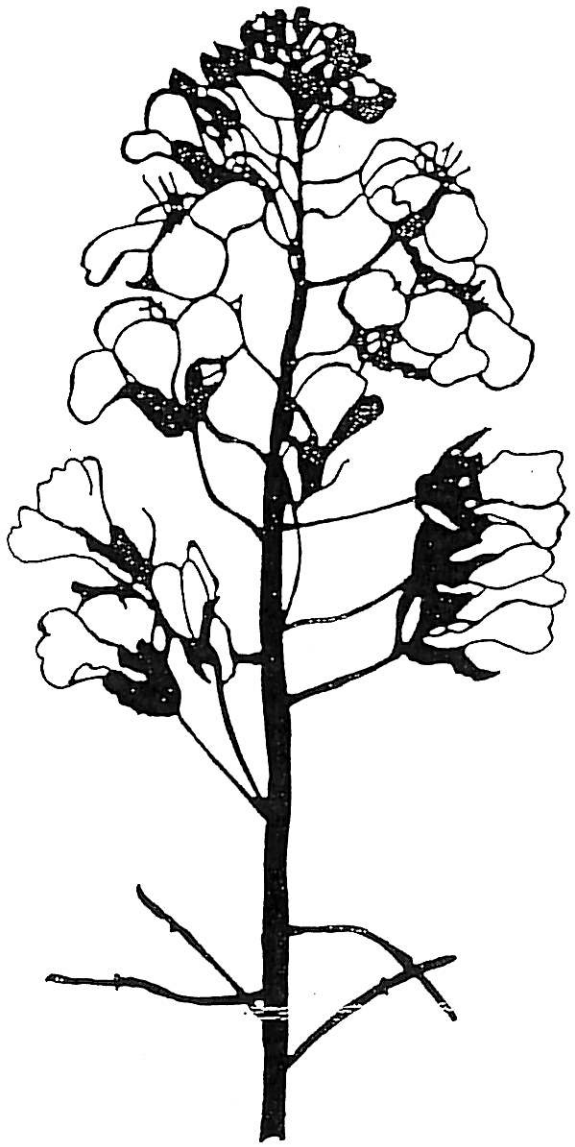
CRUCIFERAE NEWSLETTER



Nr. 24

JULY 2002

EUCARPIA

Ed. INRA Rennes

CRUCIFERAE NEWSLETTER

Address :

The Editor
INRA - ENSAR
UMR Amélioration des plantes et biotechnologies végétales
(UMR APBV)
Domaine de la Motte
BP 29
35653 LE RHEU CEDEX
FRANCE

CONTENTS – Nr.24

General contents Nr. 24

1) Genetic variability in the Brassicae tribe

2) Interspecific hybridization

3) Genome analysis and markers

4) Vitroculture studies and uses

5) Developmental and reproductive biology

6) Genetics and breeding for specific characters

7) Pest and disease studies and control

8) Agronomy and variety trials

9) General information

Book announcement :

SEED OIL CONTENT ANALYSIS OF ETHIOPIAN MUSTARD (*Brassica carinata* A. Braun) BY NEAR INFRARED SPECTROSCOPY

Font[1], R., Del Río[1], M., Fernández[1],J., Arthur[2], E., Bancroft[2],I., Chinoy[2],C., Morgan[2],C., De Haro[1], A.
[1]Instituto de Agricultura Sostenible (CSIC). Alameda del Obispo s/n. 14080 Córdoba. Spain.
[2]John Innes Centre. Norwich Research Park, Colney, Norwich, NR4 7UH, UK.

INTRODUCTION

Ethiopian mustard is an oilseed species with high potential as a crop for Mediterranean (semi-arid) conditions and as a genetic source for characters of agronomic importance. In the last 30 years, Near Infra-Red Spectroscopy (NIRS) has been widely used as a rapid and accurate method for qualitative and quantitative analysis in many fields (Williams et al., 1987).The Department of Agronomy and Plant Breeding of the Institute for Sustainable Agriculture (IAS, CSIC), has been using NIRS for the last fifteen years, to determine of seed quality components in different plant species (De Haro et al., 1989.;Velasco et al., 1992; Font et al., 1998, 2000). The most attractive features of analysis using NIRS are its speed, minimal sample preparation and its non-destructive nature thus making it possible to analyse large number of samples in a short time.

The objectives of this work are to test the potential of NIRS to determine the oil content of intact seeds of *Brassica carinata*, and to apply this technique to evaluate the protein content of a germplasm collection of this species grown in two different environments, Norwich (UK) and Córdoba (Spain).

MATERIAL AND METHOD

Ten plants from 100 accessions of *B. carinata*, chosen at random from the collections at IAS (Córdoba, Spain) and Centre for Genetic Resources (Wageningen, The Netherlands) were transplanted in March 2000 to field plots at Norwich, UK, and harvested in September 2000. The same 100 accessions were grown in field plots at Córdoba, Spain, from November 2000 to June 2001.

To perform NIRS calibration for protein content, 3g samples of intact seed from 2000 plants (100 accessions x 10 plants/accession x 2 localities), were scanned in a NIR spectrophotometer (NIRSystems model 6500, Foss-NIRSystems, Inc., Silver Spring, MD, USA) in the reflectance mode, acquiring their spectra at 2 nm. intervals over a wavelength range from 400 to 2500 nm (VIS + NIR regions). On the basis of their spectral features, a sub-set of 100 samples representative of the whole spectral variability contained in the entire set, were selected for performing NIR calibrations. Reference analytical values of the oil content for the selected samples were obtained by Nuclear Magnetic Resonance (NMR). Using the program GLOBAL v. 1.50 (WINISI II, Infrasoft International, LLC, Port Matilda, PA, USA), different mathematical treatments (0,0,1,1 (derivative, gap, first smooth, second smooth); 1,4,4,1; 2,5,5,2) were used to correlate spectral and chemical data. Cross-validation was performed on the calibration set to test the ability of the equations obtained to predict the protein content. The equation with the higher ratio of standard deviation (SD) to standard error of cross-validation (SECV) and the higher coefficient of determination (1-VR) was selected as the best equation (Williams et al., 1993). This equation was then applied to the previously acquired spectra to predict the protein content of the different accessions of *B. carinata* from both localities.

RESULTS AND DISCUSSION

The selected calibration equation resulted in a standard error of calibration (SEC) of 1.36 % dw, and a coefficient of determination (R^2) of 0.93. This indicates that the 93% of the variability contained in the reference chemical data was explained by the calibration model (Table 1). According with the ratio SD/SECV, the second derivative of the raw optical data gave the equation with the highest prediction ability, showing a good fit of the data by the model. The 1-VR coefficient was high, meaning that the calibration equation explained the 92 % of the variability contained in the data, when it was cross-validated on the calibration set (Fig. 1).

Table 1. Calibration and cross-validation statistics for oil content of *B. carinata* samples

Calibration						Cross-validation	
n	range	mean	SD	SEC	R^2	SD/SECV	1-VR
100	25.60-54.60	43.12	5.38	1.36	0.93	3.61	0.92

The main wavelengths used for performing the factors of the oil equation were chemically assigned to C-H stretching and bending of the CH_2 groups of oil (1724, 1764, 2308 and 2348 nm).
These results show that it is possible to use NIRS to determine the oil content on intact seed samples of Ethiopian mustard with enough accuracy for screening and plant breeding purposes. The use of this non destructive technique represents an important reduction of the analysis time at a low cost and without using hazardous chemicals.
Inversely to the protein content, mean seed oil content (% DW) of the *B. carinata* accessions was significantly greater ($P<0.001$) at Córdoba (44.7%) than at Norwich (33.2%), with similar range in absolute value (Córdoba: 36.60-50.90 %; Norwich: 27.00-40.30 %) (Fig. 2). Similar inverse relationship between protein and oil contents have been previously found in other oilseed crops (Robbelen, 1991).
The data obtained in this work, together with those of protein, fibre, fatty acids and glucosinolate content (unpublished results) will enable us to evaluate the stability of the most important seed storage components of *B. carinata* in different environments (G x E effects).

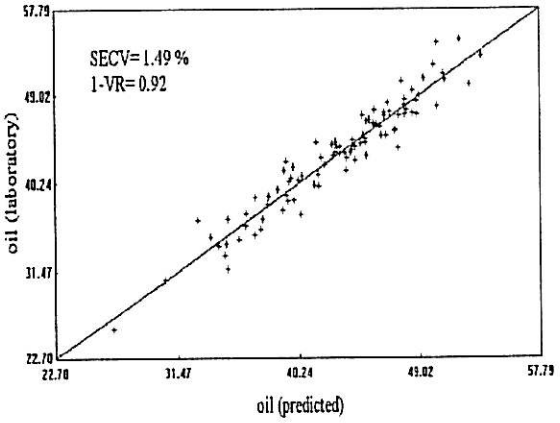


Fig. 1. Cross-validation scatter plot for oil

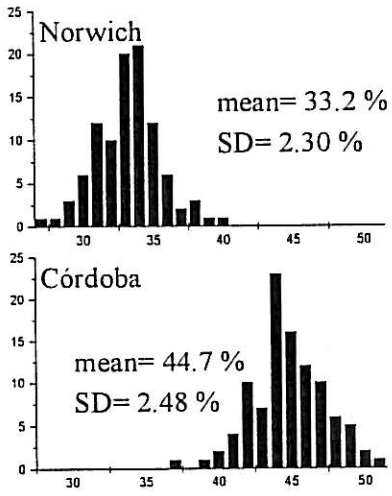


Fig. 2. Frequency distribution of oil

REFERENCES

DE HARO, A., LÓPEZ-MEDINA, J., CABRERA, A. & MARTÍN, A. (1989). In Recent Advances of Research in Antinutritional Factors in Legume Seeds. Ed. by J. Huisman, T.F.B. van der Poel and I.E. Liener. Pudoc. Wageningen. 297-300.
FONT, R., DEL RIO, M., FERNÁNDEZ-MARTÍNEZ, J.M. & DE HARO, A. (1998). Cruciferae Newsletter **20**, 67-68.
FONT, R., GUTA, B., DEL RIO, M., DOMÍNGUEZ, J., FERNÁNDEZ-MARTÍNEZ, J.M. & DE HARO, A. (2000). Cruciferae Newsletter **22**, 7-8.
RÖBBELEN, G. (1991). In Production and Utilization of Protein in Oilseed Crops. Ed. by E.S. Bunting. Martinus Nijhoff, The Hague, The Netherlands. 3-11.
VELASCO, L., MARTÍN, L.M. & DE HARO, A. (1992). In: Near Infrared Spectroscopy. Ed. by K.I. Hidrum, T. Isaksson, T. Naes and A. Tandberg. Ellis Horwood, England. 287-292.
WILLIAMS, P.C. & NORRIS, K.H. (1987). American Association of Cereal Chemists Inc.: St. Paul, MN. 1987. 330 pp.
WILLIAMS, P.C. & SOBERING, D.C. (1993). *J. NIRS*. 1, 25-32.

Acknowledgements
This work has been supported by the European project: "Brassica collections for Broadening Agricultural Use", including "Characterising and utilising genetic variation in Brassica carinata for its exploitation as an oilseed crop" (Contract no: RESGEN CT99 109-112).

USING NEAR INFRARED SPECTROSCOPY FOR DETERMINING PROTEIN CONTENT IN ETHIOPIAN MUSTARD (*Brassica carinata* A. Braun)

Font[1], R., Del Río[1], M., Sillero[2],A., Arthur[3], E., Bancroft[3], I., Chinoy[3], C., Morgan[3], C., De Haro[1], A.
[1]Instituto de Agricultura Sostenible (CSIC). Alameda del Obispo s/n. 14080 Córdoba. Spain.
[2]Laboratorio Agroalimentario. J.A. Alameda del Obispo s/n. 14080 Córdoba. Spain.
[3]John Innes Centre. Norwich Research Park, Colney, Norwich, NR4 7UH, UK.

INTRODUCTION

Ethiopian mustard is an oilseed species with high potential as a crop for Mediterranean (semi-arid) conditions and as a genetic source for characters of agronomic importance. In the last 30 years, Near Infra-Red Spectroscopy (NIRS) has been widely used as a rapid and accurate method for qualitative and quantitative analysis in many fields (Williams et al., 1987).The Department of Agronomy and Plant Breeding of the Institute for Sustainable Agriculture (IAS, CSIC), has been using NIRS for the last fifteen years, to determine of seed quality components in different plant species (De Haro et al., 1989.;Velasco et al., 1992; Font et al., 1998, 2000). The most attractive features of analysis using NIRS are its speed, minimal sample preparation and its non-destructive nature thus making it possible to analyse large number of samples in a short time.

The objectives of this work are to test the potential of NIRS to determine the protein content of intact seeds of *Brassica carinata*, and to apply this technique to evaluate the protein content of a germplasm collection of this species grown in two different environments, Norwich (UK) and Córdoba (Spain).

MATERIAL AND METHOD

Ten plants from 100 accessions of *B. carinata*, chosen at random from the collections at IAS (Córdoba, Spain) and Centre for Genetic Resources (Wageningen, The Netherlands) were transplanted in March 2000 to field plots at Norwich, UK, and harvested in September 2000. The same 100 accessions were grown in field plots at Córdoba, Spain, from November 2000 to June 2001. To perform NIRS calibration for protein content, 3g samples of intact seed from 2000 plants (100 accessions x 10 plants/accession x 2 localities), were scanned in a NIR spectrophotometer (NIRSystems model 6500, Foss-NIRSystems, Inc., Silver Spring, MD, USA) in the reflectance mode, acquiring their spectra at 2 nm. intervals over a wavelength range from 400 to 2500 nm (VIS + NIR regions). On the basis of their spectral features, a sub-set of 100 samples representative of the whole spectral variability contained in the entire set, were selected for performing NIR calibrations. Reference analytical values for the selected samples were obtained by Kjeldahl (AOAC method 920.87, 1990). Using the program GLOBAL v. 1.50 (WINISI II, Infrasoft International, LLC, Port Matilda, PA, USA), different mathematical treatments (0,0,1,1 (derivative, gap, first smooth, second smooth); 1,4,4,1; 2,5,5,2) were used to correlate spectral and chemical data. Cross-validation was performed on the calibration set to test the ability of the equations obtained to predict the protein content. The equation with the higher ratio of standard deviation (SD) to standard error of cross-validation (SECV) and the higher coefficient of determination (1-VR) was selected as the best equation (Williams et al., 1993). This equation was then applied to the previously acquired spectra to predict the protein content of the different accessions of *B. carinata* from both localities.

RESULTS AND DISCUSSION

The selected calibration equation resulted in a standard error of calibration (SEC) of 0.50 % dw, and a coefficient of determination (R^2) of 0.99, meaning that the 99% of the variability contained in the data was explained by the calibration model (Table 1). On the basis of the ratio SD/SECV, the second derivative of the raw optical data gave the equation with the higher prediction ability and a very good fit of the data by the model. On the other hand, the calibration equation explained the 98 % of the data variability when it was cross-validated on the calibration set (Fig. 1).

Table 1. Calibration and cross-validation statistics for protein content of *B. carinata* samples

Calibration						Cross-validation	
n	range	mean	SD	SEC	R^2	SD/SECV	1-VR
100	16.80-37.80	24.23	4.66	0.50	0.99	6.85	0.98

The wavelengths highly participating in the development of the protein equation were chemically assigned to stretching of S-H groups (1740 nm), protein (2052 nm) and C-H stretching and bending of the CH_2 groups of oil (1724, 1764, 2308 and 2348 nm).

