B5-2) and 1.6% agar, and then incubated at 25 ℃ under a 16 h/day photoperiod of cool white illumination at 30 $Em^{-2}s^{-1}$ for 3-4 weeks. Regenerated plantlets (or shoots) were subcultured in Erlenmeyer flask containing B5-2 medium (0.8% agar) for 2-3 weeks. Rooted plantlets were grown in vermiculite for about 2 weeks before transfer to soil in a greenhouse. The ploidy level of the regenerants was estimated by examining the flower morphology including pollen fertility, flower size and seeds set after bud pollination.

Results and discussion

The frequency of DH plants production among 45 genotypes was listed in Table 1 and summarized in Table 2. 95% of the genotypes showed the percentage of DH plants production higher than 50%. Half of the genotypes showed the DH plants production frequency higher than 70%. It is generally assumed that these diploid plants are doubled haploids generated from endomitosis, endoreduplication or nuclear fusion. It is also possible that they are derived from unreduced gametes. A genotype variation of DH plants production was observed in present study, it is considered that diploidization during microspore embryogenesis is controlled by genetic factors. On the other hand, colchicine treatment for double the chromosomes is not needed in microspore culture of Chinese cabbage.

Table 1. Frequency of doubled haploid (DH) plants derived from microspore embryogenesis in 45 genotypes of Chinese cabbage

Genotype	No. of plants investigated	No. of haploid plants	No. of DH plants	No. of plants >2n	% of DH plants
C1	34	3	29	2	85.3
C4	8	1	7	0	87.5
C8	35	2	32	1	91.4
C9	58	19	39	0	67.2
C10	15	4	11	0	73.3
C11	4	1	3	0	75.0
C12	56	43	13	0	23.2
C13	37	11	22	4	59.5
C14	12	4	8	0	66.7
C15	4	2	2	0	50.0
C16	12	0	11	1	91.7
C17	52	16	36	0	69.2
C18	52	19	30	3	57.7
C19	24	5	18	1	75.0
C20	24	8	16	0	66.7
C22	10	0	10	0	100
C23	33	7	22	4	66.7
C24	49	13	34	2	69.4
C26	7	1	6	0	85.7
C28	27	12	15	0	55.6
C29	23	5	16	2	69.6
C30	6	2	4	0	66.7
C31	39	10	29	0	74.4
C32	26	12	14	0	53.8
C33	14	4	10	0	71.4
C34	4	2	2	0	50.0
C35	4	1	3	0	75.0
C36	11	3	8	0	72.7
C38	17	1	16	0	94.1
C39	16	6	10	0	62.5
C44	11	3	8	0	72.7
C45	40	17	21	2	52.5
C46	28	12	16	0	57.1
C47	30	10	19	1	63.3
C48	31	6	25	0	80.6
C49	38	15	22	1	57.9
C50	39	4	34	1	87.2
C51	21	3	16	0	76.2
C60	30	4	26	0	86.7
C62	40	3	37	0	92.5
C63	34	3	31	0	91.2
C64	4	2	2	0	50.0
C65	55	26	29	0	52.7
C66	8	5	2	1	25.0
C67	40	16	24	0	60.0

Table 2. Distribution of genotypes with different DH plants production frequency

% of Diploids	>90	90~80	80~70	70~60	60~50	50~40	40~30	30~20	<20
No. of genotypes	6	6	9	11	11	0	0	2	0

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PRELIMINARY STUDIES ON GENETIC TRANSFORMATION OF *CRAMBE ABYSSINICA*

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INTRODUCTION

As a potential source of high erucic seed oil for industrial purposes *Crambe abyssinica* has been an object of agronomical as well as technological investigations. Products and derivatives of high erucic acid oil may be applied in a wide range of applications such as detergents, cosmetics, coatings and pharmaceuticals.

Crambe abyssinica is an annual *Cruciferae* species displaying 58 % to 62 % erucic acid (C22:1). Several cultivars are known [1, 3]. *In vitro* culture of *Crambe* should enhance breeding efficacy in this genus and constitutes a prerequisite for the increase of its C22:1 content *via* genetic transformation.

This paper reports on the first results of genetic transformation *via Agrobacterium tumefaciens.*

MATERIAL AND METHODS

Plant material: Seeds from three cultivars were used: 'BelAnn' – from Research Centre for Agriculture and Fishery (Biestow, Germany), 'Carmen' – from CEBECO Saaten GmbH (Celle, Germany) and 'Galactica' – from CPRO – DLO (Wageningen, The Netherlands).

Construct used: *Agrobacterium* strain GV 3101 C58C1 and plasmid pRE1 carrying the *KCS* gene from *B. napus* and the *LPAAT* gene from *Limnanthes douglasii* fused to the napin promoter (pNKAT) were obtained from Frentzen et al. [2].

Plant transformation and regeneration: Cotyledons from 1-2 mm petioles were cut from seedlings six-day after germination and were placed for 20 minutes in *A. tumefaciens* suspension containing Murashige & Skoog [4] – basic medium, 20 g/l Saccharose, 5 mg/l BAP, 0.5 mg/l NES and 2 mg/l $AgNO_3$. Then the explants were transferred to cocultivation medium containing the same elements as the infection medium but modified by the addition of 10 mg/l acetosyringone and 8 g/l phytagar. After cocultivation cotyledonal explants were placed on shoot induction medium. The composition of this medium was same as cocultivation medium on which 500 mg/l carbenicillin and 25 mg/l kanamycin was added.

Selection and progeny tests: The transgenic status of putative transformants was confirmed by *NPT II* expression assay. Transgenic plants (T_1) were propagated by bagging and T_2 seeds were collected. After analysing the fatty acid composition on halfseeds of the T_2 seeds as described by Thies [6] the T_2 individuals displaying the highest erucic acid content were grown in the glasshouse and propagated in the same way. Ten T_3 seeds per T_2 indiviual were analysed as mentioned above. In every generation seeds of the parent variety 'Galactica' grown under the same conditions were analysed as a standard.

RESULTS AND DISCUSSION

The results of the experiments revealed a large difference between the three genotypes regarding their regeneration response after the infection with *A. tumefaciens*. Based on the number of regenerated shoots of each genotype, regeneration efficiency ranged from 0 to 87 %. Similar differences are known in *B. napus* [5, 7]). A problem was the low transformation frequency (Table 1). After transfer to the glasshouse, the plants grew normally and developed seeds.

Tabelle 1: Regeneration and transformation efficacy after *Agrobacterium*-mediated transformation of *Crambe abyssinica* with pNKAT gene construct

Cultivar	Number of explants	Shoots regenerated	Transformation efficiency	
		n (%)	*n*	%
BelAnn	217	0 (0)	0	0
Carmen	140	31 (00)	0	0
Galactica	176	153 (87)	3	1.7

The C22:1 content of 63 T_2 half-seeds descending from three transgenic T_1 plants varied from 25.3 % to 67.8 %. The comparable 'Galactica' seeds displayed between 56.2 % and 61.0 % erucic acid. The variances of the transgenic offspring differed significantly from 'Galactica' (F-test, α=0.01) but not the mean erucic acid content (t-Test, α=0.01). The best halfseed individuals were grown in the glasshouse and propagated by bagging. T_3 seeds of a total of 11 T_2 plants were analysed by gas chromatographic. The individual C22:1 content varied from 53.2 % to 60.0 % ('Galactica': 57.4 %-60.1 %). The transgenic lines did not exeed the C22:1 content of 'Galactica' significantly. The results do not indicate any additional effect of the pNKAT gene construct regarding the erucic acid content. Further investigations of the composition of triacylglyceride will reveal wether its inner position is occupied by erucic acid as the result of the action of *LPAAT* gene.

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Effect of seed-soaking with Mg-salts on water uptake efficiency of germinating seeds of mustard.

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Proper growth of plant much depends upon the water use efficiency of the plant; effect of which starts right from the germination of seeds. It has been known that ions of the salts have different levels of water holding capacity when persent within the plants and that depends upon the chemical nature of the ion itself. However, it has been well established that Mg-salts have the capacity to enhance the various phases of growth of mustard plant starting from germination to yield when used in form of seed-soaking treatment prior to sowing (Bose and Mishra, 1997 and 1999). Therefore, in the present investigation effect of seed-soaking treatment with Mg-salts on change in water uptake of germinating seeds of mustard was put for observation and research.

Seeds of *Brassica juncea* L. Czern & Coss (Var. Vaibhav and Kranti) were obtained from the Oilseeds section of the Department of Genetics and Plant Breeding of this Institute. Hundred bold and healthy seeds were dipped in 0.1% solution of $Hg\ Cl_2$ for 5 minutes and then were washed with distilled water for 4 to 5 times. These seeds were then placed in petridishes (32 cm diameter), lined with single layer of Whatman No.1 filter paper, moistened either with 7.5 ml of distilled water (control) or with solutions (Concentrations ranging from 5 to 10 mM) of $Mg\ (NO_3)_2$ and $MgSO_4$. The experiment was conducted in normal room illumination (10 Wm^{-2}) at a temperature of $18\pm2°C$. Change in percent water uptake (WU) was noted from 12 to 96 h, during germination. The data were calculated on the basis of the following formula.

$$\text{Percent Water Uptake (WU)} = \frac{\text{Fresh Seed Weight - Initial Seed Weight}}{\text{Initial Seed Weight}} \times 100$$

Data subjected to statistical analysis are the mean value of three replicates.

With increasing hours of germination WU was observed to increase gradually in seeds of both varieties of mustard. Variety Vaibhav had significantly greater values of WU than that of Kranti at 24, 36 and 96 h except at 12 and 48 h of study; however $Mg\ (NO_3)_2$ was found to be more responsive at 12, 48 and 96 h in respect to $MgSO_4$. At 12 and 96 h of study upto 7.5 mM concentrations either of both the salts or only $Mg\ (NO_3)_2$ were observed to improve WU as compared to that of the control in germinating seeds (Table 1).

Table 1 : Effect of seed treatmnt with Mg-salts on percent - change in water uptake of different varieties of *Brassica juncea* L. Czern & Coss (Vaibhav = V and Kranti = K) during germination.

Treatments	GERMINATION HOURS									
	12		24		36		48		96	
Salts (Conc in (mM)	V	K	V	K	V	K	V	K	V	K
Control	40	55	150	91	197	216	250	273	523	472
$Mg(NO_3)_2$										
5.0	50	60	133	88	213	193	226	273	540	484
7.5	41	66	126	138	185	190	235	263	559	520
10	41	58	118	96	181	176	221	226	557	481
$Mg\ SO_4$										
5.0	48	49	126	126	193	176	230	246	- 407	429
7.5	50	51	133	128	210	183	250	221	458	416
10	48	49	126	121	206	181	248	215	449	394
C.D. at 5%										
VxS	2.79		3.62		2.46		3.13		3.33	
SxC	NS		5.12		3.48		4.40		4.72	
VxC	3.95		5.12		3.48		4.40		4.72	
VxSxC	5.58		7.25		4.93		6.23		6.67	

V = Varieties S= Salts C = Concentrations

These observations clearly indicate that in the early phase of germination, the WU in both varieties of *Brassica juncea* L. Czern & Coss were more in salt treated sets. This suggests that both salts of Mg have the capacity to enhance WU. during imbibition phase of germination. However, from 24 to 48 h of germination WU in salt treated sets were generally low as compared to that of the control. This could be due to the development of an ionic gradient in the inner and outer system of seeds, which might have checked the entry of the water inside the seeds. Scull (1916) and Ryan (1973), had reported a clear-cut decrease in rate of WU in seeds germinated in presence of salt solution. However, in the later phase of germination i.e. at 96 h again WU was observed to go up with $Mg(NO_3)_2$ treatment. This could be happened due to the presence of Mg^{++} and NO_3^- ions in $Mg(NO_3)_2$ and influx of both the ions to seeds may occur during germination (Bose and Mishra, 1999). This in turn improves the water uptake ability of seeds during later period of germination. Therefore, from these observations, it could be concluded that $Mg(NO_3)_2$ treatment to seeds of mustard during germination may enhance the water uptake ability. This could be field tested on large scale and if satisfactory could be made use by the crop growers prior to sowing to improve water use efficiency of the mustard crop plants.

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Heat unit efficiency in Indian mustard (*Brassica juncea* L.)

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Introduction

Temperature is one of the important factors that affects the growth of plant through its effect on physiological process as well as bio-chemical reactions. The structural and physiological developments of the plant vary greatly depending upon the temperature pattern of the surrounding environment. The heat unit concept, an interesting approach, for studying plant-temperature relationship has been widely used as guide in scheduling planting and prediction of harvest date. The heat use efficiency has a great importance in determining the yield potential of the crop under different environments.

Materials and Methods

A trial was carried out during 97-98 *rabi* at Dryland Research Station, Dhiansar (32^0-39'N,74^0-58'E and 332 meters amsl).Two mustard cultivars Varuna and Pusa Bahar were sown on three different dates viz; October 9(D_1),October 24(D_2) and November 8 (D_3) in radomized block design with four replications. All recommended package of practices for mustard were followed. Dry matter production and seed yield (kg/ha) were recorded in each plot. Daily temperature data recorded at Agromet Observatory Dhiansar were used for computation of growing degree days (GDD) or heat units for different phenophases using the expression:

$$\text{Accumulated HU} - \sum_{de}^{dm} \frac{MaxT+MinT}{2} - T_b$$

HU- Heat unit or growing degree days (^{0}Cd)
MaxT and MinT- Daily maximum and minimum temperature (^{0}C),respectively
T_b- Base temperature i.e 5^{0}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

Heat use efficiency (HUE) was computed as:

$$\frac{\text{Dry matter production (Kg/ha)}}{\text{Cumulative heat unit (^{0}Cd)}}$$

Results and Discussion

A perusal of data presented in Table1 showed the decreasing trend in accumulated heat unit during the periods between emergence to flower bud initiation (PS_1) and flower bud inititiation to siliqua formation (PS_2) while reverse trend was noticed during the

Table1: Influence of different treatments on accumulation and efficiency of heat unit in mustard

Treatment	Accumulated heat unit ^{0}Cd Phenophase			Heat use efficiency Kg/ha/^{0}Cd	Seed yield Kg/ha
	PS_1	PS_2	PS_3		
V_1(Varuna)					
D_1	478	297	774	2.55	862.7
D_2	421	217	811	2.76	919.5
D_3	362	198	817	2.21	543.2
V_2(Pusa Bahar)					
D_1	475	269	832	2.35	733.2
D_2	438	219	800	2.51	888.2
D_3	358	183	842	2.02	571.7

PS_1:- Emergence to flower bud initiation
PS_2:- Flower bud initiation to siliqua formation
PS_3:- Siliqua formation to maturity

D1:- (09-10-97)
D2:- (24-10-97)
D3:- (08-11-97)

period between siliqua formation to maturity. The quantification of heat unit efficiency (HUE) indicate that the efficiency of heat unit to produce dry matter is more in second date of sowing (D_2) followed by first (D_1) and third (D_3) date of sowings in both the cultivars.The variation in heat unit efficiencies were also found to be in agrement with those for seed yields which followed similar trend. Similar explaination were given by Balakrishnan and Natrajaratanam (1986) and Arjunan *et al* (1993) for pigeonpea and rice crop respectively.

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ACCELERATED AGEING-PREDICTING STORABILITY OF MUSTARD SEEDS

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Seeds are seldom used after harvest and at the places of their production. Thus they are stored for varying periods. It is impossible to judge the storability of any seed lot based on physical appearance. The seeds man is quite often faced with the decision of which lots from among many available should be marketed first and which lot should be held for possible carry over, for him then no way of knowing how much deterioration has taken place in the various seed lots. Delouche and Baskin (1973) developed "accelerated ageing technique" for predicting the storability of various crop seeds in a relatively short time. According to Desai (1976), the seed lots that maintain germination well after accelerated ageing also record good germination when stored under normal conditions. With these stated facts in view, a study was conducted to know the relative storability of mustard seeds.

Six seed lots of Indian mustard (cv. Varuna) viz., L_1, L_2, L_3, L_4, L_5 and L_6 procured from different provenances of Uttar Pradesh during 1998-99. Each lot of the seeds having 8 per cent moisture content was stored under ambient conditions for a period of 12 months and storability was observed by conducting standard germination test at three months interval as per ISTA rules (Anon, 1985). Moisture content of each seed lot was raised from 8 per cent to ten per cent and subjected to accelerated ageing at 40°C temperature and a relative humidity of 95 $\pm$ 5 per cent. After 3, 4, 5 and 6 days of exposure periods (P) the seeds were removed from the ageing chamber and subjected to standard germination test.

Seeds of L_1 lot showed highest germination percentage. Germination for lots L_2-L_6 showed significantly decreasing values and the trend continued till the end of the storage period (Table 1). The differences among seed lots pertaining to their germination may be due to the effect of provenance which affect the quality of seed. Verma *et al.* (1999) also noticed the effect of provenance on seed quality and storability in Indian mustard. After 12 months of storage period maximum reduction in seed germinability was exhibited by L_6 seed lot (19.32%) followed by L_5 (18.43%) and minimum reduction in seed germination percentage was noticed in L_1 seed lot (7.02%).