These results show that it is possible to use NIRS to accurately analyse the protein content on intact seed samples of Ethiopian mustard. The use of this non destructive technique represents an important reduction of the analysis time at a low cost and without using hazardous chemicals.

Mean seed protein content (% DW) of the *B. carinata* accessions was significantly greater (P<0,001) at Norwich (25,9%) than at Córdoba (22,7%). However, the range of protein content was greater at Córdoba (18,2-29,9%) than at Norwich (22,4-31,7%) (Fig. 2). These differences are likely to be explained by the different environments, where Córdoba were sunnier, warmer and drier that Norwich. The data obtained in this work will enable us to evaluate the stability of this important seed quality component in different environments (G x E effects).

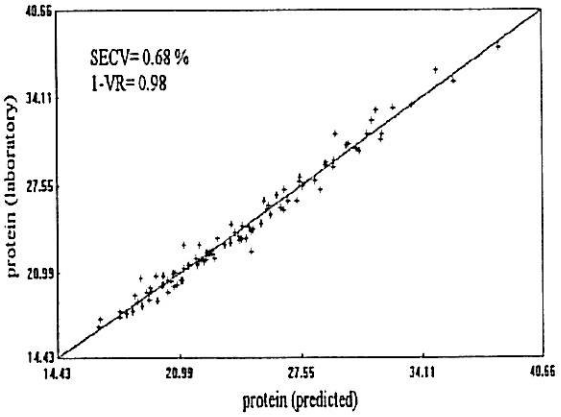


Fig. 1. Cross-validation scatter plot for protein

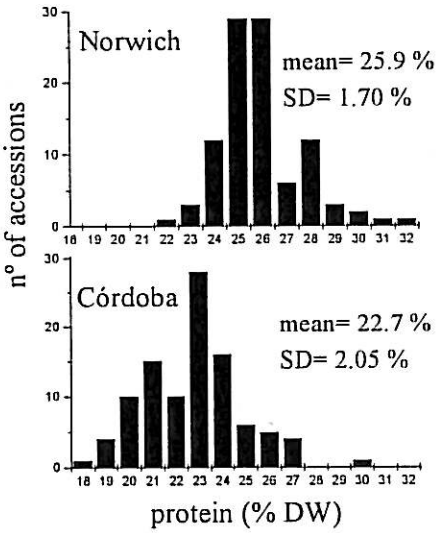


Fig. 2. Frequency distribution of protein

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. (1990). In: Official Methods of Analysis. Ed. by Kenneth Helrich.. Fifteenth edition. Arlington, Virginia, USA. Vol. 2. 781-782.

DE HARO, A., LÓPEZ-MEDINA, J., CABRERA, A. & MARTÍN, A. (1989). In: Recent Advances of Research in Antinutritional Factors in Legume Seeds. Ed. by J. Huisman, T.F.B. van der Poel and I.E. Liener. Pudoc. Wageningen. 297-300.

FONT, R., DEL RIO, M., FERNÁNDEZ-MARTÍNEZ, J.M. & DE HARO, A. (1998). Cruciferae Newsletter **20**, 67-68.

FONT, R., GUTA, B., DEL RIO, M., DOMÍNGUEZ, J., FERNÁNDEZ-MARTÍNEZ, J.M. & DE HARO, A. (2000). Cruciferae Newsletter **22**, 7-8.

VELASCO, L., MARTÍN, L.M. & DE HARO, A. (1992). In: Near Infrared Spectroscopy. Ed. by K.I. Hidrum, T. Isaksson, T. Naes and A. Tandberg. Ellis Horwood, England. 287-292.

WILLIAMS, P.C. & NORRIS, K.H. (1987). American Association of Cereal Chemists Inc.: St. Paul, MN. 1987. 330 pp.

WILLIAMS, P.C. & SOBERING, D.C. (1993). *J. NIRS.* **1**, 25-32.

Acknowledgements

Authors thank Dr. José Manuel Gálvez from Laboratorio Agroalimentario, Junta de Andalucía, for performing the chemical analyses of the reference samples.

This work has been supported by the European Union (Contract no: RESGEN CT99 109-112.

Evaluation of Cauliflower Plant Introductions for White Mold Resistance

Griffiths, P. D.[1] and Robertson, L. D.[2]

[1] Department of Horticultural Science, Cornell University NYSAES, Geneva, NY 14456
[2] USDA-ARS Plant Genetics Resource Unit, NYSAES, Geneva NY 14456

Introduction:

White mold (*Sclerotinia sclerotiorum*) is an important disease of cole crops, particularly during cool, damp seasons. The disease is of particular importance to the storage cabbage industry as it results in white mold growth on stored cabbage heads and the consequent wastage of several wrapper leaves per head. White mold is also a serious problem during seed production of broccoli and cauliflower (cottony stem rot). Previous screening of plant introductions of cabbage (*Brassica oleracea* var. *capitata*) identified resistance in accessions PI 206942, 175603, 245013, 291998, 246117 and 343628 (Dickson and Petzoldt, 1996). The current study screened the available cauliflower (*Brassica oleracea* var. *botrytis*) accessions stored at the USDA-ARS germplasm repository for white mold resistance.

Materials and Methods:

A total of 212 available accessions of cauliflower from the US collection were planted in 3 replicated plots with 5 plants per plot during the 2000 field season in New York State. Susceptible controls of cabbage, cauliflower and broccoli were also incorporated together with 9 white mold resistant breeding lines (cabbage, cauliflower and broccoli) derived from non-heading cabbage accessions. Transplants were made in early July, one month later than regular trials to avoid high temperatures during inoculation. Plants were grown to near maturity and plugs of two-day old white mold grown on PDA plates were used for inoculation in late August. The cabbages were inoculated between the wrapper leaf and head, and the cauliflower and broccoli plants were inoculated at the stem axil affixing the inoculum plug with a wooden toothpick. Overhead irrigation was set up, and following inoculation plants were irrigated three times daily for a period of 8 minutes. Two weeks after inoculation all plants not showing signs of infection were re-inoculated. Plants were evaluated (0-5) late September, and late October for severity of symptoms based on the extent of head rot for the cabbages, and the extent of stem rot for the broccoli and cauliflower. The second rating for cauliflower and broccoli included head/curd rot.

Results:

All controls rated 4.5-5, and of the 221 lines screened only ten (table 1) had a mean rating better than 2.5 (on a scale of 0-5) at the time of the first rating, and only four at the second rating. Four of the most resistant lines were selections from previous Cornell germplasm, and six were PI's (PI 462217 (2.29), PI 462216 (2.80), PI 296130 (2.87), PI 343479 (2.92), PI 244834 (2.93) and PI 462224 (2.93). While the six PI's appear to have some resistance, it is not better than the resistant germplasm derived from non-heading cabbage varieties, and may be of limited use. In follow –up greenhouse inoculations the most resistant accessions in the field test PI 462216 and PI 462217 were found to be susceptible. The remaining 4 PIs were among those that had late or no heading, suggesting that they may only have been rated at the vegetative stage, and not the curd stage. These accessions and not more resistant than current breeding material, but provide additional germplasm for breeding white mold resistant crucifers.

Accession/Line	Rating 1 Mean	Accession/Line	Rating 2 Mean
WM 002154 (Br)	1.27	WM 002154 (Br)	1.07
WM 003029 (Ca)	1.79	PI 462217 (Ca)	2.29
PI 267724 (Ca)	1.87	WM 002037 (Br)	2.29
WM 001134 (C)	1.93	WM 001134 (C)	2.36
PI 234599 (Ca)	1.93	PI 462216 (Ca)	2.80
PI 462217 (Ca)	2.14	PI 296130 (Ca)	2.87
WM 002037 (Br)	2.14	WM 002036 (Br)	2.92
PI 462224 (Ca)	2.20	PI 343479 (Ca)	2.92
PI 462216 (Ca)	2.33	PI 244834 (Ca)	2.93
WM 002037 (Br)	2.40	PI 462224 (Ca)	2.93

Table 1: Mean disease severity rating of the most resistant replicates.

*** PI = Plant Introduction, WM = White Mold breeding line, C = Cabbage, Br = Broccoli, Ca = Cauliflower.**

References:

Dickson, M.H. and Petzoldt, R., 1996. Breeding for resistance to *Sclerotinia sclerotiorum* in *Brassica oleracea*. Proc. Int. Sym on *Brassicas*, Ninth crucifer genetics workshop, ed. J.S. Dias, I. Crute and A.A. Monteiro. Acta Horticulturae 407: p103-108.

Performance of *Brassicas* under the intermediate conditions of Jammu

*S.K. Gupta and *Y.P. Khanna

SKUAST, Regional Agricultural Research Station
Rajouri.

Jammu division of Jammu and Kashmir has three different agroclimatic zones ranges from sub-tropical to temperate zone and as such the varieties grown also varies from zone to zone. Under this zone, mostly the varieties of *Brassica campestris* namely KOS-1 is predominantly grown. In order to search some other alternative of this species, an attempt has been made to identify the genotypes of other three species viz., *B. napus.*, *B. juncea* and *B. carinata* which could outyield *B. campestis*, species.

Materials and Methods

The material comprised of ten, nine and eight genotypes of three different species *B. juncea*, *B. napus* and *B. carinata* (Ethiopian mustard), respectively. These genotypes were grown in three separate experiments viz., performance of *B. juncea*, *B.napus* and *B. carinata* genotypes. Thse experiments were laid during rabi 1999 in a randomized block design with three replications at SKUAST-Regional Agricultural Research Station, Rajouri. Data was collected for yield and other yield contributing traits.

Results and Discussion

The analysis of variance was performed for all the three separate experiments. Results indicated that amongst *B. juncea* genotypes, genotype DSS-5 recorded highest seed yield of 14.3 q/ha followed by DSS-8 (13.44 q/ha) and DSS-6 (11.13 q/ha). However, DSS-8 and DSS-5 were statistically at par with each other but differed significantly from DSS-6. In second experiment on *B. napus*, DGS-1 gave the highest seed yield of 12.89 q/ha follwed by GSL-2(11.11 q/ha) and Topa (10.37 q/ha). The highest seed yield of this variety was also obtained in All India Co-ordinated trials of IVT-1 and AVT-1 during rabi 1999 (Annual Progress Report, 1999). Amongst the *B.carinata* genotypes, PHC-2 recorded highest seed yield of 12.48 q/ha followed by DIR-1519 (12.30 q/ha) and PCC (11.86 q/ha). The most important observation was negligible effect of six inches snowfall recorded during the January, 13, 1999. Ethiopian mustard which has its origin in Ethiopia also showed no or negligible effect on yield.

Performance of *B.Juncea*, *B napus* and *B campestris* genotypes

B. juncea	(q/ha)	*B. carinata*	*(q/ha)*	*B napus*	(q/ha)
Pusa Bahar	8.8	PHC2	12.48	DGS1	12.89
Pusa Basant	9.73	NPC2	11.74	Topa	10.37
DSS 5	14.36	NPC27	10.81	Gullivar	9.48
DSS8	13.34	DIR1519	12.30	Sel.001	8.57
DSS6	11.13	PC5	11.18	Sel.002	7.70
DSS1	8.53	PCC7	7.72	Excel	7.70
DSS9	9.64	PCC5	7.35	Nikalas	7.26
DS7	10.11	PCC8	11.86	GSL1	9.56
DS13	9.93	DGS1	13.09	GSL2	11.11
DSS2	7.88	GSL1	12.72		
DGS1	16.90				
C.D.	1.19		2.67		1.25

Reference

Annual Progress Report (1999). All India Co-ordinated Research Project on Rapeseed - mustard, Bharatpur, Rajasthan.

VARIABILITY STUDIES IN INDIAN MUSTARD UNDER SIX DIFFERENT ENVIRONMENTS IN ACIDIC SOIL

JAY LAL MAHTO AND Z. A. HAIDER
(DEPARTMENT OF PLANT BREEDING AND GENETICS, BIRSA AGRICULTURAL UNIVERSITY, RANCHI 834 006, JHARKHAND, INDIA.)

ABSTRACT : Environments 4th, 5th and 6th were more favourable to expression of yield attributes than that of environment 1st, 2nd and 3rd for mean, genetic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), genetic advance, heritability, and genetic advance in per cent of mean.

KEY WORDS: Mustard, environment, dates of sowing, sowing direction (North-South and East-West)

INTRODUCTION : The seed yield in Indian mustard (*Brassica juncea*) is a complex character, which is highly subjected to environmental variations. Information on nature and magnitude of variability present in a population due to genetic and non-genetic causes is an important prerequisite for a systematic breeding programme to improve the yield potential of genotypes. An attempt was made in the present investigation to asses the variability, heritability and genetic advance of some quantitative characters in a set of selected genotypes and their progenies.

MATERIALS AND METHODS: Nine parents (1. RW873, 2.PR830, 3.KRANTI, 4. RW29-6-3, 5. PR18, 6. RH843, 7.RH851, 8. VARDAN AND 9.BR40) were involved in a diallel mating design (excluding reciprocals). These lines and their 36 F_1's were grown at Birsa Agricultural University experimental area, Ranchi, in a single row in randomized block design in two replications under six environments of North-South and East-West with three dates of sowing in each directions. Distance were maintained between rows and plants at 30cm and 10cm, respectively. The area is located between 23°17'' latitude and 85''19'E longitude and altitude is 625 meters above the sea level. The p^H of the soil being 5.9. Ten plants were randomly selected in each genotypes of replication under each environment to record the observations on the 11 characters. Co-efficient of variation (Burton, 1952), heritability (Lush, 1940) and genetic advance (Johnson *et al.*, 1955) were done.

RESULTS AND DISCUSSION: Analysis of variance for most of the characters in all the environments showed the significant differences in genotypes. On the basis of mean data, 2nd environment favoured the maximum expression of number of secondary branches / plant and harvest index and earlier days 50% flowering. Third environment disfavoured the expression of number of primary branches / plant and number of siliquae / plant and favoured the expression of 1000-seed weight and oil content in percent. Plant height, number of seeds / siliqua and short duration maturity was favoured in 5th environment but did not favour to number of secondary branches / plant. Sixth environment was favoured for high days to 50% flowering, number of primary branches / plant, number of siliquae / plant, days to maturity and seed yield / plant and was unfavourable for the number of seeds / siliqua, harvest index and oil content on the basis of mean. PCV was favoured in days to 50% flowering, days to maturity, harvest index and seed yield in 4th environment. Fifth environment favoured plant height for PCV. Sixth environment was suitable for number of primary branches / plant, number of secondary branches / plant, number of siliquae / plant, number of seeds siliqua and oil content. GCV in 1st environment had positive effect for high days to maturity and 1000-seed weight, secondry branches / plant, number of siliquae / plant. Third environment favoured number of primary branches / plant and number of siliquae / plant for GCV. 4th environment was additive for more number of secondary branches / plant, plant height, harvest index and seed yield / plant. 6th environment was suitable for expression of high days to 50% flowering, number of seeds / siliqua, plant height, oil content in percent for genetic coefficient of variation. Heritability in 1st environment was favoured for expression of number of secondary branches / plant. 4th environment favoured the expression of number of secondary branches / plant, plant height, number of siliquae / plant, days to maturity, harvest index, 1000-seed weight and oil content in percent. 5th environment was suitable for days to 50% flowering as earlier, number of seeds / siliqua and seed yield / plant, harvest index, 1000-seed weight and oil content in percent for heritability. Genetic advance in 1st environment was congenial for the expression high days to maturity and 1000-seed weight. In 4th environment number of secondary braches / plant, plant height, number of siliquae / plant and harvest index was favoured for genetic advance. Sixth environment was suitable for expression of the days to 50% flowering, plant height as short stature, number of seeds / siliqua, seed yield / plant and oil content in percent for genetic advance. Genetic advance in percent of mean in 1st environment was suitable for the long days to maturity and high 1000-seed weight and unsuitable for number of secondary branches / plant and siliquae / plant. 4th environment was favoured the number secondary branches / plant, plant height, number of siliquae / plant, harvest index and seed yield / plant. 5th environment had positive effect for days to 50% flowering as earlier, days to maturity as short duration and negative effect for number of primary branches / plant, seeds / siliqua, harvest index and oil content. 6th environment favoured the days to 50% flowering, plant height as short staure, number of seeds / siliqua and oil content in percent for genetic advance in percent of mean. Low variability was due to the presence of both positive and negative alleles. Improvement in these characters can be done by hybridization. Heritability conjunction with genetic gain would give more reliable index of selection value (Johnson *et al.*,1955). To improve the character having high heritability along with genetic gain can be done selection, particularly through mass selection for different environments. The character having moderate heritability and low genetic advance, which indicates the presence of dominance and epistatic effect, may be improved by selection based on family.