With increased period of exposure to accelerated ageing showed decreasing trend in seed germination per cent. The germination values obtained after accelerated ageing were lower than the values obtained under ambient storage. The germination results of seeds stored in ambient conditions and seeds subjected for ageing showed their association. Percentage germination for different lots recorded after 3,6,9 and 12 months ambient storage coincided with those obtained after 3,4,5 and 6 days of ageing and are in agreement with Delouche and Baskin (1973).

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Table 1 : Mustard seed germination as influenced by natural and accelerated ageing

Seed lot (L)	Storage period (P) months					Mean	Accelerated ageing					Mean
	0	3	6	9	12		P_0	P_1	P_2	P_3	P_4	
L_1	95.0	94.67	92.67	90.33	88.33	92.20	95.00	93.03	92.13	89.57	87.70	91.50
	(77.08)	(76.65)	(74.29)	(71.88)	(70.02)	(73.78)	(77.08)	(74.69)	(73.71)	(71.16)	(69.47)	(73.05)
L_2	91.67	92.00	90.00	87.67	82.00	88.70	91.67	89.83	88.30	87.07	81.07	87.60
	(73.22)	(73.57)	(71.57)	(69.44)	(64.90)	(70.36)	(73.22)	(71.40)	(70.00)	(68.93)	(64.21)	(69.38)
L_3	90.00	89.00	86.33	84.00	78.33	85.53	90.00	88.17	85.47	83.23	77.80	84.93
	(71.57)	(70.63)	(68.30)	(66.42)	(62.26)	(67.64)	(71.57)	(69.88)	(67.59)	(65.83)	(61.89)	(67.16)
L_4	92.67	90.00	84.67	85.33	80.33	86.60	92.67	89.83	85.13	84.17	76.87	85.73
	(74.29)	(71.57)	(66.95)	(67.48)	(63.67)	(68.53)	(74.29)	(71.40)	(67.32)	(66.55)	(61.25)	(67.81)
L_5	88.67	87.00	82.67	78.33	72.33	81.80	88.67	86.00	81.40	77.77	71.33	81.03
	(70.33)	(68.87)	(65.40)	(62.26)	(58.26)	(64.75)	(70.33)	(68.03)	(64.45)	(61.87)	(57.63)	(64.18)
L_6	87.00	85.67	81.67	76.00	71.00	80.27	87.00	85.03	80.93	74.99	70.07	79.60
	(68.87)	(67.76)	(64.65)	(60.67)	(57.42)	(63.63)	(68.87)	(67.24)	(64.11)	(59.99)	(56.83)	(63.15)
Mean	90.84	89.72	86.34	83.61	78.72	--	90.84	88.65	85.56	82.80	77.47	--
	(72.38)	(71.30)	(68.31)	(66.12)	(62.53)		(72.38)	(70.31)	(67.67)	(65.50)	(61.66)	

	SEm ±	C.D. (P=0.05)	SEm ±	C.D. (P=0.05)
Seed lot (L)	0.23	0.63	0.09	0.25
Storage period (P)	0.21	0.57	0.08	0.24
Interaction (L x P)	0.16	0.45	0.07	0.18

Figures in parenthesis are angular transformed values.

EFFECTIVENESS AND EFFICIENCY OF PHYSICAL AND CHEMICAL MUTAGENS ON GERMINATION IN INDIAN MUSTARD (Brassica juncea L.Czern & Coss.)

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INTRODUCTION

Induced mutagenesis has become an important tool for inducing genetic variation for desirable traits in crop plants. The basic information on mutagenic sensitivity, efficiency of mutagens, method of handling the materials and treatment method, required to maximize mutation induction is essential for success in any mutation breeding program. This paper reports the results obtained on effectiveness and efficiency of physical and chemical mutagens in brown and yellow seeded Indian mustard.

MATERIALS AND METHODS

One hundred dry seeds of uniform size of mustard varieties namely, Narendra rai (brown) and NDYR 10 (yellow seeded) were treated with gamma rays with 25,50,75,100 and 125 kR doses,Ethyl Methane Sulphonate (EMS), Ethidium Bromide(EB), Acriflavin (Ac) and Malic Hydrazide (MH). The doses of chemical mutagens were 0.1,0.2,0.3,0.4 and 0.5 per cent for EMS whereas, 1000,1500 and 2000 ppm for EB, Acriflavin and MH. Dry seeds were treated with freshly prepared aquous solutions for 6 hrs in chemical mutagens. After treatment seeds were thoroughly washed in running tap water. The seeds treated with physical and chemical mutagens along with controls were put in the germinator for two days at 20^0C. Number of germinated seeds in each treatment were counted after 48 hours.

RESULTS AND DISCUSSION

The results on germination pattern with respect to effect of mutagens on seed germination are prsented in Table 1. Ethidium Bromide was found most effective mutagen followed by Malic Hydrazide, Acriflavin (medium damage) with respect to germination of these two varieties. Dose dependent relationship was noticed in all the five mutagens i.e. with increase in dose, there was corresponding increase in damage. The maximum per cent reduction in germination over control was observed in NDYR10 at 1500 ppm and 2000 ppm followed by 1000 ppm in Ethidium Bromide.

The results clearly revealed that higher concentration of mutagens showed harmful effect on germination than lower concentrations. Similar results were reported by Garg et al.(1972) in Indian colza.It was also observed that germination in all treatments of yellow seeded strain, NDYR 10 was low as compared to brown seeded Narendra rai. This may be due to thinner seed coat in yellow seeded type. The effectiveness of mutagens were EB>MH>Acriflavin>EMS>Gamma rays, which clearly showed the superiority of gamma rays and EMS over others.

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Table1. Effect of mutagens on germination in two Indian mustard varieties

Treatments	Varieties			
	Narendra rai		NDYR 10	
	Germination (%)	Per cent reduction over control	Germination (%)	Per cent reduction over control
Control	96	-	96	-
Gamma 25 kR	95	1.04	95	1.04
Gamma 50 kR	95	1.04	94	2.08
Gamma 75 kR	94	2.08	94	2.08
Gamma 100kR	93	3.12	92	4.17
Gamma 125 kR	92	4.17	91	5.21
EMS 0.1%	95	1.04	94	2.08
EMS 0.2%	94	2.08	93	3.12
EMS 0.3%	93	3.12	92	4.17
EMS 0.4%	92	4.17	90	6.25
EMS 0.5%	90	6.25	89	7.29
EB 1000 ppm	89	7.29	14	85.42
EB 1500 ppm	87	9.38	-	100.00
EB 2000 ppm	80	16.67	-	100.00
Ac 1000 ppm	95	1.04	84	12.50
Ac 1500 ppm	92	4.17	83	13.54
Ac 2000 ppm	88	8.33	73	23.96
MH 1000 ppm	91	5.21	89	7.29
MH 1500 ppm	89	7.29	73	23.96
MH 2000 ppm	70	7.08	69	28.13

Breeding and Agronomic Characters of Bt Transgenic Insect-resistant *Brassica napus* Lines

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INTRODUCTION

The *Pieris rapac* Linnaeus is mainly insect pest on rapeseed. The effect of insecticide is poor and brings environmental pollution. The breeding of Bt transgenic insect-resistant rapeseed will be efficiently. The Bt toxic protein gene transformation had some paper, but the line breeded had not reports. So the breeding of Bt transgenic insect-resistant rapeseed was carried out.

MATERIALS AND METHODS

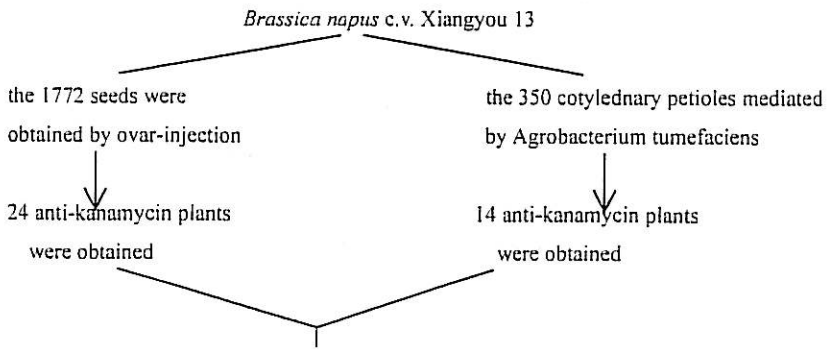
1.Using *Brassica napus* cv. Xiangyou 13(00) as receptor, Bt toxic protein gene was introduced into rapeseed via ovary-injection. The vector plasmid is pFWZ10.

2.Factors affecting transformation of cotyledonary petioles of *Brassica napus* mediated by *Agrobacterium tumefaciens* LBA4404 were explored, and transgenic plants were obtained by introducing Bt toxic protein gene into *Brassica napus* c.v. Xiangyou 13.

3.The agronomic characters and quality characters were breeded after transformation successfully.

RESULTS AND DISCUSSION

1. The anti-kanamycin plants were obtained at highest frequency of 12.8% when 0.5-1.5 μg foreign DNA was injected into the middle of ovary from 20th hour to 30th hour after pollination. Molecular hybrid analysis showed that Bt toxic protein gene was integrated into genome of rapeseed, transgenic plants were obtained at frequency of 1.6%. The result was showed: the transforming method of ovar-injection is efficient and practical for rapeseed.
2. The frequency of anti-kanamycin rapeseed plants on *Agrobacterium tumefaciens* mediating was 8.3%. The frequency of transgenic rapeseed plants was 1.18%.
3. The selection processes of Bt transgenic insect-resistant rapeseed lines were shown at Fig 1.



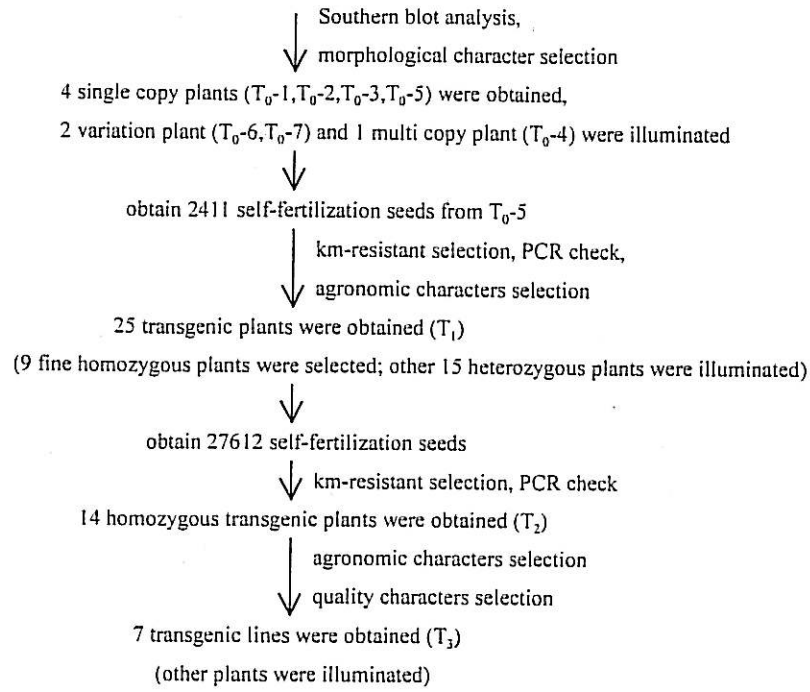


Fig 1 The selection process Bt of transgenic insect-resistant rapeseed lines

The new lines were identical to the recipient parent Xiangyou 13 except slightly larger and lighter leaves at the early stage and stronger insect resistance at the Rosette stage (Table 1,2).

Table 1 Characters of Bt transgenic rapeseed line

	Growth period (d)	Plant height (cm)	Pod No. per plant	Seeds No. per plant	1000 seeds weight (g)	Erucic acid content(%)	Glucosinolate content(umol/g)
Bt transgenic rapeseed	210	160.5	368.2	22.7	3.94	0.19	23.20
Xiangyou 13 (CK)	210	161.7	349.8	21.4	4.00	0.20	21.50

Table 2 No. of *Pieris rapac* per plant of Bt transgenic rapeseed line

Date	30 Oct.	20 Nov.	10 Dec.	30 Dec.
Bt transgenic rapeseed	0.4	1.5	1.2	0.8
Xiangyou 13(CK)	8.3	8.4	9.3	7.0

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INHERITANCE OF PURPLE PIGMENT IN FOLIAGE IN MUSTARD (*Brassica juncea* (L.) Czern. & Coss.)

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Of the several diseases of mustard, leaf blight caused by *Alternaria brassicae* (Berk.) Sacc. results in a severe damage to the crop, often being epidemic in nature and causes 40-70% reduction in seed yield in India [2,3]. Most of the cultivars show susceptible to partial tolerance reaction to leaf blight. In the germplasm collection, one line 'Ornamental Rai' (OR) shows high degree of tolerance/resistance to this disease and may constitute donor for incorporating leaf blight resistance in otherwise high yielding cultivars. But this line (OR) has purple colour foliage coupled with reduced seed-weight, poor seed yield and late maturing. Controversial reports on the inheritance of purple colour in foliage [1,4] as well as above negative attributes hamper the scope of transferring the resistance into the cultivated types. This paper deals with the inheritance of purple colour in foliage in mustard (*B. juncea* (L.) Czern. & Coss.).

Ornamental Rai was crossed with 10 diverse promising cultivars (Varuna, Kranti, Vardan, Rohini, Pus-bold, RH 8812, 8813, 8814, RLM 1359 and NDR 8501) and F_1, F_2, BC_1, BC_1 (selfed), BC_2, BC_2 (selfed) progenies were produced in each of the above 10 crosses during 1997-98 to 1999-2000. All these progenies including parents of each of the 10 crosses were raised in 2 replications following usual agronomic practices during 2000-01 at the Research farm of the University.

The F_1 had purple coloured foliage and segregation in F_2 showed good fit to 3 (purple) : 1 (green) ratio, which indicated that purple colouration in foliage was controlled by a single dominant gene (Table 1); there was no reciprocal difference in the F_1. All the crosses showed individually this same pattern of inheritance. Irrespective of the cross, this inheritance pattern was further confirmed by analyses of the back-cross progenies (BC and BC selfed). Further, the plant with green foliage in F_2 generation bred true in F_3, which indicated that the plants with

green foliage had a recessive gene.

Based on the above studies, the incorporation of tolerance/resistance gene for Alternaria leaf blight from OR is in progress at this Institute. At present, the advance generation lines (developed through several backcrossing and sib-mating of desirable types in F_2 involving same/different crosses) with high seed yield coupled with an improved level of tolerance to leaf blight and plant type have been isolated and are being evaluated against the best check 'Kranti'.

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Table 1. Segregation of Purple colour in foliage in F_2 , BC_1 , BC_1(selfed), BC_2 and BC_2(selfed) in crosses of 10 cultivars (Pooled) with 'Ornamental Rai' in mustard

Particular	Segregation-No. of plants		Segregation for ratio	χ^2 Value	P-value for Exp.ratio
	Purple-leaf	Green-leaf			
Ornamental Rai (P_1) x Cultivar (P_2)					
All 10 crosses pooled:					
P_1	All	-	-	-	-
P_2	-	All	-	-	-
F_1s	All	-	-	-	-
F_2s	2968	1015	3:1	0.483	0.30-0.40
BC_1 (F_1 x P_1)	All	-	-	-	-
BC_1 (Selfed)*	1630	265	7:1	3.781	0.05-0.10
BC_2 (F_1 x P_2)	467	428	1:1	1.788	0.10-0.20
BC_2 (Selfed)*	936	1496	3:5	1.011	0.30-0.40

* All plants of BC_1 and BC_2 selfed to raise BC_1(selfed) and BC_2(selfed) progenies.

Assessing suitable combiners in *Brassica juncea* L. for high altitude acidic soils

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ABSTRACT

RW 873 was good general combiner for seed yield/plant, oil content, 1000 seed weight, harvest index, days to maturity, plant height and days to 50% flowering and as such was the best parent. On the other hand, crosses RW 873 x PR 18, PR 830 x RH 843, RW 29-6-3 x Vardan, PR 18 x BR 40 and RH 843 x Vardan were the best specific combiners for seed yield/plant.
Key words :- *Brassica juncea*, combining ability, Mustard.

Introduction

In the present study an effort has been made to find out the lines and their crosses based on combining ability. The analysis is based on six environments so as to formulate effective breeding strategy to improve seed yield and its attributes in mustard (*Brassica juncea* L) grown at 625 meters above mean sea level in red letterite soils.

Materials and Methods

The materials for the study generated during winter 1995-96 consisted of 45 genotypes (9 parents and their diallels excluding reciprocals). The above 45 genotypes were sown on three dates (E_1 & E_4 on 27th September, E_2 & E_5 on 4th October and E_3 & E_6 on 11th October 1997) at North-South as well as in East-West sowing directions with two replications on each date during winter season at the University experimental area, Ranchi. The distance between the rows and plants was maintained at 30 and 10 cm, respectively. Cultural practices as recommended for the area were followed. The observations were recorded for eleven yield attributes (Table 1) from ten randomly selected competitive plants. Analysis was done following Griffing (1956).

Results

The analysis of varaince for combining ability revealed highly significant mean square estimates due to general combining ability for most of the characters studied. Similarly, the estimates of mean square due to specific combining ability were also high significant for all the character, except, number of primary and secondary branches/plant. This indicated that both additive and non-additive genetic components were involved in determining the expression of these characters. The magnitude of specific combining ability effects was much higher as compared to the general combining ability effects for all the characters studied, except, number of secondary branches/plant as evidenced by ratio of the two variances. The predictability ratio was less than unity reflecting and confirming preponderance of non-additive gene effects for all the characters studied, except, days to 50% flowering, number of secondary branches/plant, plant height, days to maturity, seed yield/plant and oil content (Table 1). Similarly, presence of overdominance was revealed by days to 50% flowering, plant height, number of siliquae/plant and days to maturity. The best general combiner was RW 873 which besides seed yield/plant was also a good combiner for days to 50% flowering, plant height, days to maturity, harvest index, 1000-seed weight and oil content. RH 843 was observed to be a good combiner for seed yield/plant, number of primary branches/plant, number of secondary branches/plant and number of siliquae/plant. Five cross combinations, namely, RW 873 x PR 18, PR 830 x RH 843, RW 29-6-3 x Vardan, PR 18 x BR 40 and RH 843 x Vardan, expressed highly significant sca effects for seed yield/plant. It was observed that in most of the cases the crosses showing high sca effects also exhibited high heterosis. It was also observed that in three of the above crosses (*viz.*, RW 873 x PR 18, PR 830 x RH 843 and RH 843 x Vardan), at least one parent was good general combiner for seed yield, while in rest crosses, both the parents were poor general combiner.

REFERENCE

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Table 1. General and specific combining ability in different traits of mustard.

Genotypes	1	2	3	4	5	6	7	8	9	10	11
RW 873	**	*		**			**	**	*	*	*
RW 873 X PR 830	*										**
" X Kranti						**	**				**
" X RW 29-6-3	**			*			**				
" X PR 18				**		**		*	**	**	
" X RH 843		*				**			**		
" X RH 851	*					*	**				
" X Vardan										*	
PR 830				*							
PR 830 X Kranti									*		*
" X RW 29-6-3						*		**			
" X RH 843								*		**	
" X RH 851				*						*	*
" X Vardan	**						*		**		
" X BR 40			*	**		**		**			
Kranti X RH 843	*									*	
" X RH 851							*				**
" X Vardan	*						**				
" X BR 40				*		**		**		*	
RW 29-6-3						*	*				
RW 29-6-3 X PR 18	**					*		**	**	*	
" X RH 851				*			**				
" X Vardan				**	*				**		
" X BR 40	*	*			*		*	*	*		
PR 18	**			*							
PR 18 X RH 843		**	**	**	**	*			*		
" X Vardan					**						**
" X BR 40			**		**	**		**		**	
RH 843		**	**	**	**			**		*	**
RH 843 X RH 851			*	**	**	**					*
" X Vardan		**	**							**	
" X BR 40		*									
RH 851	**		*				**				
RH 851 X Vardan		*	*	*						*	
" X BR 40							**				
Vardan	*			*		*					
Vardan X BR 40			*	*	**						
BR 40	*			**		*		**	*		

1. Days to 50% flowering, 2. Primary branches per plant, 3. Secondary branches per plant, 4. Plant height, 5. Siliquae per plant, 6. Seeds per siliqua, 7. Days to maturity, 8. Harvest index, 9. 1000 – seed weight, 10. Seed yield per plant and 11. Oil content (%).

***, ** Significant at 5% and 1% probability levels, respectively (* , ** for negative value).**

Association among seedling and yield contributing traits in mustard

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Extensive field testing of large number of hybrids is required to identify a suitable hybrids combinations which is painstaking and expensive. Screening for seedling characters in laboratory and correlating them with yield and yield contributing characters may prove a better alternative to minimize the number of hybrids for their actual field test. Keeping this in view, the correlation study among seedling and yield contributing characters was undertaken in Indian mustard.

Fifteen geographically diverse strains used as lines, three testers (Kranti, DIRA-313 and TM-22) and their 45 F_1S were studied for four seedling characters viz., germination per cent (Anon, 1985), dry weight of seedling (mg/10-seedlings), vigour index (Abdul-Baki and Anderson, 1973) and electrical conductance of seed leachate (dSm^{-1}) in Seed Technology Laboratory. Simultaneously, these 63 genotypes were raised in the field in RBD with three replications in single row of 3 m length spaced at 45 cm apart at Main Experiment Station of the University. Observations were recorded on 5 randomly selected competetive plants for number of primary branches/plant, secondary branches/plant, siliquae/plant, seeds/siliqua and seed yield/plant (g). Genotypic correlation between seedling characters with yield and yield contributing characters were computed following standard procedure.

A perusal of Table 1 showed that among seedling characters, only vigour-index manifested significant positive association with germination percentage (0.29*) and seedling dry weight (0.83**). All seedling characters, except germination percentage, exhibited significant desirable correlations with seed yield. Significant desirable associations of seedling characters with yield components were reflected between germination percentage and seeds/siliqua (0.36**), seedling dry weight and primary branches/plant (0.32**), vigour-index and primary branches/plant (0.32**), and vigour-index and siliquae/plant (0.31**). Similar results were reported by Varshney and Asin (1999).

All the four yield components viz., primary branches/plant, secondary branches/plant, siliquae/plant and seeds/siliqua exhibited significant positive association with seed yield.

Inter-relationship among component traits showed significant positive association of primary branches with secondary branches/plant (0.63**), and siliquae/plant with primary branches/plant (0.51**) and secondary branches/plant (0.55**). These findings and those of Reddy (1991) have shown that primary branches/plant, secondary branches/plant, siliquae/plant and seeds/siliqua are major yield contributing traits in mustard.

Manifestation of high order desirable correlations of seedling characters with seed yield as well as major yield components as observed in this study, may prove useful in identifying superior genotypes based on seedling characters before elaborate testing under field condition.

Table 1: Association among seedling and yield contributing characters in mustard

Characters	Seedlings dry Wt.	Vigour - index	Ec of seed leachate	Primary branches/ plant	Secondary branches/p lant	Siliquae / plant	Seeds/ siliqua	Seed yield/plant
Germination (%)	0.15	0.29*	-0.18	0.10	0.07	0.14	0.36**	0.13
Seedlings dry weight	--	0.83**	0.03	0.32**	0.14	0.11	0.13	0.48**
Vigour-index	--	--	-0.03	0.32**	0.17	0.31**	0.18	0.52**
Ec of seed leachate	--	--	--	-0.01	-0.12	0.07	-0.11	-0.24*
Primary branches/ plant	--	--	--	--	0.63**	0.51**	0.20	0.30**
Secondary branches/plant	--	--	--	--	--	0.55**	0.21	0.34**
Siliquae/plant	--	--	--	--	--	--	-0.01	0.29*
Seeds/siliqua	--	--	--	--	--	--	--	0.40**

**, significant at P = 0.01

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GERMPLASM RELEASE OF THREE WHITE FLOWERED *BRASSICA NAPUS* LINES

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An incompletely dominant white flower character was introduced into resynthesised *Brassica napus* from *Brassica oleracea* ssp *albogabra* by Chen and Jönsson (1987). The white flowered trait was shown to be linked to the presence of erucic acid in the seed (Woods and Séguin-Swartz 1997a, b), and the trait in the background of cv Westar was made available at the 10th Crucifer Genetics Workshop in Rennes in 1997. In establishing the linkage relationship zero erucic white flowered derivatives were obtained, and seed of these has been increased. As part of a study of cross pollination in self-incompatible lines of *B. napus* the white flowered trait was also introgressed into cv Topas and cv Global (Lewis *et al.* 2000). These two lines are also being released, however they have signigificant seed erucic acid.

Breeding

As described by Woods and Séguin-Swartz (1997a, b) the original white flowered introduction was crossed to cv Westar to obtain BC_3 material. At this stage all the material obtained contained erucic acid. The BC_3 material was used as a parent in the linkage study involving a further cross to cv Westar, and among the BC_4F_2 plants four plants were identified which were white flowered and contained zero erucic acid. Twenty-four BC_4F_3 plants of each line were grown, and eleven plants identified as white flowered. From each of these eleven BC_4F_3 plants 24 BC_4F_4 plants were grown, and six of the eleven produced only white flowered progenies. In two of the six BC_4F_3 derived seed lots all twenty-four BC_4F_4 plants had less than 0.3% erucic acid, (the maximum in the entire population was 2.6%). Ten BC_4F_4 plants were selected and 24 BC_4F_5 plants grown from each. All were white flowered. The harvested seed was analysed for erucic acid, any with 0.2% or above discarded (highest observed was one plant at 2.1%, predominantly those discarded were 0.2%), and the remaining 176 seed lots composited as CB 0040.

The BC_3 material was also used as a parent in producing the cv Topas and cv Global derivatives. The recurrent parent was used to produce the BC_4, and a pure white flowered line developed by single seed descent. The BC_4F_4 was grown in the field in isolation in 1995. Field harvested seed of the cv Topas derivative contains 19.3% erucic acid and is designated CB 0041, the cv Global derivative contains 16.9% erucic acid and is designated CB 0042.

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 following designations: CN 19052 for CB 0040, CN 19053 for CB 0041, and CN 19054 for CB 0042.

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AN INVESTIGATION ON LINKAGE BETWEEN THE APETALOUS CHARACTER AND ERUCIC ACID IN *BRASSICA RAPA*.

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In producing isogenous *Brassica rapa* lines with and without petals to use to evaluate the character as a means of reducing crop susceptibility to Sclerotinia, it became apparent that the presence of seed erucic acid was strongly linked with the apetalous character. This paper describes the estimation of map distance between the apetalous locus and an erucic acid locus.

Materials and methods

The original source of the apetalous character was obtained from the Crucifer Genetics Cooperative[1] as CrGC line 1-41, a rapid cycling ideotype (ie a small, rapid developing plant, not suitable for field production), with the reported genotype *apt/apt*. CrGC 1-41 has brown seed, and is assumed to be rapeseed quality, ie high in erucic acid and glucosinolates. This line was taken to back-cross one using AC Sunshine, and a line derived from AC Sunshine as the recurrent parent, to produce material suitable for field trials. Among the segregating BC_1F_2 plants 10 were identified as apetalous, and interpollinated. Seed from these was analysed by gas-liquid chromatography, and all were found to be high in erucic acid (23 to 43% C22:1). This material was used for a linkage study.

The initial step was to generate a self-compatible apetalous population. To do so a canola quality dominant self compatible breeding line was used, which was crossed to 7 different apetalous BC_1F_3 plants. This new cross was taken to F_3 with selfing each generation, with selection for the apetalous character on the F_3 plants. F_4 seed lots were tested to confirm the presence of erucic acid, and crossed again to the canola-quality dominant self-compatible line. Four plants of each of 10 F_1's were greenhouse grown and self pollinated, and 32 F_2 plants of each of 20 F_1 derived lines were planted. At flowering the plants were rated as petalled or apetalous and self-pollinated. Ten individual seeds from each plant of a representative sample of F_2 plants were assayed for erucic acid content, enabling each F_2 plant to be classed as high, low, or heterozygous for this character.

Linkage model

A simplified model for map distance in F_2 populations was used, assuming that the level of double crossovers was small enough to be ignored. Designating the proportion of crossovers as "P", the dominant petalled flower allele as "Apt" with the recessive petalless flower as "apt", and the allele for the presence of erucic acid as "E" with the recessive, no erucic acid allele, as "e" leads to the following model:

The original parents are "apt.apt.E.E." and "Apt.Apt.e.e.", and the F_1 is thus "Apt.apt.E.e."

Gametes:

"apt.E." = $^1/_2$ (1-P) "apt.e." = $^1/_2$ P ie "apt.E." + "apt.e." sum to $^1/_2$

"Apt.e." = $^1/_2$ (1-P) "Apt.E." = $^1/_2$ P

As indicated in Strickenburg (1985) an F_2 4 x 4 phenotype matrix may be derived. Since not

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all the F_2 plants were analysed for erucic acid, a combined ratio could not be used, and the matrix was used to develop independant figures for crossover probability for each petal class. Within the apetalous class the proportion of plants with crossovers would be $2P\text{-}P^2$, and these would be either low or heterozygous for erucic acid. Within the petalled class only phenotypes with high erucic acid are identifiable as the result of a crossover, and the proportion of these would be $1/3(2P\text{-}1P^2)$.

Results and discussion

Table 1. Classification of a sample of 296 F_2 plants from the cross apetalous-high erucic x petalled-low erucic.

	Erucic acid			
	High	Low	Heterozygous	Total
Petalled	39	57	122	218
Apetalous	67	3	8	78
Total	106	60	130	296

The total F_2 population grown was 576 plants, which segregated 92 apetalous : 483 petalled. This is significantly different (χ^2) from the expected single gene ratio of 144:432. This deficiency of apetalous plants was also observed at the F_2 and BC_1F_2 generations during the production of the parents used in this project.

For the apetalous class $2P\text{-}P^2 = 11/78$, P=0.0732, ie a map distance of 7.32 centiMorgans. For the petalled class $1/3(P\text{-}P^2) = 39/218$, P=0.3194, ie a map distance of 31.94 centiMorgans.

Of the two calculated map distances, the lower one is probably the better estimate. The numbers of plants classed as petalled+high erucic is probably inflated, which would result in a higher apparant crossover rate. The failure of the apt gene to act reliably as a single simple recessive allele in this and other generations means that some "apetalous" plants could have been classified as petalled, and in the absence of a crossover event these would be high erucic. Because the number of single seeds per plant examined was limited to 10 it would also be possible to classify a heterozygous (segregating) plant as a high erucic one, because of missing the low erucic segregant in this small number of seeds.

Kelly et al (1995) showed the presence of six loci in *Brassica napus* controlling the petalless character, three of which were located on the A (*rapa)* genome. This may well explain the poor fit to single gene ratios in this data set. Woods and Séguin-Swartz (1997) showed linkage between white flower colour and erucic acid in *B. napus*, probably on the C (*oleracea)* genome. It is interesting that in two cases of genes effecting petals have been found linked to seed erucic acid genes in oilseed rape.

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CHANGES IN ACTIVITIES OF OXIDATIVE ENZYMES IN *BRASSICA JUNCEA* LEAVES DURING INTERACTION WITH *ALTERNARIA BRASSICAE*

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Brassicas is an important group of oilseed crops. 30-50 per cent yield losses of Indian mustard (*B. juncea*) have been reported due to Alternaria leaf blight disease alone (Saharan, 1992). Oxidative enzymes like peroxidase (PO) and polyphenoloxidase (PPO) have been implicated in plant disease resistance (Arora and Bajaj, 1985; Kumari *et al.*, 1995). Catalase is a competitor of PO for the substrate H_2O_2 and supresses PO activity, thus exhibiting an indirect correlation to resistance. Earlier, we reported the role of phenolics as non-specific resistant factors in *B. juncea*/*A. brassicae* interaction (Gupta *et al.*, 1998). In this communication we are reporting the changes in the activities of oxidative enzymes viz. PO, PPO and catalase to ascertain their correlation with resistance/ susceptibility. Two cvs. of *B. juncea*, RC-781 (resistant) and Varuna (susceptible) were used. As described previously (Gupta *et al.*, 1998), the crop was raised in earthen pots in screen house, inoculated with the pathogen at 50 d stage and fresh leaf samples from control (uninoculated) and inoculated plants were collected at 6, 12, 24, 48, 72 and 120 h after inoculation and enzyme extracts were prepared in 0.1 M phosphate buffer (pH 7.0) centrifuged (4°C). The homogenate was strained through 4 layers of cheese cloth, the filtrate centrifuged at 10,000 g for 20 min in a cold centrifuge and the supernatant was used for assay of enzymes and protein estimations. PO and PPO were assayed by the modified methods of Shannon *et al.* (1966) and Taneja and Sachar (1974) respectively. Catalase activity was measured by the method of Sinha (1972) and protein content by Lowry *et al.* (1951).

The results presented in Table 1 indicate that peroxidase is contributing towards resistance as a post infectional factor since its activity, in general, increased more sharply in the resistant cv. RC-781 as compared to the susceptible one (Varuna). On the other hand, PPO seems to act both as pre-existing as well as post-infectional factor as the PPO activity was found to be higher in the leaves of the control plants of the resistant cv. as well as a much higher increase following inoculation was noticed as compared to the susceptible cv. Both PPO and PO enzymes mainly catalyze the oxidation of phenolic compounds through PPO-PO-H_2O_2 system whose reaction products confer resistance to the host. Peroxidase has also been reported to be involved in the biosynthesis of innert polymer lignin. A significant positive correlation between the activities of these enzymes and their role in disease resistance has been reported in various other host parasite interactions (Shimoni *et al.*, 1991; Lozkowska and Holubowska, 1992). Our results also support these findings and suggest their positive role in resistance. Not much differences were observed in the levels of activity of catalase in various samples in this interaction and thus it does not compete with PO for the substrate and allows it to carry out oxidation of phenols to more toxic quinones that enhance their role in disease resistance. However, the exact role of catalase in resistance/susceptibility of plants towards pathogens is still controversial as its increased activity has also been reported in a resistant cv. (Vir and Grewal, 1975).