REFERENCES

Burton, G. W. 1952. Quantitative inheritance in grasses. *Proc. 6th Grassland Congr.*, 1:356-363.

Johnson, H. W., Robinson, H. F. and Comstock, R. E. 1955. Estimates of genetic and environmental variability in soybeans. Agronomy Journal. 47:314-318.

Lush, J. L. 1940. Intra-sire correlation and regression of offsprings and dams as a method of estimating heritability of characters. *Proc. Am. Soc. Animal Prod.* 33: 293-301.

Table 1. Variability studies in Indian mustard for different traits in six environments.

PARAMETER		CHARACTERS										
		1	2	3	4	5	6	7	8	9	10	11
Genotypic	(N-S) E1	56.14	0.772	3.311	196.2	2030.8	4.385	59.48	0.004	0.368	7.235	5.374
variance	(N-S) E2	23.15	0.244	10.538	213.3	6587.7	1.231	14.71	0.003	0.199	6.570	3.822
	(N-S) E3	55.30	0.878	8.977	177.2	8183.8	4.546	34.68	0.003	0.190	3.715	3.637
	(E-W) E4	39.79	0.887	11.699	209.4	6726.2	4.746	32.64	0.006	0.261	5.715	5.716
	(E-W) E5	18.83	0.386	8.755	217.6	5073.2	1.261	15.42	0.002	0.180	33.61	2.099
	(E-W) E6	97.55	0.977	9.661	189.7	12399	5.237	39.26	0.002	0.281	35.92	15.95
Phenotypic	(N-S) E1	112.1	3.089	20.16	375.5	10513	7.779	90.05	0.006	0.528	7.557	5.889
variance	(N-S) E2	65.44	1.479	30.80	457.0	13496	3.561	46.66	0.006	1.007	7.115	7.150
	(N-S) E3	138.6	0.708	23.79	561.4	12467	8.134	164.9	0.006	0.469	3.875	5.686
	(E-W) E4	76.29	2.078	16.37	393.7	7876	9.083	46.19	0.008	0.370	5.959	6.189
	(E-W) E5	58.15	2.391	50.68	444.4	14455	5.912	56.93	0.004	0.587	34.69	5.544
	(E-W) E6	141.8	4.867	165.3	603.7	55855	10.39	100.0	0.004	0.531	38.14	19.61
Error	(N-S) E1	55.92	2.317	16.85	179.2	8483	2.394	40.57	0.002	0.160	0.322	0.515
variance	(N-S) E2	42.29	1.236	20.26	243.7	6908	2.330	31.95	0.003	0.908	0.645	3.328
	(N-S) E3	83.33	1.586	14.81	384.2	4283	3.588	130.3	0.003	0.279	0.160	2.049
	(E-W) E4	36.50	1.191	4.673	184.4	1150	4.337	13.55	0.002	0.109	0.244	0.473
	(E-W) E5	39.33	2.055	41.92	226.8	9382	4.651	41.51	0.002	0.407	1.087	3.445
	(E-W) E6	44.27	3.890	155.6	413.9	43455	5.153	60.74	0.002	0.250	3.814	3.659
Phenotypic	(N-S) E1	18.82	45.98	78.98	16.29	74.91	27.19	9.092	45.19	24.86	82.05	6.304
coefficient	(N-S)E2	15.76	30.07	80.25	16.91	68.79	16.92	6.517	35.56	31.85	61.35	6.891
of variation	(N-S)E3	17.73	34.24	93.39	20.87	97.29	27.58	10.76	41.43	22.84	69.78	6.142
	(E-W) E4	14.02	37.60	79.24	18.81	75.74	27.46	5.947	51.24	21.66	98.31	6.431
	(E-W) E5	14.43	34.96	148.3	16.13	61.44	20.58	7.255	30.81	24.84	92.53	6.141
	(E-W) E6	17.29	47.14	251.5	19.85	99.27	31.48	8.215	38.33	23.32	92.38	11.80
Genotypic	(N-S) E1	13.32	22.98	32.01	11.78	32.92	20.41	7.390	36.89	20.75	80.29	6.022
coefficient	(N-S)E2	9.380	12.20	46.94	11.55	48.06	9.944	3.659	28.37	14.16	58.95	5.038
of variance	(N-S)E3	11.20	25.48	57.38	11.73	78.82	20.62	4.935	27.93	14.53	68.33	4.912
	(E-W) E4	10.12	24.56	66.98	13.72	69.99	19.85	4.999	43.88	18.19	96.28	6.180
	(E-W) E5	8.210	13.10	61.64	11.29	36.40	9.504	3.776	20.17	13.75	91.07	3.778
	(E-W) E6	14.34	21.12	60.81	11.13	46.77	22.35	5.147	27.10	16.96	89.66	10.64
Heritability	(N-S) E1	50.10	24.98	16.43	52.27	19.32	56.37	66.06	66.67	69.69	95.74	91.25
	(N-S) E2	35.38	16.46	34.21	46.67	48.81	34.56	31.52	63.64	19.76	92.34	53.46
	(N-S) E3	39.89	55.36	37.74	31.56	65.64	35.89	21.03	45.46	40.51	95.87	63.86
	(E-W) E4	52.15	42.67	71.46	53.18	85.40	52.25	70.66	73.33	70.50	95.91	92.36
	(E-W) E5	32.38	14.03	17.28	48.97	35.09	21.33	27.09	42.86	30.66	96.87	37.86
	(E-W) E6	68.78	20.07	5.850	31.46	22.19	50.40	39.26	50.00	52.88	94.19	81.34
Genetic	(N-S) E1	10.93	0.900	1.520	20.87	40.80	3.239	12.91	0.106	1.043	5.422	4.562
advance	(N-S) E2	5.900	0.410	3.910	20.55	116.8	1.343	4.436	0.097	0.409	5.074	2.945
	(N-S) E3	9.680	1.440	3.790	15.41	150.9	3.283	5.562	0.069	0.572	3.888	3.142
	(E-W) E4	9.380	1.270	5.960	21.74	156.1	3.244	9.893	0.131	0.883	4.823	4.733
	(E-W) E5	5.090	0.450	2.530	21.27	86.92	1.068	4.211	0.052	0.484	11.76	1.836
	(E-W) E6	16.87	0.910	1.550	14.20	108.1	3.347	8.088	0.065	0.793	11.98	7.420
Genetic	(N-S) E1	19.43	23.66	26.73	17.54	29.81	31.57	12.37	62.06	35.69	161.8	11.85
advance in	(N-S) E2	11.49	10.20	56.56	16.26	69.17	12.04	4.232	46.63	12.97	116.7	7.588
percent of	(N-S) E3	14.57	39.05	72.61	13.57	131.6	31.75	4.661	38.79	19.06	137.8	8.093
mean	(E-W) E4	15.06	33.05	116.6	20.61	133.2	29.56	8.657	77.41	31.46	194.2	12.24
	(E-W) E5	9.630	10.11	52.78	16.27	44.42	9.042	4.049	27.20	15.69	184.6	4.789
	(E-W) E6	24.50	19.49	30.29	12.76	108.1	32.68	6.644	39.48	25.40	179.3	19.77
Mean	(N-S) E1	56.24	3.829	5.684	118.9	136.9	10.26	104.4	0.171	2.923	3.350	38.50
	(N-S) E2	51.32	4.044	6.916	126.4	168.9	11.16	104.8	0.209	3.151	4.348	38.80
	(N-S) E3	66.41	3.678	5.222	113.5	114.7	10.34	119.3	0.179	2.999	2.821	38.82
	(E-W) E4	62.31	3.833	5.107	105.5	117.2	10.97	114.3	0.169	2.806	2.483	38.68
	(E-W) E5	52.83	4.422	4.800	130.7	195.7	11.82	104.0	0.192	3.085	6.366	38.35
	(E-W) E6	68.88	4.680	5.111	124.7	238.1	10.24	121.7	0.165	3.123	6.685	37.54

N=North, S=South, E=East, W=West and En=Environments

Character 1. Days to 50% flowering, 2. Number of primary branches / plant, 3. Number of secondary branches / plant, 4. Plant height, 5. Number of siliquae / plant, 6. Number of seeds / siliqua, 7. Days to maturity, 8. Harvest index 9. 1000-Seeds weight, 10. Seed yield / plant 11. Oil content in percent

SOME CYTOLOGICAL OBSERVATION OF RADISH GROWN ON FLY- ASH AMENDED SOIL

P.K. Mishra and U. C. Mehta*
R.S. More College Gobindpur – 828109, Jharkhand , India
*Giridih Collage , Giridih, Jharkhand , India

Fly ash generated from Thermal Power Plants is posing serious problem of pollution. Very high amount of fly ash generated has made its utilization a better option then its disposal. Apart from its various industrial uses, use of fly ash as soil amendment agent has gained wide acceptance. Radish being a radio-resistant plant is a good choice to be grown on fly ash amended soil (Dayal, 1975) and up to 77.8% increase in yield has already been reported (Sarangi et. al. 1996). Growth of any organism is direct manifestation of cytological aspects like increase in mitotic index, DNA contents, chromosome volume and nuclear dry mass. Previous report has clearly indicated significant increase in mitotic index of radish grown on fly ash amended soil (Das et. al. 1998). In Present investigation, DNA content, chromosome volume and nuclear dry mass of three varieties of radish on grown on fly ash amended soil was studied.

Three varieties of radish-Japnese White, Pusa Himani and Doppel Bock were grown on 30% fly ash amended soil. For DNA estimation, fixation and staining technique was used as described by McLeish and Sunderland (1961). Photometric measurement was made as per Barr and Stroud integrated micrometer. Measurement of nuclear dry mass was made by interference microscope. Chromosome volume was estimated by measuring length and width of metaphase chromosome 't' test was used to find out significance of variations.

DNA Content, Chromosome volume and nuclear dry mass of three varieties of radish grown on 30% fly ash amended soil and their comparision with control is given in table-1. DNA of Japnese White radish increased by 6.67% over control whereas Pusa Himani and Doppel Bock registered increase of 12.16% and 6.28% respectively. So far chromosome volume is concerned, the three varieties registered increase of 1.50% 6.34% and 3.08% respectively. 5.9% increase of 1.50% 6.34% and 3.08% respectively, 5.9% increase in nuclear dry mass was observed for Japnese white variety where as increase of 5.3% and 4.2% was recorded for Pusa Himani and Doppel Bock respectively. 't' test indicated that all variations were significant at 0.05 level. The results clearly indicate that fly ash at suitable concentration (30% for radish) significantly enhance cytological parameters leading to better growth of plant. Dutta 1971 has already established that the increase in cytological parameters used in this study is directly related to better growth of plant parts.

Acknowledgement
Director, C.A.Z.R.I. was kind enough to facilitate analytical works.

Refrences

Das, R.K., Mullick, R. and Mishra, P.K. (1998): Bull Env. Sci.: 32-38
Dayal, N. (1975): Caryologia 28: 425-435
Dutta, M.K. (1971): The Nucleus 14: 152-154
McLeis, J and Sunderland N, (1961): Exp cell res 24: 527-540
Sarangi, P.K., Das M.C. and Mishra, P.C. (1996): Wat.con. poll.Res: 47-50

Table –1

DNA content , Chromosome Volume and Nuclear dry mass of three Radish variety grown on Fly- Ash amended soil.

Radish Variety		DNA Content (arbitrary units)		Chromosome Volume μ^3		Nuclear dry mass (10^{-11} gm)	
		Value	't'value	Value	't'value	Value	't'value
Japnese White	Control	25.62 ± 1.8		3.33 ± 0.5		1.01 ± 0.2	
	Grown on Fly ash	27.33 ± 2.1	1.47	3.38 ± 0.4	1.35	1.07 ± 0.1	1.34
Pusa Himani	Control	22.45 ± 1.3		2.52 ± 0.3		0.94 ± 0.05	
	Grown on Fly ash	25.18 ± 1.4	1.36	2.52 ± 0.3	1.41	0.99 ± 0.06	1.44
Doppel Bock	Control	28.62 ± 2.1		3.89 ± 0.4		1.42 ± 0.05	
	Grown on Fly ash	30.42 ± 1.7	1.35	4.01 ± 0.2	1.32	1.48 ± 0.03	1.47

A COST EFFECTIVE, SIMPLE QUALITATIVE INITIAL SCREENING METHOD FOR LOW/NEAR ZERO GLUCOSINOLATE TYPE OF RAPE SEED MUSTARD

P.C.Pande[1],R.K.Katiyar[2],P.C.Pathak[1] and R.Chamola[2].

[1]Nuclear Research Laboratory [2]N.R.C.Plant BiotechnologyIndian Agricultural Research Institute,New Delhi-110012.

A cost effective,simple qualitative method has been described for initial screening of low glucosinolate type from the segeregating population.The two step method is colorimetric and requires 25 mg seed along with checks in the first step.In the second step from advavced generations,50 mg or multiples there of the seed sample per replicate,is required along with low/near zero checks grown simultaneously,to differentiate between near zero and zero glucosinolate types.

The Indian rape seed mustard cultivars contain high content of antinutritional factor glucosinolates i.e.80-160 micro moles/g oil cake(Agnihotri and Kaushik,1999) governed by multiple recessive genes. Under these conditions, even a moderate reduction of the content of glucosinolate, might be of great interest(Johansson et al 1984), is restricted by the lack of cost-effective efficient and precise analytical methods (Agnihotri,1999). All quantitative methods require extraction and isolation of glucosinolates before they are estimated total or identified and estimated as individual glucosinolates.These additional steps make all these methods relatively time consuming,costly and cumbersome. However, the only qualitative 'Test Tape' method, suffers from low sensitivity and requires 200 mg of seed and can not distinguish among high, intermediate low and near zero glucosinolate containing segregants (Johanson et.al.1984).The present communication describes the development of a two step cost-effective and simple, initial screening method which is also capable of distinguishing between types having high,intermediary and low/near zero levels of glucosinolates in the large segregating population.