Table: 1 Peroxidase (PO)*, polyphenol oxidase (PPO)* and catalse activities in control and inoculated leaves of two *Brassica* cvs. Resistant and susceptible to *Alternaria brassicae*

HAI**	RC-781 (R)						Varuna (S)					
	PO		PPO		Catalase		PO		PPO		Catalase	
	C	I	C	I	C	I	C	I	C	I	C	I
0	32.10 ±0.69	32.10 ±0.69	5.46 ±0.05	5.46 ±0.05	3.38 ±0.02	3.38 ±0.02	32.70 ±0.18	32.70 ±0.18	5.27 ±0.13	5.27 ±0.13	3.71 ±0.08	3.71 ±0.08
6	30.00 ±0.24	42.80 ±0.24	5.70 ±0.11	11.52 ±0.24	3.64 ±0.01	3.37 ±0.11	29.32 ±0.31	41.81 ±0.31	5.05 ±0.05	7.12 ±0.14	3.26 ±0.11	3.60 ±0.12
12	34.80 ±0.28	52.38 ±0.62	5.95 ±0.07	13.25 ±0.20	3.66 ±0.02	3.54 ±0.18	34.91 ±0.44	42.90 ±0.62	5.86 ±0.07	7.35 ±0.20	3.56 ±0.12	3.48 ±0.02
24	36.36 ±0.44	53.68 ±0.56	6.07 ±0.18	12.83 ±0.24	3.37 ±0.10	3.24 ±0.08	34.07 ±0.35	44.57 ±0.42	5.34 ±0.07	7.40 ±0.05	3.25 ±0.04	3.37 ±0.03
48	37.80 ±0.65	59.10 ±0.39	6.01 ±0.12	12.77 ±0.17	3.55 ±0.12	3.49 ±0.02	37.39 ±0.65	46.42 ±0.52	5.48 ±0.06	7.40 ±0.05	3.66 ±0.01	3.54 ±0.02
72	34.93 ±0.73	59.71 ±0.34	6.39 ±0.05	12.80 ±0.20	3.52 ±0.03	3.42 ±0.01	32.56 ±0.36	47.70 ±0.92	5.32 ±0.04	7.61 ±0.08	3.52 ±0.07	3.40 ±0.03
120	36.90 ±0.38	79.53 ±0.56	6.80 ±0.06	14.67 ±0.08	3.85 ±0.08	3.76 ±0.04	35.71 ±0.34	49.73 ±0.88	5.73 ±0.10	7.98 ±0.05	3.62 ±0.04	3.76 ±0.04

*Units/mg protein, 1 unit = Δ0.1 O.D. at 430 nm/min (Values are mean ± S.D. of ten values).

**Hours after inoculation. C = Control, I = Inoculated.

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Association of a Molecular Polymorphism with Black Rot Resistance Derived from Ethiopian Mustard

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Introduction: Black rot (*Xanthomonas campestris* pv. *campestris*) is one of the most serious diseases of crucifers, especially during warm, damp seasons. It is easily spread from contaminated seed in nurseries and through mechanical transmission in the fields. Symptoms of the disease include V-shaped lesions originating from the margin of the leaf, and as the lesions enlarge the plant wilts and eventually rots. There are no effective chemicals for the control of black rot, and while copper bactericides are applied they have limited effect. The most effective approaches to controlling black rot are through good farm management practices, hot water treatment of seeds and the use of cultivars with resistance to the disease. Hot water treatment may reduce seed viability and does not fully eradicate the disease. Host plant resistance while partially effective, is not complete and can still result in spread of the disease throughout plantings. A new source of black rot resistance reported to be controlled by a single dominant gene was identified in a *Brassica napus* accession PI 199947 (later identified as *B. carinata*) and was used to transfer resistance to broccoli by protoplast fusion (Guo *et al.*, 1991; Hansen and Earle, 1995). Following identification of a somatic hybrid between PI 199947 and rapid cycling *Brassica,* additional crosses to the broccoli cultivar 'Green Comet' were made to stabilize the resistance. Lower than expected ratio's of resistant plants were observed in F2 populations generated from resistant broccoli lines, and the resistance source was further studied to better clarify the genetic control.

Materials and Methods: The black rot resistant broccoli line (11B-1-12) developed from *B. carinata* was crossed to the black rot susceptible cauliflower cultivar 'Snowball', and the hybrid was self-fertilized to form an F_2 population segregating black rot resistance. The objective of this study was to examine the relationship between RAPD polymorphisms (RAPDs) segregating within the generated F_2 population, and form associations with disease severity ratings of plants at juvenile and mature stages.

One-hundred seedlings were sown in $(3.8\ cm)^3$ 'Speedling' flats for inoculation and re-planted into six-inch pots at the 6-week stage. Four isolates of black rot were grown on plates of YDCP at 28°C for 48 hours before inoculation. Plants were wound inoculated with each isolate by puncturing the leaves with two black rot infected needles either side of the mid-rib. Plants were placed in a mist chamber for 48 hours at 28°C with a 14-hour photoperiod following inoculation to encourage infection. The inoculation was performed on the 4-week old seedlings from the generated F_2 population and 32 control plants of broccoli and cauliflower varieties 'Marathon' and 'Delira' were simultaneously inoculated to ascertain infection rate. The plants were re-inoculated at the 10-week stage to test for mature plant resistance. Plants were rated on a scale of 0 – 5, where 0 = completely resistant, and 5 = completely susceptible.

Results and Discussion: Disease severity ratings at the juvenile and mature plant stages indicated that complete resistance to black rot was being recovered in the F_2 population with some intermediate resistance to black rot in the mature plant inoculation trial. The proportion of completely resistant plants observed (10% juvenile, 8% mature) suggested that more than two genes controlled black rot resistance derived from PI 199947 differing from two previous studies of this resistance (Guo *et al.*, Zhou *et al*, 1997). Of the 10 plants rated 0 at the seedling stage, eight were still rated 0 at the mature stage suggesting that the resistance is consistent at the juvenile and mature plant stages.

A total of 16 RAPDs were scored for the F_2 population based on presence/absence of amplified bands. Mean disease severity rates were compared with RAPD band presence/absence and RAPDs exhibiting significant associations with resistance/susceptibility were determined. The most significant association with resistance was observed for the marker OP AB04-2. All plants in which the primer was amplified were rated zero at the juvenile and mature stages except for one plant which rated as 1 in the juvenile inoculation test. However, only 9 plants out of the 100 scored amplified this polymorphism, far less than the 75 that would be expected using a dominant RAPD marker. This suggests that a single dominant gene may indeed control the resistance, but the resistance to black rot in this population may not be fully stabilized at the chromosome level.

Protoplast fusion involves the somatic hybridization of protoplasts containing different numbers of chromosomes. The somatic fusion the *B. carinata* accession PI 199947 protoplast (n=17) with a *B. oleracea* protoplast (n=9) resulted in chromosomal instability for several generations of backcrossing. It is possible that the black rot resistance introgressed from PI 199947 is not fully stabilized in the plant used as a resistant parent (11B-1-12), and that further backcrossing to *B. oleracea* will be required to stabilize this resistance source before it can be used in commercial varieties. If stabilized, the apparent single dominant gene controlling resistance could be extremely valuable in the future breeding of commercial cabbage types.

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SOURCES OF RESISTANCE IN INDIAN MUSTARD AGAINST WHITE RUST AND *ALTERNARIA* BLIGHT

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Introduction

Indian mustard (*Brassica juncea*) is the most important *Brassica* oil yielding crops in Indian subcontinent because of its relative tolerance to biotic and abiotic stresses and inherent high yield potential. Amongst the major production factors, white rust (*Albugo candida*) and *Alternaria* blight (*Alternaria brassicae*) are severe menace in sustaining higher yields due to lack of genotypes with multiple disease resistance.

Materials and methods

To identify potential sources of resistance effective against white rust and *Alternaria* blight, 45 genotypes of *B. juncea* were sown in the field. The genotypes of *B. juncea* were comprised of Indian and Exotic collections. The seeds of all the genotypes were assumed to be pure and homogenous. The experimental crop was laid out in a randomized block design with three replications at the Research Farm Area of Project DTR-10, Directorate of Research, CCS HAU, Hisar. Paired rows of 6 meters long of all genotypes were planted in each replication at 30 x 10 cm spacing. The inoculation of pathogens and scoring of disease were done as described earlier (Saharan, 1997). Scoring was done only at the leaf phase of the host.

Results and discussion

The disease severity index and disease reaction of white rust and *Alternaria* blight at leaf phase on different genotypes of Indian mustard have been presented in Table 1. A critical perusal of Table 1 revealed that significant genetic variations existed for *A. candida* disease severity index at leaf phase in tested genotypes. However, the disease severity index for white rust disease ranged between 0.00 to 4.60. The genotypes namely EC-129126-1, PR-8805, Zem-1 and Shiva were completely free from *A. candida* infection at leaf phase. Hence, these genotypes were identified as rich donor source line(s) for white rust resistance. The recommended local and national cultivars, viz., RH 30, Varuna, RH 781, were observed highly susceptible to *A. candida* at leaf phase. The genotypes, viz., DHR-994, DHR-995, DHR-993, DHR-9204, DHR-9202, DHR-9102, DHR-979, PCR-28, DHR-9205, DHR-9506, DHR-9301, DHR-978, DHR-9601, DYS-25-9, DHR-19, DHR-9614, DHR-9503, DHR-9516, DHR-9781, DHR-973, DHR-9514 and DHR-9517 were resistant to *A. candida* at leaf phase.

None of the tested genotypes of Indian mustard was observed completely free from *Alternaria brassicae* infection at leaf phase. However, the significant variations in disease severity index (0-5 scale) for *Alternaria* blight on leaf phase were observed on different genotypes. The disease severity index for *Alternaria* blight ranged between 1.45 - 3.62 on tested gene pool indicating that most of the genotypes tended towards susceptibility and level of resistance was medium to low. The minimum disease score was observed on DHR-9204, EC-129126-1, PR-8805 and RC 781 and these genotypes were grouped as moderately resistant. Therefore, these source line(s) may be used for developing *Alternaria* blight resistance cultivars. The recommended cultivars for different agro-climatic zones with varying conditions of the country (RH 30, Kranti, RH 8113, RL 1359, Rajat, Varuna, RH 781, PHR-1 and Pusa bold) were found equally susceptible to *Alternaria* blight. Out of 45 genotypes/ lines of *B. juncea* screened through sequential inoculation technique only three genotypes namely, EC-129126-1, PR-8805 and RC 781 were found resistant to both *A. candida* and *A. brassicae*.

Significant genetic variations were observed amongst different genotypes for two pathogens (*A. candida* and *A. brassicae*) under test. The genotypes EC-129126-1, PR-8805, ZEM-1, Shiva and RC 781 were resistant to *A. candida* infection. These findings are in conformity with earlier reports of Kaushik and Saharan (1980) and Saharan *et al.* (1988). However, variations in the disease severity index may be due to prevailing environmental factors, inoculum level and purity level of genotypes used for screening.

Contrary to white rust, none of the genotypes of *B. juncea* was completely free from the infection of *A. brassicae*. However, critical evaluation of the genotypes revealed that greater variations existed amongst the genotypes. The genotypes, viz., EC-1291426-1, PR-8805 and RC 781 contracted lesser disease severity index as compared to other genotypes. Kumar and Kumar (1989) and Tripathi *et al.* (1980) also reported similar trend of *Alternaria* blight disease severity.

Table 1 : **Disease severity index and disease reaction of white rust (*A. candida*) and *Alternaria* blight (*A. brassicae*) at leaf phase on different genotypes of Indian mustard (*B. juncea*).**

Genotypes	LEAF PHASE			
	WR		AB	
	DSI	DR	DSI	DR
DHR-994	0.88	R	3.08	S
DHR-995	0.98	R	2.28	MS
DHR-996	1.34	MR	2.22	MS
DHR-992	1.02	MR	2.60	MS
DHR-993	0.90	R	2.26	MS
DHR-991	1.22	MR	3.06	S
PCR-10	1.72	MR	2.08	MS
DHR-9204	0.58	R	1.94	MR
DHR-9202	0.98	R	2.74	MS
DHR-9504	1.04	MR	2.62	MS
DHR-9304	1.16	MR	2.50	MS
DHR-9102	0.92	R	3.20	S
DHR-979	0.40	R	2.80	MS
PCR-28	0.22	R	3.10	S
DHR-9205	0.06	R	2.82	MS
DHR-9506	0.52	R	3.54	S
DHR-9301	0.74	R	3.24	S
DHR-978	0.16	R	3.26	S
DHR-9601	0.40	R	2.62	MS
DYS-25-9	0.18	R	3.20	S
DHR-19	0.30	R	3.18	S
DHR-9614	0.14	R	3.36	S
DHR-9503	0.16	R	3.24	S
DHR-9516	0.92	R	3.04	S
DHR-9781	0.54	R	3.18	S
DHR-973	0.42	R	3.62	S
DHR-9514	0.54	R	2.48	MS
DHR-9517	0.52	R	2.44	MS
DHR-9518	1.36	MR	3.22	S
DHR-9507	2.22	MS	2.68	MS
DHR-9738	2.24	MS	2.44	MS
EC-129126-1	0.00	HR	1.45	MR
RH 781	4.37	HS	2.71	MS
PHR-1	2.35	MS	2.05	MS
Varuna	4.29	HS	3.47	S
Kranti	3.77	S	2.61	MS
RH 8113	2.35	MS	2.52	MS
RL 1359	2.92	MS	3.00	MS
Rajat	3.41	S	2.76	MS
RH 30	4.60	HS	2.75	MS
Pusa bold	3.47	S	2.42	MS
PR-8805	0.00	HR	1.67	MR
RC 781	1.25	MR	1.68	MR
ZEM-1	0.00	HR	2.65	MS
Shiva	0.00	HR	2.22	MS
Mean	1.27		2.24	
C.D. at 5%	0.13		0.12	

Significant at P = 0.05
WR = White rust; AB = *Alternaria* blight; DSI = Disease severity index;
DR = Disease reaction; HR = Highly resistant; R = Resistant; MR = Moderately resistant;
MS = Moderately susceptible; S = Susceptible; HS = Highly susceptible

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Albugo candida induced changes in phenolics and glucosinolates in leaves of resistant and susceptible cultivars of *Brassica juncea*

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White rust caused by *Albugo candida* is an important disease of Indian mustard (*Brassica juncea*). Phenolics in higher plants have been considered as non-specific resistant factor against the pathogens while the glucosinolates are thought to contribute to resistance to pests and disease in the members of the Cruciferae including *B. juncea* and infestation of the brassica tissue results in their accumulation in the host. In order to ascertain the role of phenolics and glucosinolates if any, in expression of resistance/susceptibility in *B. juncea* to *A. candida* both pre-existing and post-infectional changes in phenolic constituents and glucosinolates were studied in leaves of *B. juncea* cvs. resistant (cv. RC-781) and susceptible (cv. Varuna) to *A. candida*.

The *Brassica juncea* crop was raised in pots following recommended package of practices of cultivation. Sixty days old plants kept in polythene enclosed chamber in screen house were inoculated with pathogen by spraying *A. candida* spore suspension (20-30 spores/microscopic field). Proper humidity conditions were created by maintaining enough moisture in the chamber. The control plants were sprayed with sterile distilled water. Leaf samples were collected from uninoculated (control) and inoculated plants of both the cvs. at 1, 3, 5, 8, 12 and 15 d after inoculation and oven dried for quantitative estimations. Total phenols were determined by the method of Swain and Hillis (1959) while flavonols and ortho-dihydroxy phenols were estimated by the method of Balbaa *et al.* (1974) and Nair and Vaidyanathan (1964) respectively. Glucosinolates contents were determined by the method of Sang *et al.* (1984). Both pre-existing as well as following inoculation, the total phenols (Table 1) in the resistant cv. (RC-781) were found to be higher as compared to those of susceptible one (Varuna) suggesting their role in disease resistance. Similar observations have been reported by other workers in different crops (Bhullar *et al.*, 1972; Keller *et al.*, 1996). Almost similar trends in changes in flavonols, orthodihydroxy phenols (ODHP) were observed as that of total phenols. However, our results suggest that ODHP seem to have less significant role as a post infectional factor compared to total phenols and flavonols in contributing towards disease resistance. Similar results in cotton v/s *Xanthomonas campestris* pv. *malvacearum* were obtained by Chawla *et al.* (1991).

Infection of the plants of the resistant cv. showed a significant increase in the content of glucosinolates while negligible increase was observed in the susceptible cv. Glucosinolates, the sulphur containing glycosides occurring in the vegetative and reproductive tissues of oilseed rape have been suggested to have a positive role in host resistance (Nashaat and Rawlinson, 1994; Giamoustaris and Mithen, 1997)

Table 1. Phenolic constituents and glucosinolates* in control and inoculated leaves of two *B. juncea* cvs. resistant and susceptible to *Albugo candida*

DAI	RC-781 (R)								Varuna (S)							
	Total phenols		O-dihydroxy phenol		Flavonols		Glucosinolates		Total phenols		O-Dihydroxy phenol		Flavonols		Glucosino-lates	
	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
1	26.15 ±0.17	32.77 ±0.20	0.89 ±0.08	0.96 ±0.04	13.06 ±0.59	14.78 ±0.96	48.04 ±0.29	50.10 ±0.32	22.30 ±0.16	24.99 ±0.24	0.68 ±0.02	0.62 ±0.05	10.41 ±0.16	9.11 ±0.08	46.20 ±0.24	46.36 ±0.42
3	26.76 ±0.32	33.62 ±0.28	1.01 ±0.07	1.09 ±0.03	12.46 ±0.25	18.54 ±0.38	47.72 ±0.33	54.81 ±1.02	21.65 ±0.28	23.86 ±0.16	0.76 ±0.07	0.72 ±0.03	11.01 ±0.22	8.91 ±0.12	45.68 ±0.71	45.90 ±0.25
5	25.95 ±0.19	36.49 ±0.22	0.96 ±0.04	1.04 ±0.06	14.65 ±0.16	27.10 ±0.24	48.30 ±0.52	50.02 ±0.52	22.80 ±0.15	23.25 ±0.42	0.72 ±0.08	0.71 ±0.02	13.33 ±0.51	9.01 ±0.09	46.10 ±0.92	47.45 ±0.85
8	26.34 ±0.38	31.28 ±0.16	0.99 ±0.10	0.98 ±0.02	17.50 ±0.51	28.18 ±0.31	51.02 ±0.18	58.28 ±0.28	23.40 ±0.12	19.49 ±0.45	0.64 ±0.04	0.61 ±0.02	13.06 ±1.02	10.19 0.35	45.96 ±0.89	47.20 ±0.62
12	27.20 ±0.38	31.05 ±0.18	0.90 ±0.07	0.95 ±0.03	15.71 ±0.28	19.79 ±0.23	57.50 ±0.23	56.85 ±0.42	20.93 ±0.14	18.39 ±0.29	0.62 ±0.06	0.64 ±0.04	11.12 ±0.16	8.75 ±0.15	46.61 ±0.48	46.50 ±0.85
15	24.96 ±0.28	27.51 ±0.24	0.92 ±0.02	0.96 ±0.04	17.93 ±0.72	21.59 ±0.25	51.28 ±1.01	55.02 ±0.92	21.02 ±0.24	19.21 ±0.18	0.65 ±0.02	0.66 ±0.03	11.86 ±0.12	9.80 ±8.23	47.28 ±0.15	47.35 ±0.85

*mg/g dry tissue (values are mean ± standard deviation of triplicates)

DAI = Days after inoculation.

C = Control, I = Inoculated

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EFFECT OF DIFFERENT FERTILIZER LEVELS ON INCIDENCE OF DISEASES AND INSECT-PESTS IN INDIAN MUSTARD STRAINS

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The yield of *Brassica* crops are reduced worldwide by major diseases namely whiterust and downy mildew and insect-pests including aphid. Four strains of Indian mustard viz. BIO-772, RL-1359(ZC), Kranti (NC) and Varuna (NC) alongwith RH-30 (LC) were evaluated against major diseases and insect-pests under different fertility levels namely 80 kg N/ha, 40 kg P_2O_5/ha, 40 kg K_2O/ha and 30 kg S/ha in north-western region of Haryana.

Higher incidence of white rust were recorded in different strains when 80 kg N/ha and 40 kg P_2O_5/ha were applied individually as compared to application of K_2O and S. White rust incidence (10.0) were maximum in Varuna (NC) with 80 kg N/ha, 40 kg P_2O_5 and 40 kg K_2O/ha. The incidence were found to be less with application of S. Incidence of white rust in RL-1359 (ZC) and Kranti (NC) were quite low at all levels of fertilizers. The results are in line with those of Roy and Saikia (1976).

Incidence of downy mildew disease were found to be higher in different strains when 40 kg K_2O/ha and 30 kg S/ha were applied alone as compared to N and P_2O_5. The incidence were minimum in RL-1359 (ZC) when 80 kg N/ha and 40 kg P_2O_5/ha were applied, while maximum in Varuna (NC) when 40 kg P_2O_5/ha, 40 kg K_2O/ha and 30 kg S/ha were applied individually.

Aphid infestation was recorded to be minimum in BIO-772 with 80 kg N/ha and 40 kg P_2O_5/ha as compared to application of K_2O and S. Maximum infestation was recorded in Varuna (NC) when 40 kg K_2O/ha and 30 kg S/ha were applied. Higher incidence of aphid were recorded in Varuna (NC) (av. 9.0) followed by Kranti (NC) (av. 17.0), RL-1359 (ZC) (av. 14.0) and BIO-772 (av. 12.0). Infestation of painted bug remain too low to record and to derive any meaningful results.

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Evaluation of different strains of Indian mustard against major diseases and insect-pests

Code	Strains	Fertilizers	Reaction to diseases		Reaction to insect-pests	
			Per cent plant infected		Per cent plant infested	
			White rust	Downy mildew	Aphid	Painted bug
MCN 114	BIO-772	80 kg N/ha	0	13	10	Very low
MCN 115	RL-1359(ZC)	80 kg N/ha	5	10	10	"
MCN 116	Kranti (NC)	80 kg N/ha	5	26	20	"
MCN 117	Varuna (NC)	80 kg N/ha	10	13	15	"
LCH-I	RH-30 (LC)	80 kg N/ha	5	16	15	"
MCN 114	BIO-772	40 kg P_2O_5/ha	10	13	10	"
MCN 115	RL-1359(ZC)	40 kg P_2O_5/ha	5	10	15	"
MCN 116	Kranti (NC)	40 kg P_2O_5/ha	0	16	15	"
MCN 117	Varuna (NC)	40 kg P_2O_5/ha	10	38	28	"
LCH-I	RH-30 (LC)	40 kg P_2O_5/ha	5	24	28	"
MCN 114	BIO-772	40 kg K_2O/ha	5	20	12	"
MCN 115	RL-1359(ZC)	40 kg K_2O/ha	0	24	18	"
MCN 116	Kranti (NC)	40 kg K_2O/ha	0	20	12	"
MCN 117	Varuna (NC)	40 kg K_2O/ha	10	43	14	"
LCH-I	RH-30 (LC)	40 kg K_2O/ha	5	22	14	"
MCN 114	BIO-772	30 kg S/ha	8	24	16	"
MCN 115	RL-1359(ZC)	30 kg S/ha	0	30	12	"
MCN 116	Kranti (NC)	30 kg S/ha	5	26	20	"
MCN 117	Varuna (NC)	30 kg S/ha	0	40	20	"
LCH-I	RH-30 (LC)	30 kg S/ha	5	28	16	"

Study on consumptive use of moisture in mustard (*Brassica juncea L.*) under rainfed conditions.

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Introduction

Irrigation scheduling services that are based on estimated soil water depletion in crop field require daily estimate of evapotranspiration (Stegman,1983). The lysimeter provides a direct and accurate measurement of evapotranspiration or consumptive use. Hence the present investigation was carried out to study the consumptive use of mositure in mustard under rainfed conditions.

Materials and Methods

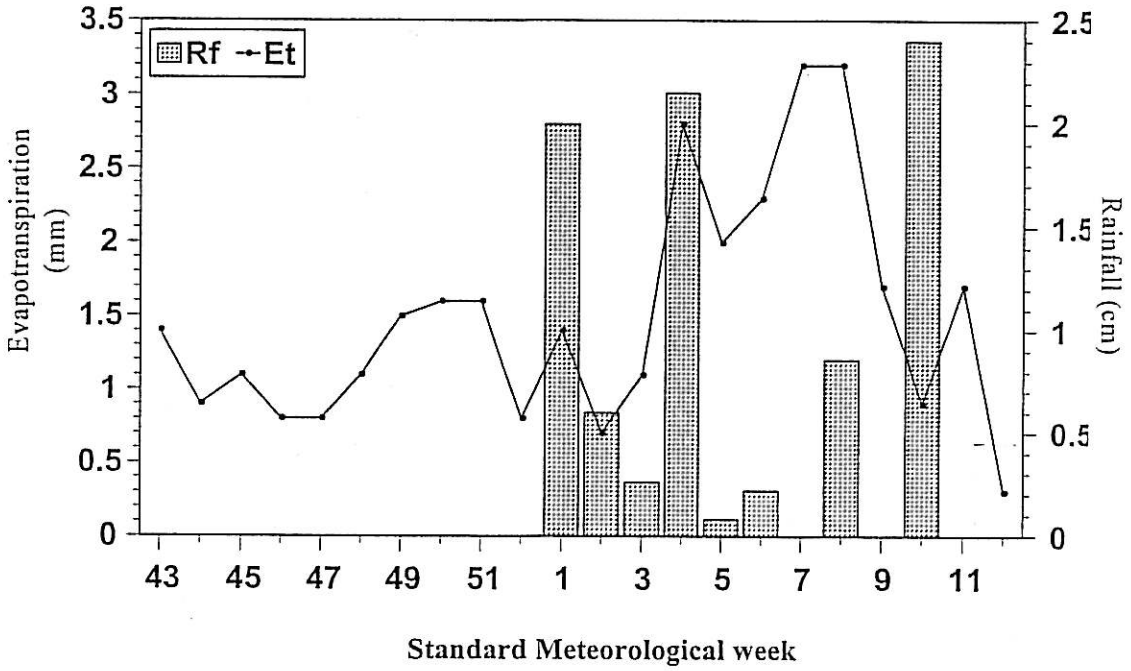
The experiment was conducted during 1998-99 *rabi* (winter) at Dryland Research Station, Dhiansar (32^0-39' N, 74^0-58' E and 332 meters amsl). The mustard Cv. Pusa Bahar was sown in lysimeter installed by Indian Meteorological Department,Pune and adjacent field under rainfed condition with standard agronomic practices .Daily data on actual evapotranspiration was recorded from lysimeter and rainfall data was used from Agromet observatory at Dhiansar.

Results and Discussion

The results so obtained from the experiments are presented in table :-1. and weekly consumptive use during crop growth against rainfall are depicted in Fig.1. The data

Table:1 Growth and yield of mustard (Cv.Pusa bahar) in Rabi 1998-99.

S.No.	Observation	Lysimeter	Field
1	Date of sowing	27.10.1998	27.10.1998
2.	Date of 50% flowering	13.12.1998	13.12.1998
3.	Date of harvesting	20.03.1999	20.03.1999
4.	Duration in days	146	146
5.	Plant height(cm)	165.5	165.5
6.	No. of pods plant^{-1}	122	99
7.	Pod Yield (q ha^{-1})	8.8	5.6
8.	Stover Yield (q ha^{-1})	28.4	18.2
9.	Consumptive use of moisture (mm)	225.1	---
10.	Moisture use efficiency (Kg ha^{-1} mm^{-1})	3.9	----
11.	Rainfall during crop growth period (mm).	85.7	85.7
12.	No. of rainy day.	13	13



reveals that the rainfall received during crop growing period was 85.7mm in 13 rainy days .The consumptive use of moisture was 225.1mm whereas moisture use efficency was 3.9 kg/ha/mm.The seed yields were recorded 8.8 q ha^{-1} and 5.6 q ha^{-1} in lysimeter and field, respectively. The data also reveals that evapotranspiration (Et) was low and erractic upto flowering thereafter it increased and reached maximum to 3.2 mm day^{-1}. The sufficient moisture availability in the soil due to heavy rains alongwith higher temperature during the period from flowering to physiological maturity might be responsible for higher rate of evapotranspiration.

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Genetic studies in *Brassica juncea* genotypes in North-South and East-West sowing direction in acidic soil at high altitude

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ABSTRACT

Studies on 45 genotypes in six environments (3 dates North-South and 3 dates East-West sowing directions) showed significant superiority most of yield attributes in East-West sowing direction.

Key words:- Additive component, *Brassica juncea* L., Dominance effect.

Introduction

Seed yield can be increased either through genetic manipulations or through appropriate management. If the genetic manipulation is coupled with proper management, seed yield can further be increased. The present work was initiated to study the performance of genetically manipulated lines of *Brassica juncea* L. in different environments.

Materials and Methods

The materials generated during winter 1995-96 consisted of 45 genotypes (9 parents and their diallels excluding reciprocals). These were sown on three dates (E_1 & E_4 on 27th September, E_2 & E_5 on 4th October and E_3 & E_6 on 11th October 1997) at North-South as well as in East-West row directions with two replications on each date at oilseed research farm of the University. The p^H of the soil being 5.9. The area is located between 23^{0}17' latitude and 85^{0}19'E longitude and altitude is 625 meters above the mean sea level. The distance between the rows and plants was maintained at 30 and 10 cm, respectively. Cultural practices as recommended for the area were followed. The observations were recorded for eleven yield attributes (Table 1) from ten randomly selected competitive plants. Statistical analysis was done as per Griffing (1956) and Hayman (1954).

Results

There were significant differences among the parental lines as well as among the crosses in respect of seed yield and its attributes. Number of primary branches/plant and plant height were controlled by additive genetic variance (D) in North-South sowing directions, whereas plant height, number of seeds/siliqua, days to maturity and harvest index were governed by additive genetic variance (D) in East-West sowing direction. Both additive and non additive genetic components ($H_1$and H_2) with preponderance for latter components were more important. Additive component (H_1) was significant for number of primary and secondary branches/plant, plant height, number of siliquae/plant, number of seeds/siliqua, days to maturity, 1000-seed weight, seed yield/plant and oil per cent of oil content in North-South sowing direction and days to 50% flowering, plant height, number of siliquae/plant, number of seed/siliqua, days to maturity, harvest index, seed yield and per cent of oil content in East-West sowing direction (Table 1). Non additive component (H_2) was significant for days to 50% flowering, number of primary and secondary branches/plant, plant height, 1000-seed weight, seed yield/plant and per cent of oil content in North-South sowing direction, for improvement of these characters for North-South sowing directions direct selection is less amenable. In the East-West sowing direction, non additive components as significant for days to 50% flowering, number of primary and secondary branches/plant, plant height, number of seeds/siliqua, days to maturity, harvest index, 1000-seeds weight, seed yield/plant and per cent of oil content. These characters are therefore less amenable for improvement through simple selection for East-West sowing directions. The ratio of $(H_1/D)^{0.5}$ as higher than one also indicated the presence of over dominance for days to 50% flowering, number of primary branches/plant, plant height, harvest index, 1000 seed weight, seed yield/plant and per cent of oil content in North-South sowing direction. In East-West sowing direction the ratio of $(H_1/D)^{0.5}$ showed the presence of

overdominance for all the characters except number of siliquae/plant. The ratio of $H_2/4H_1$ indicated the asymmetrical distribution of genes as value is greater or less than 0.25 for days to 50% flowering, number of primary branches/plant, plant height, harvest index, 1000-seed weight, seed yield and per cent of oil content, whereas in East-West sowing direction asymmetrical distribution of genes for most of the characters.

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Table 1. Genetic parameter in North-South and East-West sowing directions

Parameter	CHARACTERS										
	1	2	3	4	5	6	7	8	9	10	11
D-Variation due to additive effect (NS)		*		*							
D-Variation due to additive effect (EW)				*		*	*	*			
F-The mean of covariance of additive and dominance effect over the arrays (NS)		*		*							
F-The mean of covariance of additive and dominance effect over the arrays (EW)						*	*	*			
H_2 (NS)	*	*	*	*					*	*	*
H_2 (EW)	*	*		*		*	*	*	*	*	*
H_1-Component of variation due to the dominance effect of the genes (NS)		*	*		*	*	**		*	*	*
H_1-Component of variation due to the dominance effect of the genes (EW)	*			*	*	*	**	**		*	*
H^2-Dominance effect (NS)		*									
H^2-Dominance effect (EW)								*			
E-Environmental component of variation (NS)		*	*	*					*	*	
E-Environmental component of variation (EW)	*	*		*		*	*	*			*

1. Days to 50% flowering, 2. Primary branches per plant, 3. Secondary branches per plant, 4. Plant height, 5 Siliquae per plant, 6. Seeds per siliqua, 7. Days to maturity, 8. Harvest index, 9. 1000 – seed weight, 10. Seed yield per plant and 11. Oil content (%).

***,** Significant at 5 and 1% probability levels, respectively.**

Variability studies of different traits in *Brassica juncea* L. in North-South and East-West sowing direction in acidic soil of high altitude

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ABSRACT

Genotypic and phenotypic variance were superior for number of secondary branches/plant, number of siliquae/plant, seed yield/plant in East-West sowing direction. Genotypic and phenotypic coefficient of variation were superior for number of secondary branches and seed yield/plant than that of North-South sowing. Heritability, genetic gain and genetic gain per cent of mean were superior for seed yield/plant than that of North-South sowing direction.

Key words: *Brassica juncea*, mustard, variability, heritability.

Introduction

Mustard (*Brassica juncea* L.) is important oilseed crop. Its yield and yield attributes like other crop are more influenced by environment. Very scanty study was done heterotic variation over environments. In present study was undertaken to study the heterotic variation over different environments.

Materials and Methods

The materials for the study generated during winter 1995-96 consisted of 45 genotypes (9 parents and their diallels excluding reciprocals). The above 45 genotypes were sown on three dates (E_1 & E_4 on 27th September, E_2 & E_5 on 4th October and E_3 & E_6 on 11th October 1997) at North-South as well as in East-West sowing directions with two replications on each date during winter season at the University experimental area, Ranchi, which is located at 625 meters above mean sea level and soil is red letteritic. The distance between the rows and plants was maintained at 30 and 10 cm, respectively. Cultural practices as recommended for the area were followed. The observations were recorded for eleven yield attributes (Table 1) from ten randomly selected competitive plants. Estimation of heterosis was done as per standard method.