The reagent for colour development consists of 20 mg of KMnO4 dissolved in 100 ml of 0.1N NaoH and should be prepared fresh.The segregating seed material of *Brassica juncia* along with high and zero/near zero checks grown simultaneously and under similar conditions were obtained from the breeder every year.The apparatus consists of a 50 ml pear shaped flask with two ground glass joint acting as a inlet and an outlet. The inlet is fitted with a tube for passing N2 gas (A-grade),which is released at the bottom of the flask through the tappering end. Similarly the exit tube which takes the volatile products of the reaction from the flask along with the carrier N2 gas,releases them near the bottom of the tube which contains 2 ml KMno4 reagent for colour development reaction.Twenty five mg of seed is enclosed in a butter paper and crushed uniformly with a hammer.The crushed seed is next transferred to the bottom of the pear shaped flask with the help of a folded metal label and the surface of metal label is washed with 1.0 ml of distilled water.The reaction is started by submerging the bottom of flask containing reactants in a water bath maintained at 30°C and Nitrogen is passed at a uniform rate for 5 minutes. Three ml of distilled water is added to the tube containing the reagent for colour development and the contents of the tube are thorughly mixed. The optical density is measured at 600 nm in a spectronic-20 against the distilled water along with high and low checks. In first step, 25 mg segregant sample is run in duplicate and those found low i.e.closest to the optical density of the zero/near zero glucosinolate check are only further subjected to confirmation by using four replicates in each case. Application of the method as initial screening technique between 1998-1999 and 2001-2002 indicate considerably reduced percentage of the very low catagory. On the contrary the percentage of very high category material became nil in the year 2001- 2002 (Table 1)

Table- 1: Percentage change in the number of different categories of glucosinolate level containing samples between 1998 -99 and 2001 -2002 based on ranges of optical density at 600 nm with 25 mg seed material.

Categories of different glucosinolate levels	Corresponding range of O.D. at 600 nm and 25 mg seed	Year	
		1998 -1999 %	2001 -2002 %
Very low	0.04	0.0	4.5
Low	0.05 -0.09	3.5	64.4
Medium	0.10 -0.19	5.7	27.5
High	0.20 -0.39	44.0	3.6
Very high	0.40	46.8	0.0

In the second step,those segregants belonging to stabilised advanced generations with optical densities very close to zero glucosinolate check,are retested for their low glucosinolate levels.In this step,seed weight per replicate is increased from 25 mg per replicate to 50 mg and multiples upto 200 mg per replicate,both in the case of zero glucosinolate check and the unknown segregant.In this step, more sensitive spectrophotometer is used instead of spectronic-20,using the same procedure.The zero glucosinolate (< 18 micro mole/g seed) material does not show an increase in O.D with increase in seed weight whereas a near zero material does so.(Table 2)

Table- 2: Variation in O.D.at 600 nm by increasing sample weight differentiates between zero(below 18 micro moles/g seed) and near zero glucosinolate material.

Sl No.	Weight of seed sample(mg)	Optical density	
		Zero glucosinolate type(Parkland) (9.0 micro mole/g seed)	Near zero glucosinolate type (48.9 micro mole/g seed)
(1)	50	0.01	0.03
(2)	100	0.02	0.10
(3)	150	0.02	0.15
(4)	200	0.02	0.20

Thus, this simple, rapid and cost- effected technique under our coditions appears to be promissing for initial screening of large segregating population.

REFERENCES

Agnihotri,A.1999. Screening techniques for evaluation of double low characters in oil seed Brassica. J.oil seed Res. 16:183-195.

Agnihotri,A. and Kaushik,N. 1999. Transfer of double low characterstics in early maturing *Brassica napus*.

Johansson,S.,Svensson,R. and Uppstrom,B.1984. Simple methods for the selection *Brassica compestris* and *Brassica juncea* with low levels of erucic acid and glucosinolates. Sueriges Utsadesforenings Tidskrift. 94:191-196.

Comparative analysis of phenolic compounds among some species of the genus *Brassica* from Sect. *Sinapistrum* and Sect. *Micropodium*

M. D. Sánchez-Yélamo

Dpto. de Biología Vegetal E.T.S. de Ingenieros Agrónomos de Madrid (U.P.M.), 28040, Madrid, Spain, email: dsyelamo@bio.etsia.upm.es

INTRODUCTION

Genus *Brassica* includes near 40 taxa but only few are crops. Apart of the scientific interest by the taxonomic aspects, wild relatives of cultivated species can be particularly interesting as potential source of useful genes to transfer to the cultivated forms. Present work is a continuation of previous paper (Sánchez-Yélamo, 2000) and represents a chemotaxonomic analysis of some wild species belonging to Sect. *Sinapistrum* and Sect. *Micropodium* of genus *Brassica* using phenolic compounds.

MATERIAL AND METHODS

Seeds of studied species were 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 following Gómez-Campo (1999).

Table 1. Plant material

TAXON	Gametic number
B. oxyrrhina (Box)	9
B. tournefortii (Bto)	10
B. barrelieri (Bba)	10
B. maurorum (Bma)	8
B. spinescens (Bsp)	8
B. fruticulosa subsp. *fruticulosa* (Bff)	8
B. fruticulosa subsp. *mauritanica* (Bfm)	16
B. cossoniana (Bco)	16

Phenolic compounds were extracted, from adult leaves of plants cultivated in the green house, and isolated 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 (Rolf, 1994). The similarity matrix was employed to construct the dendrogram by UPGMA using the Jaccard's index.

RESULTS AND DISCUSSION

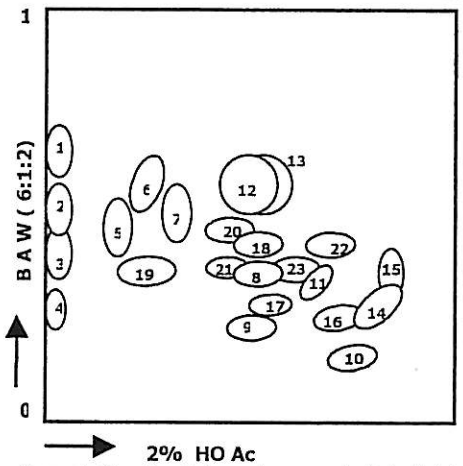


Figure 1. Combined chromatogram of all studied taxa.
BAW=BuOH-HOAc-H_2O; HO Ac= Acetic acid

As a whole 23 flavonoids were isolated from the taxa analysed. The combined chromatograms of all taxa are presented schematically in Figure 1. Table 2 shows the presence of compounds within each taxon. The compounds were identified as kaempferol, quercetin and isorhamnetin mono-, di- and triglycosides, those which are quite frequent in the *Brassicaceae*. Apart from the role that they could play in plants as secondary metabolites, flavonoids have demonstrated to be useful molecular markers to contribute to clarify taxonomic and systematics questions.

In the present work, the UPGMA cluster analysis using phytochemical data shows the distribution of species in two groups (Figure 2) that are concordant with the taxonomy of this taxa. Group I is formed by the species belonging to Sect. *Micropodium* (*B. fruticulosa/spinescens/ maurorum* complex as well as *B. cossoniana* -formerly subspecies of the first one-). Group II is composed by the taxa that constitute completely Sect. *Sinapistrum: B.tournefortii* and *B.barrelieri* (both n=10) and *B. oxyrrhina* (n=9).

Table 2. Flavonoids identified in studied taxa (*)

	Compound	Box	Bto	Bba	Bma	Bsp	Bff	Bfm	Bco
1	K 7-Gal	-	+	+	-	-	-	-	+
2	Q 7-Gal	-	+	+	-	-	-	-	+
3	K 7-Glc	+	-	-	+	-	+	+	-
4	Q 7-Glc	+	-	-	+	-	+	+	-
5	Q 3-Glc	-	-	-	+	-	+	-	+
6	K 3-Glc	-	-	-	-	-	+	-	+
7	I 3-Glc	-	-	-	-	-	+	-	+
8	I 3-Digal	-	-	-	+	+	+	+	+
9	Q 3-Digal	-	-	-	+	+	+	+	+
10	K Trigly	-	-	-	+	-	+	+	+
11	K 3-Digal	-	-	-	+	+	+	+	+
12	K 3-Gal-7-Rha	-	+	+	-	-	-	-	-
13	I 3-Gal-7-Rha	-	+	+	-	-	-	-	-
14	I Trigly (Rha+Gal)	-	-	-	+	+	+	+	+
15	K Trigly (Rha+Gal)	-	-	-	+	-	+	+	+
16	Q 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; I= isorhamnetin; Glc= glucose; Gal= galactose ;
Rha= rhamnose; -gly: glycoside; (+) presence (-); absence; (* For Abbreviations see Table 1)

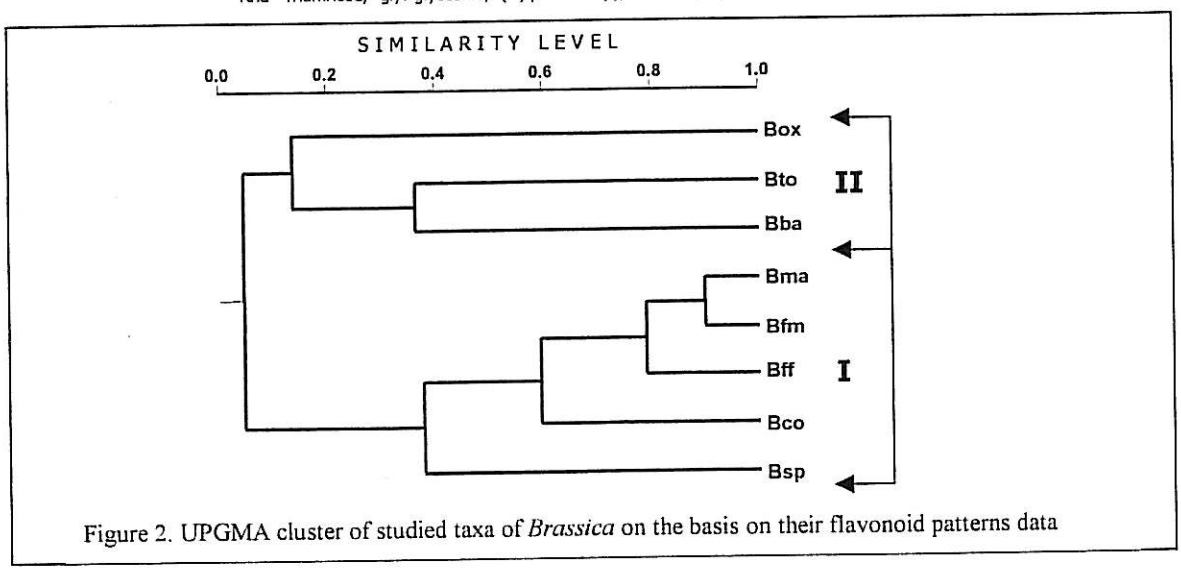


Figure 2. UPGMA cluster of studied taxa of *Brassica* on the basis on their flavonoid patterns data

References

Gómez-Campo, C. 1990. A germplasm collection of Crucifers. Catálogos I.N.I.A. no 22. Ed. Ministerio de Agricultura Pesca y alimentación, Madrid, Spain.

Gómez-Campo, C. 1999. Taxonomy. In: Biology of Brassica Coenospecies. Amsterdam: Elsevier.

Hansen, S.A. 1975. Thin-Layer chromatographic method for the identification of mono-, di- and trisacharides. *Journal of Chromatography*. 107: 224-226.

Harborne, J.B. 1988. The Flavonoids. Advances in Reseach since 1980. London: Chapman and Hall.

Harborne, J.B. 1989. Methods in Plant Biochemistry. Vol. 1. Plant Phenolics. London: Academic Press.

Marham, KR. 1982. Techniques of Flavonoid identification. London, Academic Press.

Rolf, J. 1994. NTSYS-pc, version 1.8. Copyrigth Applied Biostatistics, Inc.

Sánchez-Yélamo, M.D. 1994. A chemosystematic survey of flavonoids in the *Brassicinae*: *Diplotaxis*. *Bot. Jour. Linn.Soc.* 115:9-18.

Sánchez-Yélamo, M.D. 2000. Occurrence of Phenolic Compounds in *Erucastrum* and *Brassica (Brassicinae)*. Cruciferae Newsletter (22) 9-10.

Lb siliqua mutant in taramira (*Eruca sativa* Mill.)

Yash Pal Yadav

CCS Haryana Agricultural University, Regional

Research Station,Bawal-123501, Rewari (India).

Genetic variability is vital for any crop improvement programme. Spontaneous mutations, source of genetic variability are natural phenomenon in crop plants. These mutants can be used directly or indirectly for incorporation of desirable traits in the cultivars. Taramira or rocket salad is an important crop of the rapeseed - mustard group of oilseeds. In this crop, there is no report where a spontaneous mutant has been found of direct use i.e. tested and released as a cultivar. The normal siliqua length in taramira is short (2.7 cms) and having small breadth (5.7mms)

In the present study, a spontaneous mutant with Lb(long & bold) siliqua was observed in a taramira field planted with the cultivar T-27 during *rabi* 1999-2000. The spontaneous mutant was picked up and compared for various morpho- physiological characters with the parent cultivar and cv. RTM-314 which is being used as a check in the national taramira improvement programme under rainfed conditions. The characters studied were :-

Plant height (cms)

Number of primary branches/plant

Number of secondary branches/plant

Number of tertiary branches /plant

Internode length : Average length of five nodes from the bottom (cms)

Total siliquae/plant

Number of siliquae on main shoot

Siliqua length (cms)

Siliquae breadth (cms)

Seeds per siliqua

Seed size (1000- seed weight in g)

Seed yield /plant (g)

The mutant was found distinguishable for a number of characters.The mutant was comparable in height with the parent but was shorter as compared to the check RTM 314 (Table 1). The mutant produced significantly higher number of secondary and tertiary branches

as compared to the parent and the check. But in respect of primary branches, it was comparable with the parent cultivar and the check . Internode length (cms) and siliquae number on main shoot were also comparable with the parent and the check. However, for the total number of siliquae per plant, the mutant was conspicuously superior to the parent and the check. The highlight of the mutant was its longer and broader siliqua than its parent and the check. Similarly, the mutant produced more number of seeds/ siliqua without any reduction in seed size. Seed yield per plant (g) of the mutant was significantly superior than its parent and the check.

The foregoing observations showed that the long – bold siliqua mutant has the desirable combinations of the key components of seed yield such as number of secondary & tertiary branches, total number of siliquae/ plant, siliqua length & breadth, seeds per siliqua and seed size. This was supported by its higher seed yield per plant than the parent and the check. The mutant is being further investigated and will be tested as a elite genotype in a multilocation trial. The mutant holds a promise in giving further impetus to the production and productivity of taramira, an important rapeseed- mustard crop of oilseeds.

Table 1. Distinguishing characters of the spontaneous mutant, the parent and the check in Taramira

Characters	Mutant	Parent(T-27)	Check (RTM 314)
Plant Height (cms)	100	103	134
Number of Primary branches	11	14	11
Number of Secondary branches	73	39	35
Number of Tertiary branches	186	45	29
Internode Lengh(cms)	4.2	4.5	4.6
Total no. of siliqua/ plant	1084	389	589
Siliqua on main shoot	26	27	22
Siliqua Length (cms)	3.2	2.7	2.4
Siliqua breadth (mm)	7.6	5.7	6.0
Seeds/ siliqua	28.2	21.0	23.9
Seed size (1000- s.w (g)	3.4	3.4	3.6
Seed Yield/ plant (g)	51.9	22.3	38.5

Germplasm release of root rot resistant, canola quality summer turnip rape (*Brassica rapa* L.)