RESULT

The analysis of variance showed significantly differences for the characters. Genotypic variance of East-West sowing direction showed the superiority to North-South sowing direction for number of secondary branches/plant, number of siliquae/plant, number of seeds/siliqua, 1000 seed weight and seed yield/plant. Whereas North-South sowing direction had higher genotypic variance for days to 50% flowering, number of primary branches/plant, plant height, days to maturity, harvest index and per cent of oil content. Phenotypic variance of East-west sowing direction had superiority to North-South sowing direction for number of primary and secondary branches/plant, plant height, number of siliquae/plant and seed yield/plant. Error variance was higher than that of North-South sowing direction for number of primary and secondary branches/plant, plant height, number of seeds/siliqua, seed yield/plant and per cent of oil content (Table 1). Phenotypic coefficient of variation was higher in number of primary and secondary branches/plant, plant height, number of siliquae/plant and seed yield/plant than that of North-South sowing direction. Genotypic coefficient of variation was higher in number of secondary branches/plant, number of seeds/siliquae, 1000-seed weight and seed yield/plant than that of North-South sowing direction. Heritability had higher value for number of seeds/siliqua, 1000-seed weight and seed yield/plant than that of North-South sowing direction. Genetic gain was higher in number of secondary branches/plant, number of seeds/siliqua,1000-seed weight and seed yield/plant than that of North-South sowing direction. Genetic gain per cent of mean was higher in number of secondary branches/plant, plant height, number of seeds/siliqua,1000-seed weight and seed yield/plant than that of North-South sowing direction. Mean value of East-West sowing

direction was higher for days to 50% flowering, number of primary branches/plant, plant height, number of siliquae/plant, days to maturity, 1000-seed weight and seed yield/plant than that of North-South sowing direction.

Table 1. Variability studies of different characters in North-South(NS) and East-West(EW) sowing directions

Parameter	CHARACTERS										
	1	2	3	4	5	6	7	8	9	10	11
Genotypic Variance (NS)	18.99	0.20	2.68	107.80	2450.60	0.71	28.34	0.00	0.06	1.41	3.82
Genotypic Variance (EW)	15.16	0.18	6.32	98.34	2873.70	4.77	9.28	0.00	0.07	7.52	2.99
Phenotypic Variance (NS)	105.38	2.02	25.10	436.90	12157	13.16	119.44	0.01	0.65	6.70	7.05
Phenotypic Variance (EW)	92.09	3.23	77.20	469.70	26206	11.93	67.71	0.01	0.49	26.31	5.54
Error Variance (NS)	86.39	1.82	21.90	309.20	9706.70	12.45	91.10	0.01	0.59	5.29	3.33
Error Variance (EW)	76.93	3.05	70.60	382.30	23332	7.16	58.42	0.00	0.43	9.05	3.45
Phenotypic Coefficient of Variantion (NS)	17.70	40.45	83.50	17.47	73.22	34.02	9.77	47.58	26.64	73.93	6.89
Phenotypic Coefficient of Variantion (EW)	15.64	40.90	175.5	18.23	87.48	31.26	7.21	40.41	23.04	99.06	6.14
Genotypic Coefficient of Variantion (NS)	7.51	20.25	27.53	8.68	32.87	7.88	4.76	29.13	7.89	33.86	5.04
Genotypic Coefficient of Variantion (EW)	6.35	5.68	50.21	8.24	28.97	19.77	2.67	18.07	8.67	52.95	3.78
Heritability in per cent (NS)	18.02	25.05	10.88	24.67	20.16	5.36	23.73	37.50	8.77	20.97	53.46
Heritability in per cent (EW)	16.46	1.93	8.19	20.46	10.97	39.99	13.71	20.00	14.14	28.57	37.86
Genetic Advance (NS)	3.81	0.80	1.11	10.62	45.79	0.40	5.34	0.07	0.15	1.12	2.95
Genetic Advance (EW)	3.25	0.07	1.48	9.24	36.57	2.85	2.32	0.03	0.21	3.02	1.84
Genetic Advance in per cent of mean (NS)	6.37	20.88	18.71	8.88	30.40	3.76	4.77	36.75	4.82	31.94	7.59
Genetic Advance in per cent of mean (EW)	5.31	1.62	29.59	7.68	19.76	25.76	2.04	16.64	6.71	58.31	4.79
Mean (NS)	57.99	3.85	5.94	119.60	150.58	10.66	111.89	0.19	3.02	3.5	38.80
Mean (EW)	61.34	4.31	5.01	120.00	185.05	11.04	114.16	0.18	3.06	5.18	38.35

1. Days to 50% flowering, 2. Primary branches per plant, 3. Secondary branches per plant, 4. Plant height, 5. Siliquae per plant, 6. Seeds per siliqua, 7. Days to maturity, 8. Harvest index, 9. 1000 – seed weight, 10. Seed yield per plant and 11. Oil content (%).

LENGTH OF THE VEGETATION PERIOD AND MORPHOLOGICAL CHARACTERISTICS OF CAULIFLOWER *(BRASSICA OLERACEA VAR. BOTRITIS* L.) CULTIVARS AND HYBRIDS GROWN AS SPRING CROPS (BY AUTUMN PLANTING)

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ABSTRACT

The purpose of the present investigation was to study some basic traits of cauliflower cultivars and hybrids grown as a spring crop by autumn planting under the conditions of Bulgaria. Length of the vegetation period and some morphological characteristics were investigated in the studied cauliflower cultivars and hybrids White Ball, Celesta, Batsman F_1, Horseman F_1 and Torina F_1. All the studied cultivars and hybrids are appropriate for growing as spring crops by autumn planting. Celesta was the earliest cultivar with length of the vegetation period of 231 days.

INTRODUCTION

The cauliflower *(Brassica oleracea* var. *botritis* L.) cultivars and hybrids introduced in Bulgaria are used mainly for obtaining produce in the period September – November when grown by a technology for late field production (Mihov and Antonova, 2000). When there are appropriate cultivars and hybrids (Houghton, 1980; Ruffio-Chable and Harve, 1987) and optimum conditions for production (Booij, 1987; Pearson et al., 1994; Wurr and Fellows, 1998) it is possible to obtain production in different seasons of the same calendar year. The purpose of the investigation is to study the vegetation period and some traits of the morphological characteristics of cauliflower cultivars and hybrids when grown as spring crops by autumn planting.

MATERIAL AND METHODS

During the period 1996-1998 five cauliflower cultivars and hybrids – White Ball, Celesta, Batsman F_1, Horseman F_1 and Torina F_1, grown by autumn planting were tested. The trial was conducted by the block method in 4 replications in the experimental plots with size of 12.48 m^2. The sowing was carried out on 5 – 10 September and the planting was conducted on a high flat bed on 20 – 25 October by the scheme of 90 + 70/60 cm. Determined was the degree of the influence of the variation factors (%) upon the studied indices: number and weight of the rosette leaves, weight (kg), length and diameter of the curd (cm), length of the vegetative period (days from planting to ripeness). Data were processed using the two-factor dispersion analysis and the Duncan's multiple range test.

RESULTS AND DISCUSSION

The tested cauliflower cultivars and hybrids have a significant ($P < 0.001$) strong effect (from 44.99 % to 96.57 %) upon all the investigated indices (Table 1). The rest of the variation factors have a very weak ($P \leq 0.01$, $P \leq 0.05$) influence only upon some of the indices. The trial year and the interaction of cultivar x year have a weaker influence upon the length of the vegetation period - 11.25 % and 12.51 %, respectively.

There is a significant variation of the morphological characteristics and the length of the vegetation period of the studied cauliflower cultivars and hybrids (Table 2). Regarding

the number of the rosette leaves the variation is from 18.42 in White Ball and Torina F_1 to 22.94 in Batsman F_1. The lowest mean weight of the leaf rosette - 1.099 kg, is registered in White Ball, while the highest one - 1.864 kg, was in Horseman F_1.

The mean curd weight varies from 1.079 kg in Batsman F_1 to 1.991 kg in Torina F_1; the curd length is from 12.17 cm in Celesta to 14.72 cm in Batsman F_1, and the curd diameter is from 16.90 cm in Batsman F_1 to 23.28 cm in Torina F_1. The vegetation period is shortest in Celesta – 231 days. It is latest in Torina F_1 – 240 days.

The investigated cauliflower cultivars and hybrids preserve stable morphological characteristics when grown as spring crops by autumn planting under the conditions of Bulgaria. In this respect Celesta is the best cultivar with mean curd weight of 1. 221 kg.

CONCLUSIONS

The investigated cauliflower cultivars and hybrids – White Ball, Celesta, Batsman F_1, Horseman F_1 and Torina F_1, can be grown as spring crops by autumn planting under the conditions of Bulgaria.

It is possible to carry an earlier cauliflower production by using the cultivar Celesta which has the shortest vegetation period (231 days).

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TABLE 1.

TWO-FACTOR DISPERSE ANALYSIS OF THE LENGTH OF THE VEGETATION PERIOD AND THE MORPHOLOGICAL CHARACTERISTICS OF CAULIFLOWER CULTIVARS AND HYBRIDS

VARIATION FACTORS	DEGREE OF FREEDOM	NUMBER OF ROSETTE LEAVES		WEIGHT OF ROSETTE LEAVES		CURD WEIGHT		CURD LENGTH		CURD DIAMETER		VEGETATION PERIOD	
	df	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
Block	3	0.883		0.003		0.014		0.300		0.297		7.378	
Cultivar	4	*** 41.908	93.03	*** 1.220	89.46	*** 1.546	93.50	*** 61.539	93.54	*** 69.878	96.57	*** 114.596	44.99
Year	2	* 0.649	0.72	* 0.031	1.12	NS 0.001	–	NS 0.302	–	NS 0.360	–	** 57.325	11.25
Cultivar x Year	8	NS 0.27	–	* 0.022	3.24	NS 0.002	–	* 0.393	0.31	** 0.379	1.04	* 15.93	12.51
Left	42	0.154		0.008		0.009		0.139		0.126		7.053	

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; NS – not significant

TABLE 2.

MORPHOLOGICAL CHARACTERISTICS AND LENGTH OF VEGETATION PERIOD

CULTIVAR (HYBRID)	ROSETTE LEAVES	WEIGHT OF ROSETTE LEAVES	CURD WEIGHT	CURD LENGTH	CURD DIAMETER	VEGETATION PERIOD
	MEAN SD	MEAN SD	MEAN SD	MEAN SD	MEAN SD	MEAN SD
White Ball	18.42 ± 0.41^{c}	1.099 ± 0.045^{d}	1.258 ± 0.063^{b}	13.4 ± 0.72^{b}	18.83 ± 0.51^{d}	237 ± 1.92^{b}
Batsman F_1	22.94 ± 0.55^{a}	1.369 ± 0.010^{c}	1.079 ± 0.072^{c}	14.72 ± 0.83^{a}	16.90 ± 0.68^{e}	236 ± 2.58^{bc}
Celesta	19.33 ± 0.39^{b}	1.104 ± 0.057^{d}	1.221 ± 0.057^{b}	12.17 ± 0.88^{c}	20.92 ± 0.73^{b}	231 ± 4.34^{d}
Torina F_1	18.42 ± 0.22^{c}	1.542 ± 0.087^{b}	1.991 ± 0.122^{a}	14.05 ± 0.69^{ab}	23.28 ± 0.45^{a}	240 ± 2.06^{a}
Horseman F_1	19.43 ± 0.43^{b}	1.867 ± 0.142^{a}	1.271 ± 0.097^{b}	13.81 ± 0.53^{b}	19.28 ± 0.78^{c}	235 ± 5.08^{bc}
Mean values	**19. 71**	**1.369**	**1.364**	**13.63**	**19.84**	**236.05**

SOME MORPHOLOGICAL CHARACTERISTICS OF BROCCOLI *(BRASSICA OLERACEA* VAR. *ITALICA* PL.) HYBRIDS GROWN AS SPRING, SUMMER AND AUTUMN CROPS (UNDER VHE CONDITIONS OF BULGARIA)

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ABSTRACT

The purpose of the present investigation is to trace the changes in some morphological characteristics of broccoli *(Brassica oleracea* var. *italica* Pl.) hybrids, grown as spring, summer and autumn crops with a view to determination of their appropriateness for the respective production. Length of plants, as well as curd weight and diameter of the tested broccoli hybrids Kermit F_1 and Fiesta F_1 were investigated. Under the growing conditions as a spring crop the two studied hybrids does not reveal well their biological potential. Extension of plant length and formation of untypical, small in size and weight curds were observed. Kermit F_1 and Fiesta F_1 are appropriate for growing as summer and autumn crops, because they form standard curds with weight of over 0.365 kg and a diameter larger than 16.7 cm.

INTRODUCTION

Broccoli *(Brassica oleracea* var. *italica* Pl.) production in Bulgaria is limited and rare, as the areas planted with this vegetable crop according to our observations reach 20 ha with mean yield of 8-12 t/ha.

The economic production from the grown in our country introduced cultivars and hybrids is obtained mainly in the period September – October. In a number of countries the different growing conditions combined with the biological requirements of this crop give possibilities for marketing of broccoli production during various seasons. Quantity and quality of the obtained standard curds are influenced by the conditions of growing (Wurr et al., 1991; Greven, 1998), as well as the specific production appropriateness of the used broccoli cultivars and hybrids (Titley, 1987;Mihov and Antonova, 2000).

The purpose of the present investigation was to study the changes in some morphological characteristics of broccoli hybrids grown as spring, summer and autumn crops with a view to determine their appropriateness for the respective production.

MATERIAL AND METHODS

During the period 1997-1998 studied were two broccoli hybrids – Kermit F_1 and Fiesta F_1, grown as spring, summer and autumn crops with sowing dates 10 September, 10 March and 10 June and planting dates – 25 October, 20 April and 20 July, respectively. The trial was conducted by the block method in 4 replications (26 plants per replication). The plants were planted on a high flat bed by the scheme of 90 + 70/60 cm. The investigated indices were determined in the period of ripeness in April, August and October for the spring, summer and autumn production, respectively. The following indices were studied: plant length (cm), curd weight (kg) and curd diameter (cm). Data were processed using the two-factor dispersion analysis.

RESULTS AND DISCUSSION

The investigated morphological characteristics are influenced mostly by the production trend – from 83.55 % to 97.81 % ($P \leq 0.001$) (Table 1). The genetic differences of the tested broccoli hybrids ($P \leq 0.001$) and their interaction with the growing variants ($P \leq 0.05$) have an influence only upon the index curd weight - 10.13 % and 2.32 %, respectively.

In growing of the two broccoli hybrids as a spring crop (by autumn planting) there is a significant extension of the plant length combined with unsatisfactorily in size curds with weight from 0.153 kg (Fiesta F_1) to 0.203 kg (Kermit F_1) and a diameter from 10.7 to 10.9 cm (table 2). The unfavourable growing conditions in this production trend combined with the low temperatures and a short day have a negative effect upon the plants and the productive behaviour of the tested hybrids, which is the reason for their inappropriateness for this production trend. Growing of Kermit F_1 and Fiesta F_1 as a summer and autumn crop is favourable for the existence of the morphological characteristics typical for the two hybrids. The plants have mean length from 38.7 to 39.7 cm in Fiesta F_1 and 40.3 cm in Kermit F_1, with curd diameter from 16.7 to 18.4 cm in Fiesta F_1 and from 17.2 to 18. 4 cm in Kermit F_1. The plants from the hybrid Fiesta F_1 form smaller curds with mean weight from 0.365 to 0.450 kg, while the curds formed by Kermit F_1 have mean weight from 0.482 to 0.525 kg.

CONCLUSIONS

The hybrids Kermit F_1 and Fiesta F_1 are not appropriate for growing as a spring crop by an autumn planting in Bulgaria.

The two investigated hybrids are recommended for production by growing as summer or autumn crops when the climatic conditions in our country are favourable for the formation of standard curds with diameter of more than 167 cm and mean weight of more than 0365 kg.

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TABLE 1.

TWO-FACTOR DISPERSE ANALYSIS OF THE LENGTH OF THE MORPHOLOGICAL CHARACTERISTICS OF BROCCOLI HYBRIDS GROWN IN THREE PRODUCTION TRENDS

VARIATION SOURCES	DEGREE OF FREEDOM	CURD LENGTH		CURD WEIGHT		CURD WEIGHT	
	df	DISPERSION	INFLUENCE OF THE VARIATION FACTORS	DISPERSION	INFLUENCE OF THE VARIATION FACTORS	DISPERSION	INFLUENCE OF THE VARIATION FACTORS
Block	3	1.708		0.0009		0.674	
Hybrid	1	NS 5.041	–	*** 0.061	10.13	NS 0.01	–
Production trend	2	*** 301.167	88.85	*** 0.251	83.55	*** 177.978	97.81
Hybrid x Production trend	2	NS 1.167	–	* 0.007	2.32	NS 0.431	–
Left	15	**4.208**		**0.0014**		**0.339**	

*** **P ≤ 0.001**
** **P ≤ 0.01**
* **P ≤ 0.05**
NS – not significant

TABLE 2.