D. L. Woods and **S.R. Rimmer**, Research Farm, Agriculture and Agri-Food Canada, Box 29, Beaverlodge, Alberta, Canada T0H 0C0, and Research Station, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2 respectively.

A line of *Brassica rapa* with some resistance to brown girdling root rot, and with canola quality traits was developed and tested. This material is being made available as possible parental germplasm.

Breeding

Lines showing resistance to root rot were developed using three years of mass selection (1983-85) in a root rot nursery in Manitoba. The parent pool consisted of various accessions from China, selections from co-operative trials of the period, and cv Tobin planted every fourth row. From these, a single line (R835-363) was selected on the basis of field evaluation for root rot resistance in Beaverlodge in 1986. Thirteen single plants from this line were grown in the greenhouse, the progeny seed was evaluated in the field for root rot resistance in 1989 and 1990, and reserve seed of a single selection was retained as a resistant parent. The canola quality parent was AC Sunshine (Woods 1995).

Initial crosses and growth of the F_1 plants were done in 1991 and 1992, and single seeds F_2 were selected for zero erucic acid (1/2 seed technique, Downey and Harvey 1963). A population of 119 F_2 plants were interpollinated, and the F_3 rows evaluated for root rot resistance in the field in 1993. Based on these field data reserve seed of three single F_2 plants was retained further development.

F_3 plants from each of the three selected F_2 plants were grown in the greenhouse and interpollinated in 1994. Harvested F_4 seed was screened for glucosinolates (TesTape, McGregor and Downey 1975, 102/168 retained) and for erucic acid (90/102 retained). The 90 F_4 seed lots were evaluated in the field in 1995 for root rot resistance and row glucosinolates (Gas chromatography of tri-methyl-silyl de-sulphonated glucosinolates, based on Daun and McGregor 1983), and six superior seed lots identified. F_5 plants were grown in the greenhouse from these six seed lots, and the seed from the harvested single plants screened for glucosinolates (TesTape, 62/115 retained). F_5 derived F_6 rows were grown in isolation in the field in 1996 to provide seed for subsequent testing in 1997 and 1998. Based on field performance and other data one of these lines was assigned the identification CB 9919, and the F_5 derived F_6 bulk further increased in 1999 for testing at multiple locations in 2000, and for root rot evaluation trials in 2000 and 2001.

Root rot resistance data

Trials (6 replicates) to evaluate root rot resistance were conducted in the field at Beaverlodge in 2000 and 2001. Alternate rows of test line and the susceptible check Tobin were planted, and 20 plants per row scored on a zero to five scale at flowering time (Harper and Berkencamp (1975) growth stage 4.1 to 4.4). The difference between the average test line score and the average score from the two adjacent Tobin check rows was used as the reported test line value. The 0-5 scale was as reported by Woods *et al.* 2000, ie 0, clean; 1, fleck lesions or small slightly discoloured lesions on taproot or small,

water-soaked lesions, taproot not girdled or sunken; 2, lesions distinct, may be sunken but do not girdle the taproot at or above the main lateral roots; 3, lesions girdle the taproot at or above the main lateral roots, lesions may be sunken; 4, root rotted off at or above the main lateral roots, plant is still green; 5, root rotted off at or above the main lateral roots, plant is prematurely ripened. The test average root rot score for the Tobin check was 2.45 in 2000 and 3.14 in 2001, and the CB 9919 value was -0.70 in 2000 and -0.71 in 2001; in both years CB 9919 was significantly better than Tobin at P=0.05.

Agronomic performance and other data

In 2000 CB 9919 was entered into co-operative trials in Western Canada, at 8 locations with three check varieties. Average yields (kg/ha), oil content (% dry basis), protein content (% dry basis), and glucosinolates (µmoles total glucosinolates/g seed at 8.5% moisture) were as indicated below;

Entry	Yield	Oil	Protein	Glucosinolates
Maverick	2041	47.4	23.8	15.3
AC Parkland	1732	46.9	23.8	17.2
Reward	2000	47.5	23.2	27.0
CB 9919	1754	45.2	24.3	19.0
LSD 5%	100	0.3	0.4	1.5

In addition to the agronomic trials the planted seed was analyzed for erucic acid content (0.84%). The line was also tested for susceptibility to race 7a of white rust (78% susceptible versus 36% susceptible for the check Tobin).

Availability

The root rot resistant *B. rapa* line CB 9919 may be obtained from DLW, or from Plant Gene Resources of Canada, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2 under the designation CN19156.

References

Daun, J.K., and **McGregor, D.I.** 1983. Glucosinolate analysis of rapeseed (Canola). Method of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg. 1981 (revised 1983).

Downey, R.K., and **Harvey, B.L.** 1963. Methods of breeding for oil quality in rape. Can. J. Plant Sci. **43:** 271-275.

Harper, F.R., and **Berkenkamp, B.** 1975. Revised growth-stage key for *Brassica campestris* and *B. napus*. Can. J. Plant Sci. **55:** 657-658.

McGregor, D.I., and **Downey, R.K.** 1975. A rapid and simple assay for identifying low glucosinolate rapeseed. Can. J. Plant Sci. **55:** 191-196.

Woods, D.L. 1995. AC Sunshine summer turnip rape. Can. J. Plant Sci. **75:** 467-468.

Woods, D.L., Turkington, T.K., McLaren, D., and **Davidson, J.G.N.** 2000. Breeding summer turnip rape for resistance to brown girdling root rot. Can. J. Plant Sci. **80:** 199-202.

Production and cytogenetics of intergeneric hybrids between *Ogura* CMS *Brassica napus* and *Raphanus sativus*

Bangquan Huang, Youqi Liu, Wenhua Wu, Xiaoqiao Xue
Biology Department of Hubei University, Wuhan 430062, P.R.China

Abstract: Crosses between *Ogura* CMS *Brassica napus* (AACC, 2n=38)and *Raphanus sativus* (RR, 2n=18) were made and many intergeneric hybrids were produced. The F_1 seedlings did not show chlorosis at low temperature. All the F_1 plants had white flowers and normal nectary glands. Some of the F_1 plants had well-developed anthers but the male gametes were highly sterile while the female gametes were partly fertile. Cytological studies indicated that chromosome number of the F_1 was 2n=28 as expected, the mean chromosome pairing pattern was 6.12 I +6.29 II +2.16III+0.49IV +0.08 V +0.08 VI. Genetic and breeding implications of these hybrids and their progenies were discussed.

Key words: *Ogura* CMS *Brassica napus*; *Raphanus sativus*; intergeneric hybrids; cytogenetics

The cytoplasmic male sterility found by Ogura (1968) in radish (*R.sativus*) was transferred to *Brassica oleracea* and then to *B.napus* (Bannerot et al, 1974). Resultant male sterility was highly stable but three major problems arose because of the incompatibility of the radish cytoplasm and the *Brassica* nucleus, i.e., chlorophyll deficiency, low nectar production, and lack of male fertility restoration in Brassicas (Rouselle and Renard, 1978). Pelletier et al (1983) regenerated cybrids combining chloroplasts of *B.napus* and the CMS trait from *R.sativus* and produced *Ogura* CMS lines with normal leaf color and nectary glands. Heyn (1976) crossed *Ogura* CMS rapeseed with the *Raphanobrassica* (*R.sativus*×*B.napus* amphidiploid, 2n=56) and obtained *B.napus* with restored male fertility. In the present study *Ogura* CMS *B.napus* was crossed directly with *R.sativus* and many hybrids with normal leaf color and nectary glands were produced. In some combinations there appeared some F_1 plants with well-developed anthers.

Materials and Methods

Seeds of *Ogura* CMS *B.napus* were from Prof. Luo Peng of Sichuan University and seeds of *R.sativus* were bought from vegetable market of Wuhan. In September of 1999 *Ogura* CMS *B.napus* and *R.sativus* were planted in Hubei University and crosses were made in the next spring. The seed set rate was calculated as that follows: hybrid seeds/pollinated flowers. In September of 2000 the hybrids were sown in Hubei University and the pollen mother cells were squashed with acetocarmine in the next spring. Anthers were dissected one day before flowering and stained with I_2-KI solution to determine the pollen fertility.

Results and Analysis

Crosses of nine combinations were made and many intergeneric hybrids were produced. Some combinations such as *Ogura* CMS *B.napus*×*R.sativus* cv.*zao* had an extremely high seed set rate (25.60%), and some combinations such as *Ogura* CMS *B.napus*× *R.sativus* cv.*nanpanwan* had lower seed set rate (6.38%). In general the seed set rate in present study was rather high (17.67%) compared with previous results (Lelivelt et al,

1993).

The F_1 seedlings did not show chlorosis when the temperature was below15℃. All the F_1 plants had white flowers and normal nectary glands. In some combinations such as *Ogura* CMS *B.napus*×*R.sativus* cv.*hongxin*, *Ogura* CMS *B.napus*×*R.sativus* cv.*korea white*, *Ogura* CMS *B.napus*×*R.sativus* cv.*nzao* and *Ogura* CMS *B.napus*×*R.sativus* cv.*hongbao* there appeared some F_1 plants with well-developed anthers but the male gametes were unstainable with I_2-KI solution while the female gametes were partly fertile. Backcrosses with *B.napus* were carried out and some progenies were produced.

Cytological studies indicated that chromosome number of the F_1 was 2n=28 as expected, the mean chromosome pairing pattern was 6.12 I +6.29 II +2.16III+0.49IV+0.08 V +0.08VI, suggesting that genome A and C were partly homologous with genome R. At anaphase I some chromosome laggards were observed.

Discussion

Ogura CMS has been extensively explored as it is very stable and many male sterile lines can be obtained just by repeated backcrossing. *Ogura* CMS lines with dark green leaf color and normal nectary glands were obtained through protoplast fusion and male fertility restored by crossing *Ogura* CMS rapeseed with the *Raphanobrassica*. The present study indicated that it is also possible to overcome chlorophyll deficiency and low nectar production and restore the male fertility by directly introducing *R.sativus* nucleus into the *Ogura* cytoplasm. Backcrosses will be continued for the generation of recombinants or addition and translocation lines with normal leaf color and nectary glands and restored male fertility.

In the present cross between *Ogura* CMS *B.napus* and *R.sativus* the seed set rate was extremely high (17.67%). Similar result was observed in crosses between *Ogura* CMS *B.campestris* var. *purpuria* and *R.sativus* (Huang et al, 2001). Probably the presence of maternal radish cytoplasm facilitated the paternal radish pollen tubes to penetrate into the stigma.

References

[1] Bannerot H, Boulidard L, Cauderon Y, Tempe J. Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. Proc. EUCAPIA Meeting—Cruciferae, 1974, 52-54

[2] Heyn F W. Transfer of restorer genes from *Raphanus* to cytoplasmic male sterile *Brassica napus*, Cruciferae Newsletter, 1976, 1:15-16

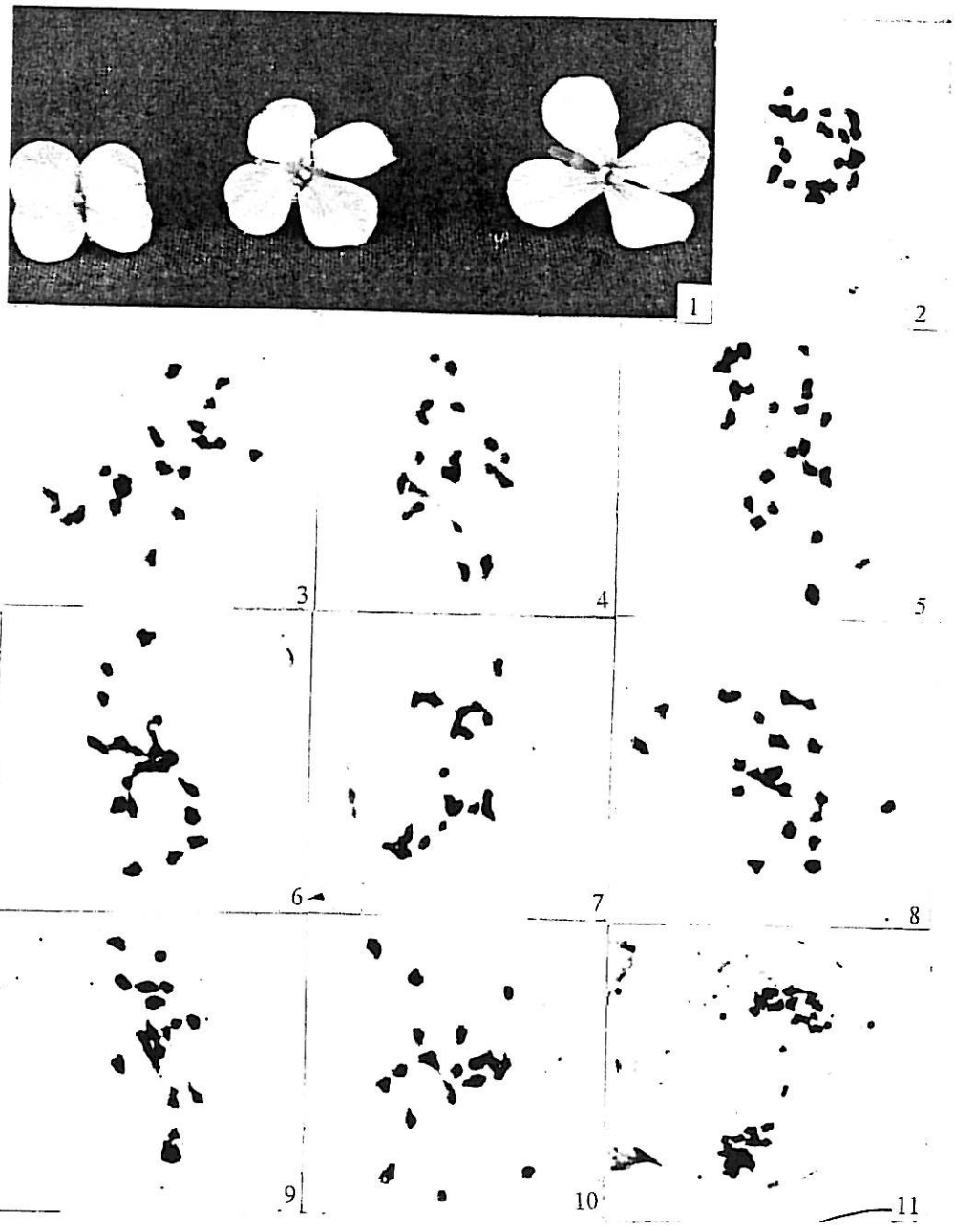
[3] Huang B, Chang L, JU C, Chen J. Production and cytogenetics of intergeneric hybrids between *Ogura* CMS *Brassica campestris* var.*purpuraria* and *Raphanus sativus*, Acta Genetica Sinica, 2001, 28 (6): 556-561

[4] Lelivelt CLC, Lange W, Dolstra O. Intergeneric crosses for the transfer of resistance to the beet cyst nematode from *Raphanus sativus* to *Brassica napus*. Euphytica, 1993, 68:111-120

[5] Ogura H. Studies on the new male sterility in Japanese radish, with special references to the utilization of this sterility towards the practical raising of hybrids. Mem Fac Agric Kagoshima Univ. 1968, 6:39-78

[6] Pelletier G, Primand C, Cedal F, Chetrit P, Remy R, Rousselle P, Renard M. Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol Gen Genet,1983, 91:244-250

[7] Rouselle P, Renard M. Study of a cytoplasmic male sterility in rapeseed. Cruciferae Newsletter, 1978, 3:40-41


1
2
3
4
5
6
7
8
9
10
11

Production of intergeneric hybrids between *Brassica napus* and *Diplotaxis virgata* and meiotic chromosome association of the F_1 hybrids

Inomata, N.