MORPHOLOGICAL CHARACTERISTICS OF BROCCOLI HYBRIDS GROWN IN THREE PRODUCTION TRENDS

CULTIVAR (HYBRID)	PRODUCTION TREND	WEIGHT OF ROSETTE LEAVES	CURD WEIGHT	VEGETATION PERIOD
		MEAN SD	MEAN SD	MEAN SD
Kermit F_1	SPRING	47.8 ± 3.6	0.203 ± 0.030	10.7 ± 0.44
	SUMMER	40.3 ± 1.3	0.482 ± 0.104	17.2 ± 0.78
	AUTUMN	40.3 ± 1.3	0.525 ± 0.030	18.4 ± 0.53
Fiesta F_1	SPRING	46.5 ± 2.4	0.153 ± 0.020	10.9 ± 0.25
	SUMMER	38.7 ± 1.0	0.365 ± 0.027	16.7 ± 0.44
	AUTUMN	39.7 ± 2.9	0.450 ± 0.036	18.4 ± 0.48

RESPONSE OF ETHIOPIAN MUSTARD *(Brassica Carinata* (A.) Braun) TO VARIOUS FERTILITY LEVELS UNDER LATE SOWN CONDITIONS.

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Introduction

Higher productivity of Ethiopian mustard *(Brassica Carinata* (A.) Braun) than that of Indian mustard was reported by Katiyar et al. (1986) and Labana et al. (1987), and especially under delayed sowing by Ganga Saran and Giri (1987). It possesses greater degree of resistance to mustard aphids and white rust, indicating a greater prospect in India and more so under multiple irrigated cropping systems. Sufficient in formation regarding fertilizer requirement of Indian mustard is available but in formation regarding response of *Brassica Carinata* to different nitrogen and phosphorus levels is meagre. The present study was therefore undertaken to assess the response of Ethiopian mustard to different nitrogen and phosphorus levels by keeping Indian mustard as standard under late planting conditions.

Material and methods

A field experiment was conducted during rabi 1999-2000, at the oil seeds research Area of CCS Haryana Agricultural University, Hisar (India). The soil of the experimental plot was sandy loam in texture, slightly alkaline and medium in fertility status. The crop was soon at 30cms. row spacing using 5kg. seed per hectare on December 2, 1999. All the agronomic operations and plant protection measures was followed as per recommendations of Indian mustard. The experiment was laid out in randomized block factorial design with three replications. There were twenty treatment combinations comprising five mustard cultivars and four fertility levels. (details in table 1).

Results and Discussion

Ethiopian mustard *(Brassica Carinata)* cultivar JTC-1 recorded significantly higher seed yield (1968 kg. 1ha) over all cultivars except HC-9606. (Table I). Cultivar HC-9606 also recorded significantly higher seed yield (1538 kg./ha.) over variety varuna (1366 kg./ha.) of *B. Juncea* but remained at par with Kranti and IGC-01. Highest oil content (42.5%) was recorded in Kranti, which was significantly higher over all other cultivars. Application of 150%. of the recommended in fertilizer dose (table I) fertility level recorded significantly higher. Seed yield (1646 kg./ha) but was at par with 125% of recommended fertility (100kg. N+ 37.5 kg. $P_2 0_5$kg./ha.) level (1576 kg./ha.) Significant increase in yield of Brassica carinate with increase in dose of nitrogen upto 100kg./ha. have been reported by Pramanik et. al. (1995). Highest oil content (41.8%) was found at recommended fertility level, test weight (1000-seed wt.) was found to differ significantly among various cultivars. Varuna & Kranti cultivars of *B. Juncea* recorded significantly higher test weight. over *B. carinata* cultivars.

Conclusion

Ethiopian mustard cultivar. JTC-I and HC-9606 with application of, 100kg. N+37.5kg.P_2 0_5/ha. can be grown successfully under late planting conditions.

Table I- Effect of fertility level on seed yield, oil content (%) and 1000 seed wt. of *Brassica cultivars.*

Treatment	Seed yield (kg./ha.)	Oil content (%)	1000seed wt. (G)
Cultivars			
JTC-I	1668	40.9	3.91
IGC-DI	1416	42.0	4.10
HC-9606	1538	40.5	4.05
Varuna	1366	41.6	4.95
Kranti	1482	42.5	4.86
CD at 5%	126	0.30	0.42
Fertility Levels			
150% of Recommended fertility	1646	41.5	4.08
125% of Recommended fertility	1576	41.3	4.28
*Recommend fertility	1452	41.8	4.40
75% of Recommend fertility	1301	41.4	4.70
CD at 5%	130	0.32	0.40

* Recommended Fertility = 80 kg. N+30kg. p_2o_5/ha.

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NITROROGEN REQUIREMENTS OF MUSTARD GROWN IN DIFFERENT CROP SEQUENCES

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Pearlmillet-mustard, jawar-mustard, fallow-mustard and moong-mustard sequence has gained popularity in semiarid climatic conditions of south western region of Haryana with the invent of high yielding and fertilizer responsive varieties of these crops. Now under irrigated conditions, cotton-mustard sequence is being followed by some farmers. All these crops grown in sequence are quite exhaustive and require heavy fertilization for higher and sustainable yield level. Information on nitrogen management in mustard as individual crop is available but the optimum dose of nitrogen in mustard grown in different crop sequences is lacking. keeping this consideration in view, the present field investigation was planned.

Materials and Methods

A field experiment was conducted for two consecutive years (1998-99 and 1999-2000) on sandy loam soil at oilseeds Research Area of CCS HAU, Hisar. The soil was low in organic carbon (0.24%) medium in available P (10.5kg/ha) and high in available K (325kg./ha). The experiment was laid out in split plot design with replicates. Five crop sequences viz green gram-mustard, bajra-mustard, fallow-mustard, cotton-mustard and jawer-mustard were kept as main treatments and five nitrogen levels 0, 40, 80, 120 and 160 kg N/ha were considered as subtreatments. Crops preceeding to mustard were grown as per recommended package of practices of CCS HAU, Hisar. In mustard half dose of nitrogen, full dose of phosphorus sulphur were applied at sowing time and remaining half dose of nitrogen was applied after first irrigation.

Results and Discussion

Different crop sequences had significant effect on mustard seed yield. During 1998-99, significantly higher seed yield of mustard (1551 kg/ha) was obtained in cotton-mustard sequence while during the year 1999-2000, significantly higher seed yield of mustard (1933 kg/ha) was obtained in fallow-mustard sequence which was at par with moong-mustard sequence (Table I). Lowest seed yield of mustard was obtained in bajra-mustard sequence. This confirms the findings of research carried out at Navgoan (Rajasthan) (Anonymous, 1999). During first year, seed yield of mustard increased significantly with the application of 120 kgN/ha in different crop sequences except in moong-mustard and cotton-mustard sequence whereas in second year significant effect of nitrogen application on seed yield increase was also upto 120 kg N/ha except in follow-mustard sequence.

During both the years, oil content (%) diffeused significantly with nitrogen levels cropping sequence had no significant impact on oil content (Table 2).

Table 1. Seed yield (kg/ha) and oil content (%) of mustard as influenced by various crop sequences and nitrogen levels during 1998-99

Cropping sequences	Nitrogen levels (kg/ha)				Mean seed yield (kg/ha)	Mean oil content (%)
	40	80	120	160		
Green gram-mustard	939 (43.4)	1520 (43.4)	1638 (43.0)	1764 (42.2)	1523	42.8
Bajra - mustard	1063 (43.7)	1290 (43.3)	1421 (42.8)	1987 (42.3)	1490	- 42.9
Cotton - mustard	1276 (43.2)	1559 (42.9)	1642 (42.7)	1552 (42.4)	1551	42.8
Fallow - mustard	981 (43.7)	1167 (43.5)	1459 (43.3)	1585 (42.8)	1350	43.2
Jowar - mustard	953 (43.2)	1395 (43.2)	1495 (43.2)	1610 (42.6)	1417	42.9
Mean Seed yield (kg/ha)	1042	1390	1531	1699	-	-
Mean oil Content (%)	43.4	43.2	43.0	42.4	-	-

CD at 5% (seed yield) - Sequences (29), N levels (108), Interaction (128), Oil (%)- Sequences (NS), N levesl (0.42) Interaction (0.28).

Note : Figures in parenthesis denote oil content (%).

Table 2. Seed yield (kg/ha) and oil content (%) of mustard as influenced by various crop sequences and nitrogen levels

Crop sequences	Nitrogen levels (kg/ha)					Mean seed yield (kg/ha)	Mean oil content (%)
	0	40	80	100	120		
Green gram-mustard	530	840	1212	1993	1850	1285	42.8
Bajra - mustard	579	803	878	937	1086	856	42.9
Fallow - mustard	1075	1788	2058	2307	2439	1933	43.2
Jowar - mustard	481	863	1082	1015	1128	914	42.9
Mean Seed yield (kg/ha)	666	1073	1307	1563	1626	-	-
Mean oil Content (%)	43.8	42.9	42.4	41.5	40.5	-	-

CD at 5% (seed yield) - Sequences (723), N levels (277), Interaction (NS), Oil (%)- Sequences (NS), N levesl (0.48) Interaction (NS).

Note : Figures in parenthesis denote oil content (%).

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EVALUATION OF INDIAN MUSTARD STRAINS UNDER DIFFERENT FERTILIZER LEVELS

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INTRODUCTION

A differential response of fertilizer application on phenological characters like plant height and maturity days, oil content and seed yield was studied in four strains of Indian mustard alongwith local check. The present study was aimed to assess the response of different fertilizers applications in different strains in developing agronomy of the crop which would be helpful for the breeder to plan futuristics approach in breeding programmes

MATERIALS AND METHODS

A field experiment was conducted at Krishi Vigyan Kendra, Sadalpur in CCS Haryana Agricultural University, Hisar during *rabi* season of 1998-99. The treatments consisted of different fertilizers viz. 80 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1}, 40 kg K_2O ha^{-1} and 30 kg S ha^{-1} in four strains namely BIO-772, RL-1359, Kranti, Varuna and RH-30 as local check. The experiment was laid out in RBD design with three replications.

RESULTS AND DISCUSSION

The results in Table 1 revealed that plant height was found to be significantly higher in all strains except Kranti when nitrogen was applied at the rate of 80 kg/ha. In all the strains when different fertilizers were applied, maturity days were found to be non-significant. Although Varuna shows the earliest maturity. Oil content was estimated maximum (42.2%) in BIO-772 when S was applied. In different strains BIO-772, RL-1359 and Kranti application of P_2O_5 and S gave higher oil content. Sandhya and Gupta (1998) observed similar results, but among different fertilizer applications, it was found to be non-significant in the strains under study. Seed yield was found to be maximum (1000 kg/ha) in RL-1359 which was closely followed by 990 kg/ha in RH-30 but do not differ significantly when nitrogen (N) was applied at the rate of 80 kg/ha. Seed yield differs significantly among all the treatments. Seed yield in all the four strains was highest when N at the rate of 80 kg/ha was applied as compared to other fertilizers. Yadav and Kumar (1984) observed the higher seed yield under N fertilizer applications.

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Table 1. Response of N, P_2O_5, K_2O and S in different strains of Indian mustard

Code	Strains	Fertilizers	Final Plant height (cm)	Days to maturity	Oil contents (%)	Seed yield (kg/ha)
MCN 114	BIO-772	80 kg N/ha	169.3	126.3	41.0	850
MCN 115	RL-1359(ZC)	80 kg N/ha	172.0	126.3	41.4	1000
MCN 116	Kranti (NC)	80 kg N/ha	100.1	124.0	40.6	880
MCN 117	Varuna (NC)	80 kg N/ha	160.0	126.0	41.7	790
LCH-I	RH-30 (LC)	80 kg N/ha	170.5	128.0	40.8	990
MCN 114	BIO-772	40 kg P_2O_5/ha	116.5	126.3	42.1	610
MCN 115	RL-1359(ZC)	40 kg P_2O_5/ha	145.3	124.0	41.1	880
MCN 116	Kranti (NC)	40 kg P_2O_5/ha	105.5	123.8	41.8	700
MCN 117	Varuna (NC)	40 kg P_2O_5/ha	107.8	122.0	41.2	640
LCH-I	RH-30 (LC)	40 kg P_2O_5/ha	135.0	126.0	41.1	760
MCN 114	BIO-772	40 kg K_2O/ha	142.8	125.3	41.4	660
MCN 115	RL-1359(ZC)	40 kg K_2O/ha	138.5	125.3	40.5	590
MCN 116	Kranti (NC)	40 kg K_2O/ha	106.5	123.0	40.7	740
MCN 117	Varuna (NC)	40 kg K_2O/ha	145.3	125.3	41.5	480
LCH-I	RH-30 (LC)	40 kg K_2O/ha	135.7	124.1	41.3	690
MCN 114	BIO-772	30 kg S/ha	148.0	124.0	42.2	520
MCN 115	RL-1359(ZC)	30 kg S/ha	147.3	124.8	41.8	550
MCN 116	Kranti (NC)	30 kg S/ha	103.0	125.3	41.1	510
MCN 117	Varuna (NC)	30 kg S/ha	103.5	122.0	41.7	500
LCH-I	RH-30 (LC)	30 kg S/ha	125.7	123.4	41.5	525
CD at 5%			8.4	N.S	N.S	115.7

ASSESSSMENT OF CAULIFLOWER (*Brassica oleracea* var. *Botrytis*) VARIETIES FOR SUMMER PRODUCTION IN TEMPERATE CLIMATE

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ABSTRACT : In a preliminary trial ten varieties of cauliflower were tested during 1997. Out of these five varieties /selections were again assessed during 1998 and 1999 at Baragraon farm of IARI, Regional Station Katrain (Kullu Valley) H.P., India situated at an altitude of 1560 m a.s.l. Selection SWI-1gave the highest yield of 387.16 q/ha having good curd quality as well as size. The number of outer leaves were also lowest. This was followed by RS-119, but it was discarded due to poor curd quality.

INTRODUCTION : Cauliflower is one of the important vegetables grown under large area not only in autumn-spring but also during summer as off season crop in temperate climatic regions of-India. Hence this work was donc to identify the best varieties/selections which can be grown successfully in temperate climate prevalent in hilly regions of India . The high yielding varieties/selections with good curd quality will be an asset to the farmers to enhance their economic returns.

MATERIALS AND METHODS : Ten varieties/selections were tested in a preliminary trial in the year 1997. Out of these five promising varieties were again planted in a randomised block design having four replications for two consecutive years. Each plot measured 7.29 m^2. Plants were spaced 45x45 cm apart. Nursery sowing was done in the last week of May in 1998 and 1999. Seedlings were transplanted in field 25 days after sowing. Standard cultural practices were adopted to raise a good crop. The experimental farm is situated at an altitude of 1560 m a.s.l. Average maximum temperature ranged from 29.03°c to 32.06 °c and minimum from 4.45°c to 14.69°c from June to October for last twenty three years (1975-1998) . Observations were recorded for yield and related traits including curd quality.

RESULTS AND DISCUSSION : Analysis of the data recorded in both the years revealed significant differences among varieties for all the characters (Table1). Curd formation remained uniform inspite of heavy rains in the month of July-August. Selection SWI-1 topped by giving yield of 387.16 q/ha followed by RS-119 (354.51 q/ha). Vanparys (1998) found Escale, Cortes Liberty, Pavilion and Thalessa the highest yielders out of fifteen varieties of cauliflower assessed in June-October crop in Belgium. Rooster (1998) also reported that Cosmos was the best cultivar out of five tested for June-October production. The number of outer leaves were lowest in RS-119 and SWI-1 in first and second years of regular trials. Curd size was maximum for White Fox followed by SWI-1 and RS-119 . Average net curd weight was also maximum in the best selection SWI-1 . Days to fifty percent curd formation were also lowest for top ranking selection. Curd quality of SWI-1 is good. It is concluded that SWI-1 is the high yielder with good curd quality hence most suitable for summer production in temperate climate of India.

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Vanparys , L. 1998. Proefluinnieuws 8 (6) :139-40

Table-1. Mean performance of cauliflower varieties during summer in temterate climate of India

Varieties/ Selections	Number of outer leaves		Curd size (DxH)		Gross wt. per curd (kg)		Net wt. per curd (kg)		Days to 50% curd formation		Yield q /ha	Curd quality
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	Average	
RSK-1301	36.5	31.0	150.3	126.8	2.803	1.358	0.560	0.421	86.0	92.2	242.24	White, compact
White Fox	35.0	35.0	218.6	204.2	2.021	1.656	0.743	0.650	81.5	87.0	343.95	White, compact
RS-119	21.9	27.5	190.5	167.3	2.138	1.252	0.774	0.662	62.5	80.2	354.51	Yellow, Compact
SWI-1	26.7	25.1	202.4	164.4	1.193	1.221	0.897	0.671	61.0	82.0	387.16	Shining White, compact
PHJ-Check	30.6	30.2	159.0	113.4	1.769	0.873	0.692	0.432	60.2	82.5	277.49	White, compact
CD at 5%	3.5	2.9	40.4	31.9	0.318	0.220	0.137	0.108	4.9	3.4	-	-
CD at 1%	4.8	4.0	55.7	44.0	0.438	0.300	0.189	0.150	6.7	4.6	-	-

The Effect of the Technology of Growing Over the Biochemical Characteristic of Some Hybrids of Brussels Sprouts

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The Brussels sprouts is slightly spread in Bulgaria. Its high nutritious and dietetic properties, 2-3 times bigger contents of proteins, amino acids and absorbic acids, in comparison with the Headed cabbage (3), are in the basis of the raised interest in this crop in our country.

Some authors notify for the influence of different factors over the qualitative characteristics of the producte (1,4).

The purpose of this research is the determination of the influence of the breaking of the plants over the bio-chemical indexes of some hybrids of Brussels sprouts.