Biological Laboratory, Konan Women's University, Kobe 658-0001, Japan

Study of the phylogenetic relationships between crop plants and the wild relatives is important. The wild relatives are also often variable source of important genes. Though the production of interspecific and intergeneric hybrids is difficult, many interspecific and intergeneric hybrids had been obtained by embryo rescue techniques among the tribe *Brassiceae*. As a result, the studies for their meiotic chromosome behavior were reported (Harberd and McArther, 1980; Inomata, 1997; Prakash *et al.*, 1999). This study produced intergeneric hybrids between *Brassica napus* and *Diplotaxis virgata* and showed the behavior of meiotic chromosome association in the F_1 hybrid. The crossability of the progenies was further investigated.

The materials used in the experiment were *Brassica napus* ssp. *oleifera* cv. Westar ($2n$=38; AACC) and *Diplotaxis virgata* (Cav.) DC. ($2n$=18; D^vD^v), which seed was provided by Gómez-Campo. When emasculated flowers of *B. napus* bloomed, the conventional cross was made with the fresh pollen grains of *D. virgata*. The crossed ovaries were cultured in according to the previous paper (Inomata, 1990). The medium used in the experiment was MS medium (Murashige and Skoog, 1962) with 300mg/*l* of casein hydrolysate (CH). When growing embryos were obtained in the ovaries, the embryos were further cultured in the 1/2 MS medium with 300mg/*l* of CH. Somatic chromosomes and chromosome associations in the first meiotic division were checked by using the method of Inomata (1994).

Eighty-seven ovaries were cultured at 3 days after pollination. No seeds were obtained but 21 full-grown embryos and two walking stick-shaped embryos were obtained. They were further cultured and 22 plants matured in the field. All plants consisted of 28 chromosomes except one that showed 56 chromosomes. The leaves and flowers 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 of the F_1 hybrids resembled each other. The mode of chromosome association was $8_{II}+12_I$ and $9_{II}+10_I$. The bivalent association ranged from 4 to 11, with a mean of 7.5. High chromosomal homology existed between the genomes. The bivalent association ranged from 0 to 6, with a mean of 1.5, in the F_1 hybrid of *D. virgata* x *B. rapa* (Takahata and Hinata, 1983), and in the reciprocal cross, the bivalent association ranged from 0 to 7, with a mean of 2.8 (Inomata, 1999). The bivalent association ranged from 4 to 11, with a mean of 7.6, in the F_1 hybrid of *B. carinata* x *D. virgata*, and from 1 to 8, with a mean of 5.0 in the F_1 hybrid of *D. virgata* x *B. juncea* (Harberd and McArther, 1980). It is assumed that the chromosomal homology between C genome in *B. napus* and D^v genome in *D. virgata* was much greater than that between A genome in *B. napus* and D^v genome of *D. virgata*. No seeds were obtained from both self- and open pollination of the F_1 hybrids in 980 flowers and in 4494

flowers, respectively, and from the F_1 hybrids backcrossed with *B. napus* in 717 flowers.

Acknowledgement

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

References

Harberd, D. J., and E.D. McArther, 1980. In: Tsunoda, S., K. Hinata, and C. Gómez-Campo, (eds.), *Brassica* Crops and Wild Allies. Japan Sci. Soc. Press Tokyo, pp. 65-87.

Inomata, N., 1990. In: Bajaj, Y.P.S. (ed.), Biotechnology in Agriculture and Forestry. Legume and Oilseed Crop I. Springer, Berlin, Heidelberg, pp. 367-384.

Inomata, N., 1994. Chromosome Infor. Serv. 57: 38-39.

Inomata, N., 1997. In: Kalia, H.R., and S. Gupta, (eds.), Recent Advances in Oilseed Brassica. Kalyani Pub. Luhiana, New Delhi, pp. 53-76.

Inomata, N., 1999. Cruciferae Newslet. 21: 39-40.

Murashige, T., and F. Skoog, 1962. Physiol. Plant. 15: 473-497.

Prakash, S., Y. Tkahata, P.B. Kirti, and V.L. Chopra, 1999. In: Gómez-Campo, C. (ed.), Biology of *Brassica* Coenospecies. Elsevier Sci. B.V. Amsterdam, pp. 59-106.

Takahata, Y., and K. Hinata, 1983. Tohoku Jour. Agri. Res. 33: 111-123.

Table 1. Pollen fertility and chromosome associations in the first meiotic division of the F_1 hybrids of *Brassica napus x Diplotaxis virgata*

Plant number	Chromosome number in root tip	Pollen fertility (%)[a]	Number of PMCs observed	Mean chromosome association per cell at metaphase I (range in parenthesis)					
				VI	V	VI	III	II	I
1	28	0	37	0.03 (0-1)	0.03 (0-1)	0.43 (0-4)	0.03 (0-1)	7.51 (4-10)	10.86 (8-20)
2	28	0	30	0 (-)	0 (-)	0.13 (0-1)	0 (-)	8.8 (6-11)	9.77 (6-12)
3	28	0	34	0 (-)	0 (-)	0.41 (0-3)	0.06 (0-1)	7.59 (5-9)	10.74 (8-16)
4	28	0	30	0 (-)	0 (-)	0.3 (0-2)	0.07 (0-1)	7.63 (5-10)	10.43 (7-18)
5	28	0	25	0 (-)	0 (-)	0.6 (0-2)	0 (-)	6.68 (4-10)	12.24 (8-20)
6	56[b]	0	33	0 (-)	0 (-)	0.12 (0-1)	0.06 (0-1)	7.88 (6-9)	11.58 (8-16)
7	28	0	33	0 (-)	0.03 (0-1)	0.42 (0-2)	0.03 (0-1)	6.45 (3-9)	12.91 (8-18)
8	28	0	30	0 (-)	0 (-)	0.33 (0-2)	0.13 (0-2)	7.53 (5-9)	11.2 (8-14)
Total or range		0	252	0.004 (0-1)	0.008 (0-1)	0.341 (0-3)	0.048 (0-2)	7.519 (4-11)	11.202 (6-20)

[a]: 500 pollen grains were counted.

[b]: PMCs were 28 chromosomes.

EFFECT OF EMS ON CONSTITUTIVE HETEROCHROMATIN OF DIPLOID AND TETRAPLOID FORMS OF TURNIP

Shubhra Basak (Saha) & Chandreshwar Prasad

University Department of Botany, Vinoba Bhave University, Hazaribag - 825 301, Jharkhand, India

ABSTRACT

The tetraploid form of turnip (*Brassica rapa* L.) is more heterochromatized than its respective diploid. EMS treatment causes a gradual decrease in mean number of chromocentres/nucleus (Cfr) from lower to higher doses in the diploid as well as tetraploid forms of turnip in M1 generation. A considerable recovery occurs in M2, but not to the extent of control. Besides there is a wider range of distribution of the chromocentres in the nuclei in the tetraploid than the diploid.

INTRODUCTION

The constitutive heterochromatin is present in the shape of chromocentres in various plant species. They are dark-staining heteropycnotic bodies and have been attributed considerable role in taxonomy and evolution (1-2). Shape and size of chromocentres are quite variable due to differential DNA replication and endoploidy (3). 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. At present the study of heterochromatin has quarried a special attention in view of its possible role in gene regulation, chromosome pairing, genetic recombination and evolution (2,4-8). Turnip is a suitable material for genetic study of heterochromatin as all kinds of its cells exhibit chromocentres in the interphase nuclei. Present work has been undertaken to know the impact of Ethyl Methane Sulphonate (EMS) on the chromocentres of the diploid and tetraploid forms of cultivated turnip.

MATERIALS AND METHODS

A diploid 'Rose Red' variety of turnip and one of its promising tetraploid line T-76, raised by Prasad and Kumari (13), constituted the materials for present investigation. EMS solutions of different concentrations were prepared by adding required amount of distilled water (v/v). Seed treatment was given only for 6 hours. Then the treated seeds were sown in the field at Hazaribag immediately along with the control (untreated) to raise M_1 plants. M_2 plants were grown from the seeds collected from M_1 through selfing. Methods of slide preparation and chromocentre counts were the same as described earlier (9). Counting was made in 50 receptive cells of stigma in each treatment and control. The results are presented in Table 1.

RESULTS AND DISCUSSION

The control tetraploid line (T-76) had higher (about one and half times) mean number of chromocentres/nucleus (Cfr) than the diploid. There was a marked effect of EMS treatment on Cfr of both the forms of turnip. The treatment caused a gradual decrease in mean number of chromocentres/nucleus from lower to higher doses in the diploid as well as tetraploid forms in M_1 generation. A considerable recovery in this nuclear phenotype took place in M_2 generation in both the forms at all the doses, but not to the extent of control. Besides there was a wide range of distribution of the chromocentres within the nuclei in the tetraploid than the diploid at all doses. It was of particular interest to note that the wide range of distribution of the chromocentres within the nuclei was more distinct at the higher doses in comparison to the lower ones in both the forms.

Number of chromocentres within the nuclei is the characteristic for different varietal populations of turnip (10). In the hybrids, Cfr averages mid way between their parents (11). Amount and distribution of chromocentres in turnip are under the control of genotype and are regulated polygenically (11). Air pollution causes in an increase in the amount of heterochromatin which can be utilized in the measurement of pollution level of different places (12). The chromocentre counts can be exploited as a quicker, easier and reliable cytological method for determining the

ploidy level (13). Different varietal populations of turnip react differently to different doses of gamma rays in turnip (14). Aqueous leaf extracts of neem (*Azadirachta indica* A. Juss.) have retarding effect on constitutive heterochromatin of turnip (15). Reduction in the number of chromocentres, particularly at the higher doses, is perhaps due to their fusion as a consequence of mutagenic effects of Ethyl methane sulphonate.

Table 10 : Effect of EMS on the amount and distrbution of chromocentres of diploid and tetraploid forms of turnip

Dose (%)	Generation	2X												4X									
		Distribution of chromocentres										Chromocentres/nucleus		Distribution of chromocentres								Chromocentres/nucleus	
		7	8	9	10	11	12	13	14	15	16	Mean±SE	CV (%)	7	8	9	10	11	12	13	14	15	16	Mean±SE	CV (%)
Control	M_1	-	5	5	13	18	9	-	-	-	-	10.4±0.17	11.26	-	-	-	3	17	5	17	2	6	-	12.3±0.20	11.64
	M_2	-	6	7	11	15	6	5	-	-	-	10.5±0.20	13.82	-	-	-	4	16	11	12	2	5	-	12.1±0.19	11.42
1.2	M_1	-	6	10	15	14	5	-	-	-	-	10.0±0.16	11.61	-	-	-	13	13	9	12	2	1	-	11.6±0.19*	11.31
	M_2	-	7	8	10	13	10	2	-	-	-	10.3±0.20	13.76	-	-	-	5	15	13	10	3	4	-	12.1±0.19	11.29
1.3	M_1	-	14	5	20	8	3	-	-	-	-	09.6±0.17	12.63	-	2	4	14	15	5	8	2	-	-	10.9±0.20**	13.17
	M_2	-	5	6	18	12	7	2	-	-	-	10.3±0.18	12.19	-	-	-	6	15	12	11	4	2	-	11.9±0.18	10.83
1.4	M_1	-	19	10	10	9	2	-	-	-	-	10.0±0.18**	13.48	-	8	5	12	13	2	6	4	-	-	10.6±0.25**	16.77
	M_2	-	7	9	13	12	9	-	-	-	-	10.1±0.18	12.78	-	-	-	9	15	10	11	3	2	-	11.8±0.19	11.37
1.5	M_1	3	19	8	10	7	3	-	-	-	-	09.2±0.19**	14.51	-	6	7	13	15	6	3	-	-	-	10.3±0.19**	13.06
	M_2	-	8	3	11	12	7	9	-	-	-	10.7±0.23	15.26	-	-	-	3	19	10	13	4	1	-	11.9±0.17	09.80
1.6	M_1	11	20	6	7	13	3	-	-	-	-	08.6±0.20**	16.61	-	6	6	13	13	6	4	2	-	-	10.5±0.20**	14.72
	M_2	-	6	8	19	9	7	1	-	-	-	10.1±0.15	10.48	No plant survived										-	-

REFERENCES

1. Stebbines, G.L. (1971). Chromosomal Evolution in Higher plants. Edward - Arnold, London.
2. Sharma, A.K. (1971). In "The Cell Nucleus" (ed. II Bush). Acad. Press, London - New yourk, pp. 269-291
3. Nagl, W. (1979). *Z. Pflanzenphysiol.* **95** : 283-314.
4. Bennet, M.D. (1982). In "Genome Evolution" (eds. G.A. Daver & R.D. Flavell). Acad. Press, New York, pp. 234-256.
5. Lavania, U.C. & Sharma, A.K. (1982). *Curr. Sci.* **51** : 175-180.
6. Nagl, W. (1983). In "Geneties : New Frontier's" (eds. V.L. Chopra et al.), Vol. I, pp. 195-204, Oxford IBH, New Delhi.
7. Nicolini, C.A. (1979). In "Chromatin Structure and Function" (ed. C.A. Nicolini), Plenum, New York, pp. 613-666.
8. Rees, H. & Jones, R.N. (1977). Chromosome Genetics. Edward - Arnold, London.
9. Khag, S. & Prasad, C. (1999). *Eucarpia Cruciferac Newsletter.* **21** : 77-78.
10. Prasad, C. & Shrivastava, M. (1993). *Geobios new Reports.* **12** : 160-163.
11. Prasad, C. (1993). *Columban J. Life Sci.* **1** : 7-10.
12. Prasad, C. (1995). *Geobios new Reports.* **14** : 29-32.
13. Prasad, C. & Kumari, N. (1996). *Eucarpia Cruciferae Newsletter.* **18** : 20-21
14. Kumari, R. (1999). *Ph.D. Thesis,* V.B. niversity, Hazaribag (India).
15. Prasad, C. (2001). *Eucarpia Cruciferae Newsletter.* **23** : 29-30.

Dynamic Chromosome Morphology in Meiosis of Pollen Mother Cells of *Brassica napus*

Changming Lu · Masahiro Kato
(Laboratory of Plant Breeding, Agricultural Faculty, Ehime University, Matsuyama, Japan)

B. napus is one of the most important oil producing crops in the world. Its cytological studies began since Morinaga (1934) first established its chromosome number (2n=38). A lot of evidence has showed that the chromosomes of *B. napus* are very small and it is difficult to identify homologues and to distinguish between different pairs in the complement. Few studies have been made on the characterization of the chromosomes. No complete set of microscopic photographs and description has been available on the dynamic chromosomal morphology in meiosis of pollen mother cells in *B. napus*. We present here a set of microscopic photographs to show the behavior of chromosomes and the pollen formation in meiotic process of *B. napus*.

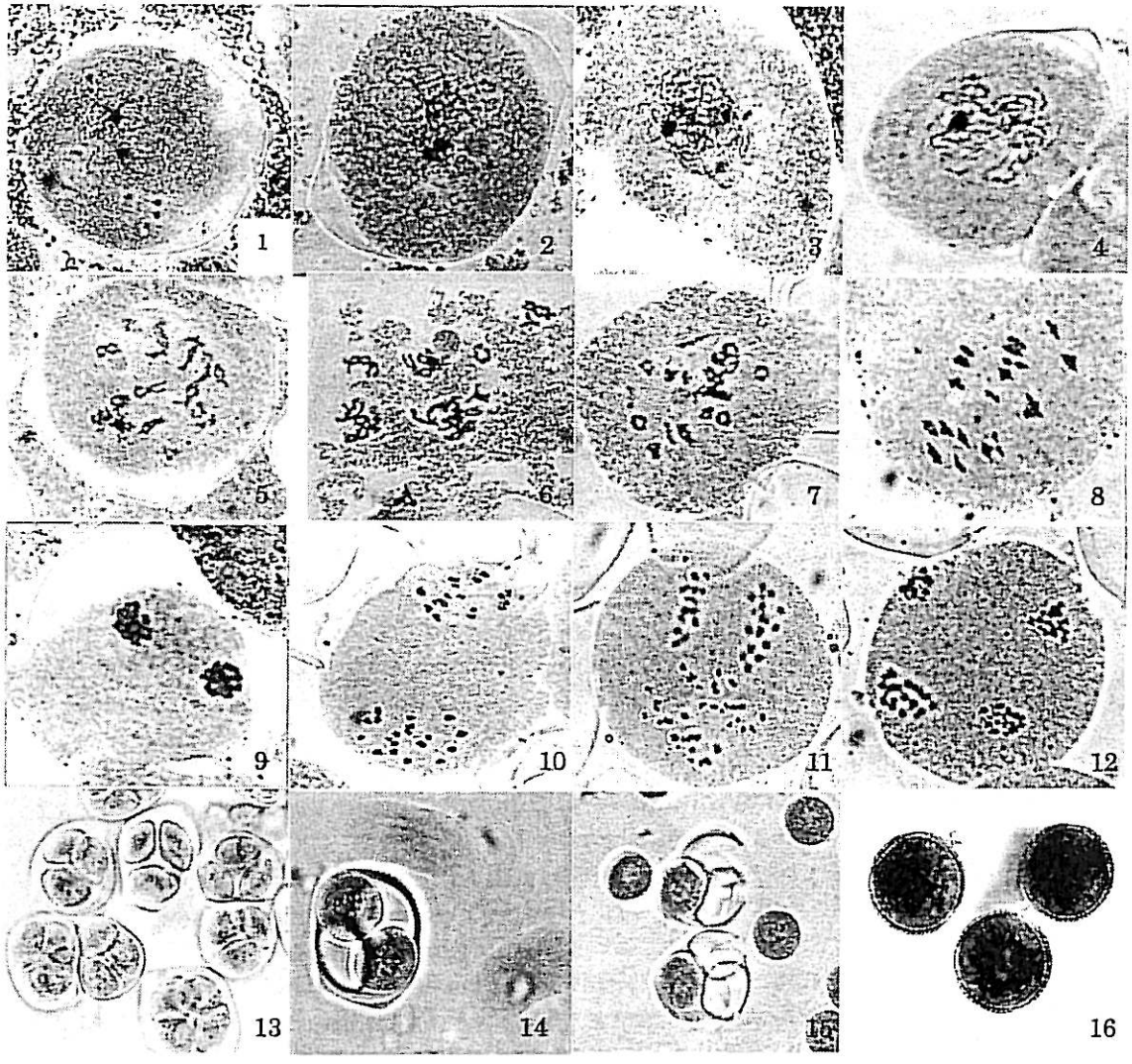
Materials and methods

Plants of *Brassica napus* cv. Westar were grown in green house until flowering. Meiotic preparation was made using young flower buds at the initial flowering stage. Anthers were squashed and pollen mother cells observed after aceto-carmine staining. Photographs were taken by Nikon/PFX camera linked to Nikon/OPTIPHOT microscope.

Results and Discussions

Pollen mother cells (PMC) of *B. napus* looked global (pic.1, 2), of which the nuclear occupied almost all the space, with only thin layer of transparent cytoplasm surrounding it. At the beginning of meiosis, two nucleoli could be seen, and the chromosomes spreaded evenly over the whole nuclear area (pic. 1). In the nuclear mass of the early primary meiocytes, the chromosomes gradually became visible as very thin threads (pic.2). Homologues chromosomes in some way managed to find each other and starting from a few initial points paired along their entire length (pic.3, 4). The condensation of the bivalents has proceeded so far that the individual bivalents could be distinguished (pic.5) and sometimes even recognized (pic. 6). Conspicuous difference of bivalents in length and morphology was found (pic. 6). 4 long bivalents with 3 chasmata, 10 medium sized bivalents with 1 chasmata and 5 small bivalents with 2 chasmata could be distinguuished (pic. 6). In diakinesis stage, the nuclear membrane and nucleoli were still intact and 6 ring-like, 6 bar-like, 2 chain-like and 4 C, or Y like bivalents was visible (pic.7). The nuclear membrane and nucleoli disappeared when the spindle was organized. At this stage, chromosomes experienced considerable stretch followed by another condensation at metaphase proper where the bivalents lined up in the equator (pic.8). The centromeres were well on their way to the poles but the chiasmata prevented them from moving further. The bivalents

were the most condensed and no conspicuous difference was found in bivalent length in metaphase I. The chiasmata slipped off and the chromosomes were free to move to the poles. Soon after telophase I (pic.9), second division proceeded directly. No membrane formation was found in the cell plate at the end of the first meiotic division. The whole second division closely resembled a mitotic division (pic.10, 11, 12). The result of the second meiotic devision was a tetrad of four cells (pic.13), and each daughter cell produced a microspore (pic.14, 15) which developed into mature pollen grains (p.16).



Reference
Morinaga T (1934) On the chromosome number of *Brasssica juncea* and *B. napus*, on the hybrid between he two, and om the offsprintg line of the hybrid. Jpn J Genet 9: 161-163.

INFLUENCE OF LEAF EXTRACTS ON CULTIVATED TURNIP VII. MEIOSIS AND POLLEN VIABILITY

Chandreshwar Prasad
University Department of Botany, Vinoba Bhave University, Hazaribag - 825 301, Jharkhand, India

ABSTRACT

Aqueous leaf extracts of periwinkle (*Catharanthus roseus* Don.), carrot weed (*Parthenium hysterophorus* L.) and neem (*Azadirachta indica* A. Juss.) cause a gradual increase in meiotic chromosome abnormalities from lower to higher doses in turnip (*Brassica rapa* L.). Contrary to this, a progressive decrease in the pollen viability is seen.

INTRODUCTION

Study of meiosis in allogamous populations may be exploited as an important tool for understanding the genetics of chromosome behaviour. The nature of recombination, reduction in chromosome number and degree of fertility largely depends upon the chromosome behaviour during meiosis. The biological significance of meiosis lies on the ability of the chromosomes to pair. Present study was carried out in order to know the effect of aqueous leaf extracts of periwinkle, carrot weed and neem on meiosis and pollen viability of turnip.

MATERIALS AND METHODS

The material used, method of preparing the solutions of different concentrations from the mother solution, mode of seed treatment and raising the plants from them were the same as described earlier (1). For meiotic studies, the flower buds from five plants in each concentration and control were collected and fixed in Carnoy's fixative. The anthers were stained and squashed in phenol fuchsin solution. Scoring was accomplished at metaphase - I and anaphase - I in 200 PMCs in each case. For pollen viability test, the technique suggested by Ferreira (2) was exploited. The results are presented in Table 1.

RESULTS AND DISCUSSION

Meiosis did not occur synchronously in the treated as well as control plants of turnip. PMCs were found at different stages of meiosis. There were typically 10 bivalents in the control, but abnormal metaphases were found in the treated plants. Bad orientation of bivalents, stickiness of chromosomes and univalents were observed. Maximum cases of bad orientation of bivalents were seen in M_1 plants at the higher doses under all treatments. Stickiness of chromosomes was observed at all the doses, but in small frequencies. However, there was a lack of any definite pattern of stickiness under all the treatments. Even the individual plants under the same treatment differed in this regard. Univalents were of rare occurrence. They were seen at the higher doses in small frequencies under all treatments. On the whole, there was a gradual increase in meiotic abnormalities at metaphase - I from lower to higher doses under all the treatments. Anaphase - I was normal with 10 chromosomes at each pole in the control. Laggards, bridges and other minor abnormalities were found in the treated plants, particularly at higher doses. There was a gradual increase in the number of laggards from lower to higher doses under all the treatments. Bridge formation was relatively rare. Other types of minor abnormalities were normally confined to the higher doses. On the whole, meiotic abnormalities at anaphase - I progressively increased from lower to higher doses. However, meiosis was more normal in M_2 in comparison to M_1 under all the treatments. So far as the effect of the leaf extracts on pollen viability was concerned, a gradual decrease from lower to higher doses was seen under all the treatments in M_1 generation. Though a considerable recovery took place in M_2 at all the doses, but not to the extent of control.

All the 20 - chromosome species of *Brassica* exhibit the same frequency of chromosome types (3). Asynchronous meiosis in the characteristic of turnip (4). A negative correlation between meiotic chromosome abnormalities and pollen viability in turnip has been reported (4). Among several alkaloids found in periwinkle, the most active are vinblastine and vinecristine (5). They produce effects similar to those observed after colchicine treatment at the cellular level (6). According to them (6), there is accumulation of mitotic figures. Metaphase becomes arrested with highly coiled chromosomes. There are multipolar anaphases with lagging chromosomes in the interpolar region. There is a tendency to polyploidy by doubling of chromosomes but failure of chromatid separation and at very high doses they can cause pycnosis. The alkaloids are also known to interfere with some metabolic reactions related to DNA and RNA synthesis (7). *Parthenium* produces a non-alkaloidal and non-glycosidic substance called parthenin (8), along with some soluble inhibitors (9-10). Parthenium extracts exhibited retarding effect on yield and yield components of several crops including turnip (9,10,13). Obiviously, it is related with the meiotic irregularities. Neem leaf extract elaborates a vast array of biologically active and chemically diverse constituents (11-12). All or a few of these are concerned with the meiotic abnormalities and consequently cause a fall in the pollen viability. However, further biochemical investigations are required for an adequate explanation.

Table 1 . Effect of botanical extracts on meiosis and pollen viability of turnip.

Leaf extract	Dose	Gen-eration	Meiotic abnormalities (%)								Pollen viability (%)
			Metaphase - I				Anaphase - I				
			Bad ori-entation	Stick-mess	univa-tents	Total Mean±SE	Lagards	Brid-ges	Minor ab-normalities	Total Mean±SE	Mean±SE
Periwinkle	Con-trol	M_1	3.5	1.5	0.0	5.0±1.54	3.0	0.0	2.5	5.5±1.61	90.0±0.95
		M_2	2.5	1.5	0.0	4.0±1.39	3.0	0.0	2.5	5.5±1.61	90.2±0.94
	20%	M_1	3.5	1.5	0.0	5.0±1.54	3.5	1.0	1.0	5.5±1.61	85.6±1.11**
		M_2	3.0	1.0	0.0	4.0±1.39	3.0	1.0	1.0	5.0±1.54	90.2±0.94
	40%	M_1	4.0	1.5	1.0	6.5±1.74	4.5	0.0	1.5	6.0±1.68	70.5±1.44**
		M_2	3.5	1.5	1.0	6.0±1.63	4.0	1.0	1.0	6.0±1.68	80.0±1.26**
	60%	M_1	4.0	2.0	1.0	7.0±1.80	4.0	1.0	2.5	7.5±1.86	56.0±1.57**
		M_2	4.0	2.0	1.0	7.0±1.80	4.5	0.0	2.0	5.5±1.74	60.0±1.55**
	80%	M_1	4.5	2.0	1.0	7.5±1.86	4.5	1.5	2.5	8.5±1.97	65.2±1.51**
		M_2	5.0	1.5	0.5	7.0±1.80	4.5	0.0	2.0	6.5±1.74	70.0±1.45**
	100%	M_1	5.5	2.5	1.0	9.0±2.02	5.5	2.0	3.5	11.0±2.21	52.2±1.58**
		M_2	4.5	2.5	1.0	8.0±1.92	5.5	1.5	2.5	9.5±2.07	60.2±1.55**
Carrot weed	Con-trol	M_1	3.0	1.5	0.0	4.5±1.46	3.0	0.0	2.5	5.5±1.61	90.2±0.94
		M_2	2.5	1.5	0.0	4.0±1.38	2.0	0.0	2.5	4.5±1.46	92.1±0.85
	20%	M_1	1.0	1.0	2.0	4.0±1.38	2.5	1.0	1.5	5.0±1.54	84.2±1.15**
		M_2	1.0	1.5	2.0	4.5±1.46	1.5	2.5	0.0	4.0±1.38	87.0±1.06**
	40%	M_1	3.0	3.0	0.0	6.0±1.68	3.5	1.0	3.5	8.0±1.92	83.0±1.19**
		M_2	2.0	1.5	0.0	3.5±1.30	2.0	0.0	2.0	4.0±1.38	85.7±1.11**
	60%	M_1	3.0	3.5	0.0	6.5±1.74	3.5	1.5	2.5	7.5±1.86	82.2±1.21**
		M_2	2.5	2.5	0.0	5.0±1.54	3.0	0.0	4.0	7.0±1.80	85.1±1.13**
	80%	M_1	4.5	5.5	0.0	10.0±2.12*	4.0	1.5	5.5	11.0±2.21*	79.6±1.27**
		M_2	4.5	3.5	1.0	9.0±2.07*	3.0	1.5	5.5	10.0±2.12*	83.0±1.19**
	100%	M_1	6.0	7.0	1.5	14.5±2.49**	6.5	2.0	7.5	16.0±2.59**	34.6±1.50**
		M_2	5.0	5.0	1.0	11.0±2.21*	4.5	1.5	3.5	9.5±2.07	67.2±1.48**
Neem	Con-trol	M_1	3.5	1.5	0.0	5.0±1.47	3.0	0.0	2.0	5.0±1.54	90.0±2.12
		M_2	3.0	1.5	0.0	4.5±1.47	2.5	0.0	2.0	4.5±1.47	92.5±1.86
	20%	M_1	3.5	1.5	0.0	5.0±1.54	3.0	0.0	2.5	5.5±1.61	78.0±2.93**
		M_2	3.0	1.0	1.0	5.0±1.54	2.5	0.0	2.0	4.5±1.47	82.0±2.72**
	40%	M_1	5.0	1.0	0.0	6.0±1.68	4.5	0.0	2.0	6.5±1.74	75.0±3.06**
		M_2	4.5	1.0	0.0	5.5±1.61	4.0	0.0	1.5	5.5±1.61	76.0±3.01**
	60%	M_1	4.0	2.0	1.0	7.0±1.80	4.5	1.5	1.0	7.0±1.80	66.0±3.51**
		M_2	4.5	2.5	1.0	8.0±1.92	3.0	1.0	0.5	4.5±1.47	68.0±3.44**
	80%	M_1	4.5	2.5	1.5	8.5±1.97	3.5	1.0	3.0	7.5±1.86	51.0±3.54**
		M_2	3.0	1.5	0.0	4.5±1.47	3.0	0.0	2.0	5.0±1.54	48.0±3.53**
	100%	M_1	5.5	2.5	1.0	9.0±2.02	3.5	2.0	3.5	11.0±221*	47.5±3.53**
		M_2	5.0	2.0	0.0	7.0±1.80	5.0	1.5	3.0	9.5±2.07	51.0±3.54**

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

REFERENCES

1. Prasad, C. (2001). *Eucarpia Cruciferae Newsletter.* **23** : 27-28.
2. Ferreira, J. (1985). Pollen Viability Test for Brassica. In *'CrGC Resource Book'* (ed. P.H. Williams), Wisconsin, U.S.A.
3. Sikka, M. (1940). *J. Genet.* **40** : 441-509.
4. Prasad, C. & Shrivastava, M. (1996). *Bionature* **16** : 27-31.
5. Johnson, I.J., Wright, H. & Sovobods, G. (1959). *T. Lab. Clin, Med.* **54** : 830.
6. Degraeve, N. (1978). *Mutation Research.* **55** : 31-42.
7. Shrivastava, S., Rao, S. & Shah, V. (1973). *Cytologia.* **38** : 1-9.
8. Arny, H.V. (1897). *Ameri. J. Pharm.* **69** : 168.
9. Kanchan, S.D. (1975) *Curr. Sci.* **44** : 358-359.
10. Laxmi Ranjan (1973). *Curr. Sci.* **42** : 729-730.
11. Devakumar, C. & Mukherjee, S.K. (1983). *I.A.R.I. Res. Bull.* **40** : 1-17.
12. Singhal, K.K. & Mudgal, V.D. (1983) *J. Punjab Agricultural University,* Ludhiana, p. 165.
13. Priyadarshani, S. (1997) *Ph.D. Thesis,* Vinoba Bhave University, Hazaribag (India).