Materials and Methods

During the 1994-1995 period there have been tested the following hybrids: Rider, Diablo, Rampart, Cor/Valiant and Prince Marvel, grown by the technology for a late field production (pricking off – between 25-30 July). The growing tops of the plants have been broken when the diameter of the sprouts has reached 15-20 mm.

An average sample of 50 sprouts there have been analyzed the following indexes: the dry matter content (determined by weight), the ascorbic acid (using Tillman's reaction) (2), the monosaccharides (using the Shoorl-Regenbogen's method) (2) and the nitrate content (using the potentiometric method).

Results and Discussion

The technology of the growing effects the tested biochemical indexes in different ways. As a result of the breaking the content of the dry matter is increased insignificantly in Rider, Rampart and Prince Marvel, while in the other two hybrids it is decreased (Figure 1). Except Rider , the monosaccharides are in bigger quantity in the unbroken plants (Figure 2). A firm tendency to increasing of the ascorbic acid is observed in all the hybrids after the breaking (Figure 3). The increase varies from 7,09% (Rider) to 11,11% (Cor/Valiant).

The nitrate content in the broken plants is strongly reduced (Figure 4). Most clearly it is expressed in Rampart and Rider, in which the decrease is accordingly 50,77 and 48,42 %.

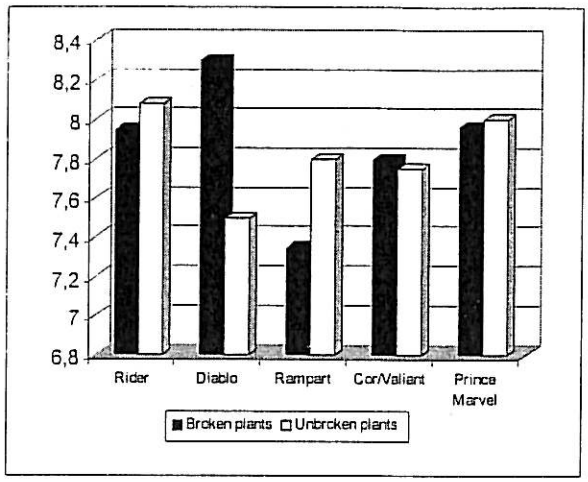

Figure 1. Dry matter content

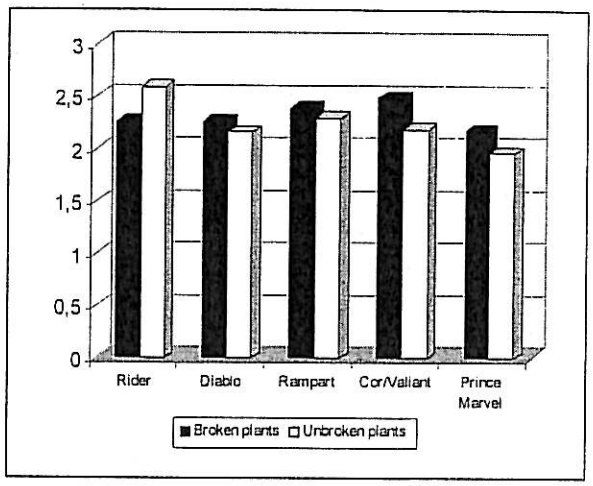

Figure 2. Monosaccharide content

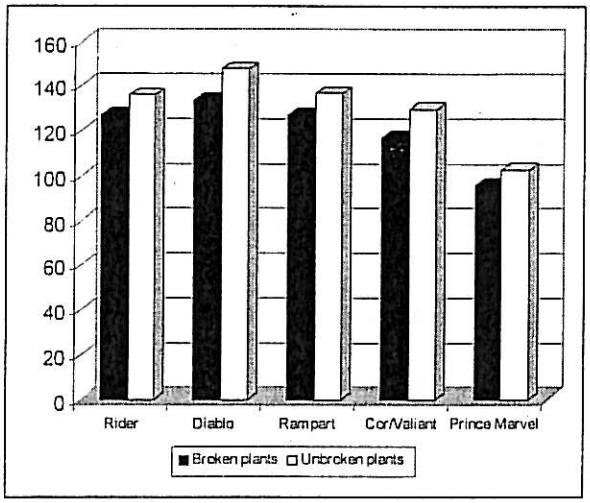

Figure 3. Absorbic acid content

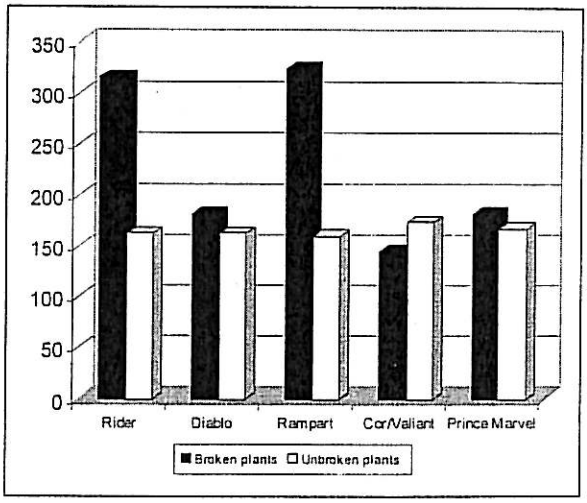

Figure 4. Nitrate comtent

A diversion of this tendency is observed in Cor/Valiant, which probably is due to the hybrid specification.

The breaking of the plants appears as an effective agro-technical solution for the improvement of the biochemical composition of the Brussels sprouts.

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Technological Elements and Suitable Hybrids of Brussels Sprouts for the Conditions of Bulgaria

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Summary

During the 1994-1995 period there was implemented a probation of the adaptive capacities of the following hybrids: Rider, Diablo, Cor/Valiant and Prince Marvel, grown up for a late field production. Quite good effect of the breaking of the plants was found out, which was expressed in the increase of the yields from 8,0% up to 46,2% and in shorten vegetative period with 3 to 8 days.

With the highest yields and quality were Rider and Prince Marvel.

Brussels sprouts has been grown on larger fields (30 ha) in Bulgaria for the first time in 1966 (2), as the climatic and soil conditions are suitable for that.(1)

In countries with wider distribution of this crop complex investigations of new varieties and technologies for growing are held. (3,4,5)

The purpose of the present work is the optimization of the varieties' structure and the offer of some technological solutions for a late field production of Brussels sprouts.

Materials and Methods

During the1994-1995 period in the Maritsa Vegetable Crops Institute, Plovdiv the Brussels sprouts hybrids Rider, Diablo, Rampart, Cor/Valiant and Prince Marvel have been tested. The plants have been grown up on high flat bed following the scheme 90-70/60 cm. The seeds have been sown on 5-10 June. The field pricking off has been done on 25-30 July. Fertilizer rate N_{15} P_{15} K_{10} has been used. When the sprouts have reached diameter of 15-20 mm., the tops of the plants were broken.

There have been reported the indexes of average mass of the sprouts, the thickness (in grades of 1 to 5), the yield, the vegetative period (days between the growing and the ripeness)

.

This was set through 4 replications held by the block method on testing plots of 10 m^2.

Results and Discussion

Through the testing there have not been noted some rapid diversions in the development of the separate hybrids, which shows their good adaptive capacities in our conditions.

The average mass of the sprouts from plants without breaking is 5,8 g (Cor/Valiant) to 14,0 g (Prince Marvel) – table 1. As a result of the breaking is determined an increase of the average mass from 13,8% to 36,6%.

The sprouts of Prince Marvel and Rider are with best thickness.

Table 1. Agro-biological characteristics of Brussels sprouts

Hybrids	Average weight, g		Thickness of sprouts, grades	Yield, kg/ha		Vegetative period, days	
	unbr*	br**		unbr*	br**	unbr*	br**
Rider	8.2	11.2	4.6	9030	13200	136	132
Diablo	11.5	14.5	4.4	8320	9270	131	127
Rampart	8.9	9.4	3.8	7730	8350	134	126
Cor/Valiant	5.8	6.6	3.5	6190	6850	134	131
Prince Marvel	14.0	16.4	5.0	9170	11350	141	136
CV	32.6	33.7	14.2	14.9	25.5	2.7	3.1

unbr*– unbroken plants ; br** - broken plants
CV - coefficient of variability, %

Unexpectedly good is the effect of the breaking of the plants which is expressed in the increase of the yield of the standard production from 8,0% (Rampart) to 46,2% (Rider).

The vegetation period varies from 131 to 141 days and is shorten with 3 to 8 days as a result of the breaking of the plants.

Conclusions

Quite good effect of the breaking of the plants is determined which is expressed in the increase of the yield from 8,0% to 46,2% and in shortening of the vegetative period with 3 to 8 days.

In the conditions of late field production with the highest yield and quality are the hybrids of Rider and Prince Marvel.

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INTERNATIONAL CLUBROOT WORKING GROUP - CLUBROOT 2000

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In 2000 the International Clubroot Working Group (ICWG) was fortunate to convene *Clubroot 2000* as a Satellite Meeting to the *Brassica 2000* Symposium held at Horticulture Research International (HRI), Wellesbourne, Warwickshire CV35 9EF, UK in September.

In the first part of *Clubroot 2000* active workers from around the world provided oral contributions describing their research. This Session was chaired by Professor Ian Crute, Director, Arable Crops Research Institute (IACR), Rothamsted. The second part provided an round table discussion of aspects of clubroot disease and its causal organism, *Plasmodiophora brassicae* when all those present were encouraged to make their views known.

Paul Scholze described the development of somatic hybrids using wild relatives of *Brassica oleracea* and other species within the Brassicaceae family. Resistance to several pathogens including *P. brassicae* was tested against wild isolates obtained from throughout Germany. For resistance testing the use of mixtures of races was advocated. (Scholze, P., Krämer, R., Ryschka, U., Klocke, E. & Schumann, G. Expression of resistance to fungal diseases and turnip mosaic virus in somatic hybrids; Federal Centre for Breeding Research on Cultivated Plants, Quedlinburg, D-06484, Germany).

The application of anther culture to obtain lines of Chinese cabbage resistant to *P. brassicae* was outlined by Yong Pyo Lim. This also provided an opportunity to discuss the importance of clubroot in Korea. The pathogen was first recorded in 1920, the entire peninsula is now severely infested especially since Chinese cabbage is the prime horticultural crop. Cultivars from Japan tend to be resistant while locally bred material is susceptible. Resistance is thought to be controlled by a single dominant gene. (Lim, Y. P., Jang, C. S., Piao, Z. Y. & Ahn, S. N. Development of clubroot resistant plants using anther culture in Chinese cabbage; Department of Horticulture, Chungnam National University, Taejon 305-764, Republic of Korea).

Molecular characterisation of the virulences in single spore isolates of *P. brassicae* was highlighted by Johannes Siemens. The single spore isolates were derived from *Arabidopsis* hosts inoculated with collections originally made by Elke Diederichsen. Extraction of *P brassicae* DNA was eventually successful despite problems with contamination from the host. Subsequently hosts were infected with mixtures of characterised single spore isolates. Re-analysis indicated that new RFLP bands were present suggesting recombination events during meiosis within the root which could be explained either by crossing over events between the original single spore genotypes or by mutation. It was postulated that this occurred immediately before resting spore formation. (Seimens, J., Klewer. A., Graf, H., Sacristán, M. D. & Luerßen, H. RFLP - characterisation of *Plasmodiophora brassicae*; Institute of Biology - Applied Genetics, Berlin 14195, Germany).

Chinese cabbage is a major crop in Japan and the control of clubroot is a priority issue as emphasised by Yasuhisa Kuginuki. The genetic diversity of the pathogen in Japan has been mapped using F_1 hybrids and lines of *B. rapa*. Four population groups have been recognised. Results suggest there are several major genes for clubroot resistance in *B. rapa*. Pyramiding these would help to delay the erosion of resistance in new cultivars of Chinese cabbage. (Kuginuki, Y., Masaoki, S., Tsukazaki, H. & Kageyama, K. Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot - resistant cultivars of Chinese cabbage; National Research Insitute of Vegetables, Ornamental Plants & Tea, Mie 514-2392, Japan).

The results of extensive studies of the incidence and host - pathogen biology of *Plasmodiophora brassicae*, in spring sown oil seed rape in Sweden, were explained by Ann - Charlotte Wallenhammar. From these studies the half life of the pathogen at 3.6 years for heavily infested fields was estimated, infestation fell below the detection level after 17.3 years. Molecular PCR based detection methods allowed the identification of DNA from *P brassicae* extracted from highly infested field soil. (Wallenhammar, A - C. Monitoring and control of *Plasmodiphora brassicae* in spring

oilseed brassicas; Swedish Federation of Rural Economy & Agricultural Societies, Orebro 701-45, Sweden).

Detection and control systems for clubroot which is rated as the most serious disease of vegetable *Brassica* crops in Australia were described by Caroline Donald. Control may be improved by using injection machines that place fertiliser and fungicides into the root zone. This has the triple advantage of increasing product efficacy, reducing soil residues and lowering costs. A PCR based system has been developed which detects *P. brassicae* in soil, water and plant material. By this technique some sources of infestation have been tracked to irrigation and bore hole water. An alternative to the use of the European Clubroot Differential (ECD) pathotyping system was proposed involving the use of microsatellite - primed PCR (MP-PCR). (Donald, C., Faggian, R., Porter, I. J. & Lawrie, A. C. Current status of clubroot detection in Australia; Institute for Horticultural Development, Victoria 3176, Australia).

The interaction of *P. brassicae* with the soil environment especially the effects of nutrient elements such as boron, calcium and nitrogen affecting resting spore germination, penetration and colonisation of root hairs and the resistance of hosts was discussed by Geoffrey Dixon. (Dixon, G. R. & Page, L. V. The impact of calcium in *Brassica* genotypes to *Plasmodiophora brassicae* Wor. (clubroot); University of Strathclyde, Glasgow, UK). He also presented a description of research into clubroot in West Bengal on behalf of Indrabrata Bhattacharya. Clubroot has become a major scourge especially in oil seed and cole brassicas and a research programme is established to identify the distribution of pathotypes. (Bhattachary, I. & Dixon, G. R. Study of race variation, characterisation and behaviour of clubroot of crucifers caused by *Plasmodiophora brassicae* (Woronin) in West Bengal; Bidhan Chandra Krisi Viswavidyalana, West Bengal, India).

The round table discussion that followed was led by:- Ann - Charlotte Wallenhammar (Sweden), Mats Gustafsson (Swedish Agricultural University, Alnarp), Gabriele Engqvist (Svalöv, SE-26881, Sweden) and Geoff Dixon (UK). Issues which emerged were:-

- The recombination rate in *P. brassicae* was judged to be very high.
- The need for a reconstructed ECD series and that this may be superseded by PCR techniques.
- Stronger sources of host resistance and sources of marker genes are urgently needed.
- Queries were raised as to the efficacy and reliability of inoculation techniques.
- Evidence for meiosis in the latter part of the life - cycle had been demonstrated.
- Dikaryotic stages were identified in epidermal cells and these may be the infective form for secondary infection.
- Does *P. brassicae* behave preferentially as an inbreeding or out breeding organism?
- There could be several pathotypes distributed spatially in different parts of the root system.
- The need for a central bank of single spore isolates.
- Mode of impact of calcium on resistance expression.
- The importance of calcium in providing field control was emphasised from Australia and Canada.
- A wider range of cultural and biological control options is required.

Generous sponsorship for this meeting was given by: SKW, Trostberg, Germany in partnership with PP Products, Norwich, UK - manufacturers and distributors of Perlka® (calcium cyanamide) and by Hydro Agri, Humberside, UK and Oslo, Norway - manufacturers of calcium nitrate.

Grateful thanks are also due to the organisers of Brassica 2000 for their help with *Clubroot 2000* notably:- Dr David Pink, Dr Graham King and Ms Michelle King. *Brassica 2000* was held under the auspices of Horticulture Research International (HRI), the International Society for Horticultural Science (ISHS) and the Crucifer Genetics Workshops. Approximately 60 delegates attended *Clubroot 2000* and the organisers are grateful to all those who provided formal and informal contributions for making this a very successful event. The organisers of the 8th International Plant Pathology Congress to be held in Christchurch, New Zealand, 2nd to 8th February 2003 have agreed that ICWG may hold a Workshop linked to their event In the intervening period there may be another meetings of ICWG in Australia.

EUCARPIA CRUCIFERAE NEWSLETTER Nr. 24

Instructions to authors
2001

Deadlines : **december 31st 2001**

Next Cruciferae Newsletter Nr 24 will be produced and edited at the end of 2001. 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.

1 – Contributions should be en English.

2 – The scripts will not be retyped and must be produced with the best typing quality. **Laser printing is expected with a clear black character**.

3 – As previously contributions must not exceed **2 pages**, including tables and figures. Whole pages are preferred (eventually reduce the format through computer printing). Consecutive pages must be numbered in pencil. **Times 12** character is expected.

4 – The heading of the paper must include the title (**1st line in bold letter)**, followed by the authors names (lines below).

5 – Single spacing is required. If not, it will be published at the editors discretion. The **A 4 format (21 x 29,7)** is strictly required with margins (not less than 3 cm on the left, 2 cm above and below). No other format will be accepted.

6 – Tables and figures must be included in, or at the end of the text. Photographs cannot be included and reproduced.

7 – Please do not fold the script and do not clip the pages together.
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