An Efficient Plant Regeneration Protocol from Seedling Explants of *Brassica juncea* RH-781, a Freeze Tolerant Cultivar

A. K. Dhawan, Amit Jain, Inderjeet and Jaipal Singh
CCS Haryana Agricultural University, Regional Research Station, Uchani, Karnal-132001, India

Introduction

Brassica juncea is a major oilseed crop in India. There is a heavy loss of yield because of frost during flowering season. Two of the possible strategies considered to overcome this are transfer of genes responsible for frost tolerance and selection of variant cells or *in vitro* raised plants using low temperature as selection pressure. Both of these require efficient regeneration protocols specific for each genotype. Here we present shoot regeneration protocol direct from seedling explants, which is being used in this laboratory.

Material and Methods

Seeds of *Brassica juncea* RH-781, a var. with lesser susceptibility to frost, were washed under running tap water, surface sterilized by dipping in 0.2% $HgCl_2$ for 8 minutes and finally washed 3-4 times with sterilized water to remove excess of $HgCl_2$. These seeds were inoculated on modified MS (Murashige and Skoog, 1962) media (MS +0.8% agar +3% sucrose) and allowed to germinate at $25\pm1^{\circ}$ C under light intensity of 4000 lux and 16 h photoperiod. Cotyledons and hypocotyls from 4-5 days old seedlings were excised and inoculated on different regeneration media listed in the Table 1. Observations on percent shoot formation and number of shoots formed were taken at 10-day intervals.

Results and Discussion

The frequency of shoot formation from cotyledon explants was higher in the media that were tried, as compared to hypocotyl explants. When cotyledons were used as explants, all the media produce shoots and R_4 medium showed highest (nearly 100%) regeneration followed by R_3. Hypocotyl showed much less regeneration capacity. R_2 medium was found to be the best for regeneration from hypocotyls followed by R_4. Statistically there was no significant difference between response of hypocotyls to R_2 and R_4 medium. Therefore, R_4 was concluded to be the best medium as maximum shoot formation was promoted in this combination from both cotyledon and hypocotyl explants.

From the above study it is clear that cotyledons are the best explant for shoot regeneration in *Brassica* in the media listed in Table 1. Similar observations have been made by Yadav *et al* (1991) ; Shrama *etal* (1991) ; Arora *et al* (1996) in contrast to the observations of Lazzeri and Dunwell (1986) and Dash *et al* (1995) who concluded that hypocotyl were the most regenerative explant . This might be due to differences in culture conditions, genotype, age of the explant and different media combinations.

Table 1. Response of seedling explants of *Brassica juncea* var. RH-781 for shoot and root formation in different media

Medium (ppm)	Cotyledons		Hypocotyls	
	%Shoot formation	No of Shoots	%Shoot formation	No of Shoots
R_1 MS+BAP(1.0)+IAA(0.2)	70 ± 5.2	M	20 ± 4.7	2 – 5
R_2 MS+BAP(2.0)+IAA(0.2)	65 ± 7.8	2 – 10	50 ± 8.3	2 – 8
R_3 MS+BAP(1.0)+IAA(1.0)	90 ± 6.2	M	20 ± 5.9	2 – 4
R_4 MS+BAP(2.0)+IAA(0.5)	97 ± 2.7	M	45 ± 7.9	2 – 5
R_5 MS+BAP(1.0)+IAA(0.2) +Kn(1.0)	70 ± 5.8	M	20 ± 2.1	1 – 3

M= Multiple shoots; BAP= banzyl amino purine; IAA= Indole-3-acetic acid

In view of percent shoot regeneration and number of shoots/explant, cotyledons as explant and MS+BAP(2.0)+IAA(0.5) as the media for raising *in vitro* plants are in use for further freeze stress experiments.

References

1. Arora ,N. , Yadav ,N.R. ,Chowdhury ,J.B.(1996). **Cruciferae News Letter** 18:27
2. Dash , P.K., Sharma , R.P., Kumar ,P.A. (1995). **Cruciferae News Letter** 17:26
3. Lazzeri ,P.A. , Dunwell ,J.M. (1986). **Annals of Botany** .58:689
4. Murashige, T. and Skoog, F. (1962). **Physiol. Plant.** 15: 473
5. Sharma ,K.K. , Bhojwani ,S.S., Thorpe ,T.A.(1991). **Plant Cell Tissue and Organ Culture.** 24:55
6. Yadav ,R.C., Kamada ,H., Kikuchi ,F.(1991). **Annals of Biol.**.7:119

Somatic embryogenesis from hypocotyl-derived calli of three varieties of genus *Brassica*

J.P. Martín[1] and A. Mohanty[2]

[1]*Departamento de Biología Vegetal, E.T.S. de Ingenieros Agrónomos de Madrid (U.P.M.),28040-Madrid, Spain*

[2]*International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, India*

Introduction

Somatic embryogenesis has been reported by several authors in the genus *Brassica* from different explants directly (Bagga *et al.* 1983; Narasimhulu *et al.* 1992; Koh and Loh 2000) or through an intermediate callus phase (Pareek and Chandra 1978; Gupta *et al.* 1990; Leroy *et al.* 2000).

In this study, we have used hypocotyl explants of three varieties of *Brassica* to induce an embryogenic response through an intermediate callus phase. The investigation shows two different developmental stages in the process of somatic embryogenesis; first the callus formation and then development of somatic embryos. The analyses of biochemical and molecular changes during these stages, will provide better insight into the embryogenic process as previously shown during organogenesis of cultured melon cotyledons (Leshem and Sussex 1990) or during microspore embryogenesis in *Brassica napus* (Cordewener *et al.* 2000).

Material and Methods

Seeds from three commercial varieties of genus *Brassica* were used for this study: a cultivar of cauliflower [*Brassica oleracea* L. var. *botrytis* L. subvar. *cauliflora* (Gars.) DC., "cauliflower large Christmas"] (CC; 2n=18); and two cultivars of rapeseed [*Brassica napus* L. var. *oleifera* DC.] (AACC; 2n=38), a Canadian cultivar of spring (cv. Oro), and a French cultivar of winter (cv. Jet Neuf).

Hypocotyl explants were obtained from 10 days-old seedlings growing *in vitro* and placed in Petri plates containing 25 ml of the MS medium (Murashige and Skoog 1962) supplemented with 2,4-D (1 mg l^{-1}), kinetin (0.1 mg l^{-1}), sucrose (3.5%) as carbon source and Difco Bacto agar (0.8%). After four weeks of culture, the induced calli were subcultured on MS medium during 3-6 weeks to facilitate the development of previously induced embryogenic structures.

The cultures were maintained in a culture room under a 16 h photoperiod, irradiance of 40 µmol m^{-2} s^{-1} and a temperature of 25 ± 1 °C. The experiment was repeated thrice, and each replicate consisted of 50 callus tissues. The embryogenic response was analysed in the second subculture and the data shown represent the mean of the three replicates.

Histological studies have been done to confirm the induction of embryogenic structures. Callus tissues were fixed in FAA (formalin: glacial acetic acid: 70% ethanol, 5:5:90, v/v/v) for 48 h, dehydrated through a graded series of ethyl alcohol and then embedded in paraffin (m.p. 51-53° C). Serial sections 12µm thickness were obtained using rotary microtome and were stained with toluidine blue for microscopic observation.

Results and Discussion

Hypocotyl explants of the three cultivars of *Brassica* formed calli and developed groups of proembryogenic cells simultaneously, which was confirmed by histological studies. During the second subculture two different morphological types of callus were

obtained: soft callus with a dark-yellow colour (type A) and hard callus with a green-yellow colour (type B). The frequency of type B callus was greater than type A callus in the three cultivars studied (Table 1).

Embryogenic response was obtained in type B callus from *B. oleracea* and *B. napus* cv. Oro, but no embryogenic structures were observed in any calli of *B. napus* cv. Jet Neuf (Table 1). The subculture on MS medium seems to induce the loss of the embryogenic capacity in cultivar Jet Neuf. After 5-6 weeks on MS medium, calli of the two embryogenic cultivars showed embryogenic structures in different stages. The frequency of type B calli showing embryogenic response was higher than 30% in both cultivars (Table1). Considering both callus types, the frequency of embryogenic response was higher in the cultivar Oro (27.6%) than in *B. oleracea* (18.1%).

This marked difference in embryogenic response at interspecific and intraspecific levels may be due to the genotypic differences. Pua (1990) and Ferrie *et al.* (1999) have also reported a strong influence of genotype on embryogenic frequency in different accessions of *B. juncea* and *B. oleracea*, respectively.

Table 1. Frequency of embryogenic response from hypocoty-derived calli of the three *Brassica* cultivars studied

	Callus		Frequency of embryogenic calli	
	Type	Frequency		
B. oleracea var *botrytis*	A	43	0.0	18.1
	B	57	31.7	
B. napus var *oleifera* cv. Oro	A	19	0.0	27.6
	B	81	34.2	
B. napus var. *oleifera* cv. Jet Neuf	A	21	0.0	0.0
	B	79	0.0	

References

Bagga, S., Bhalla-Sarin, N., Sopory, S.K. and Mukherjee, S.G. (1983). Comparison of *in vitro* plant formation from somatic tissues and pollen grains in *Brassica oleracea* var. *botrytis*. Phytomorphology, 32: 152-156.

Cordewener, J., Bergervoet, J. and Liu, C.M. (2000). Changes in protein synthesis and phosphorylation during microspore embryogenesis in *Brassica napus*. J. Plant Physiol., 156: 156-163.

Ferrie, A.M.R., Taylor, D.C., MacKenzie, S.L. and Keller, W.A. (1999). Microspore embryogenesis of high sn2 erucic acid *Brassica oleracea* germplasm. Plant Cell Tiss. Org. Cult., 57: 79-84.

Gupta, V., Agnihotri, A. and Jagannathan, V. (1990). Plant regeneration from callus and protoplasts of Brassica nigra (IC257) through somatic embryogenesis. Plant Cell Rep., 9: 427-430.

Koh, W.L. and Loh, C.S. (2000). Direct somatic embryogenesis, plant regeneration and *in vitro* flowering in rapid-cycling *Brassica napus*. Plant Cell Rep., 19: 1177-1183.

Leroy, X.J., Leon, K., Charles, G. and Branchard, M. (2000). Cauliflower somatic embryogenesis and analysis of regenerant stability by ISSRs. Plant Cell Rep., 19: 1102-1107.

Leshem, B. and Sussex, I.M. (1990). Polypeptides of cultured melon cotyledons serve as markers of root and shoot organogenesis. J. Plant Physiol., 137: 155-159.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Narasimhulu, S.B., Kirti, P.B., Prakash, S. and Chopra, V.L. (1992). Somatic embryogenesis in *Brassica nigra* (L) Koch. J. Exp. Bot., 43: 1203-1207.

Pareek, L.K. and Chandra, N. (1978). Somatic embryogenesis in leaf callus from cauliflower (*Brassica oleracea* var. *botrytis*). Plant Sci. Lett., 11: 311-316.

Pua, E.-C. (1990). Somatic embryogenesis and plant regeneration from hypocotyl protoplasts of *Brassica juncea* (L.) Czern and Coss. Plant Sci., 68: 231-238.

Influence of plant growth regulators on the fatty acid composition of mature seeds of Brassica napus L. (cv. GSL-1)

Navita Ghai, R.C. Setia and Neelam Setia
Department of Botany, Punjab Agricultural University
Ludhiana - 141004 (India)

INTRODUCTION

Oleiferous Brassicas play a very important role in agricultural and industrial economy of India. Oil is a major constituent of brassica seed and quality of oil depends upon its fatty acid composition. Linolenic and erucic acid which constitute about 9 and 53% of the total fatty acids, respectively, are undesirable for human consumption. There is thus a need to improve the oil quality in terms of fatty acids composition. In recent years plant growth regulators (PGRs) have been profitably exploited to boost production and uplift the quality of produce in several crops (Malik 1995, Malik et al 1986). The present investigation was, therefore, conducted to study the effect of PGRs viz; paclobutrazol (PBZ), salicylic acid (SA) and triacontanol (TRIA) on changes in fatty acid composition of oil from mature seeds of Brassica napus L.(cv. GSL-1).

MATERIALS AND METHODS

Seeds of Brassica napus L. (cv. GSL-1) were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana and the crop was raised in the experimental area of Department of Botany, Punjab Agricultural University, Ludhiana, following standard package of practices. The crop was foliarly sprayed with paclobutrazol (10 & 20 μgml^{-1}) at green floral bud stage and with salicylic acid (10, 20 & 50 μgml^{-1}) and triacontanol (1 μgml^{-1}) at anthesis stage followed by a repeated spray after one week interval. The crop was harvested at maturity (152 days after sowing). Oil content of mature harvested (treated and control) seeds as determined by NMR was used for determination of fatty acid composition. Method of Luddy et al. (1968) was employed for the preparation of methyl esters of fatty acids, which were subsequently estimated by GLC.

RESULTS AND DISCUSSION

The results showed that all the PGRS used, modified the fatty acid composition of oil in Brassica napus L. (cv. GSL-1) (Table 1). While the level of palmitic and linoleic acids remained unchanged, that of stearic acid increased with all treatments, except TRIA which caused its decrease. However, maximum increase in stearic acid level was recorded with SA application. The level of oleic acid increased with 20 μgml^{-1} PBZ, 10 & 20 μgml^{-1} SA and TRIA but decreased with 50 μgml^{-1} SA; however it remained unchanged with 10 μgml^{-1} PBZ treatment. All the PGRs treatments, in general, decreased the levels of linolenic, eicosenoic and behenic acids. Erucic acid content also exhibited decline with all the treatments except 50 μgml^{-1} SA which recorded a slight increase. Interestingly, a decrease in eicosenoic, behenic and erucic acids level following PBZ and TRIA applications has also been reported by Bhathal (1994) in B. carinata. The low level of erucic acid in seed oil of B. napus is desirable from the nutrition point of view, the lower level of linolenic acid is essential to improve the storage characteristics of oil (Kimber and McGregor, 1995). In the present studies a decrease in the level of both these acids following PGRs treatment has been obtained.

Alternations in fatty acid composition of oil in response to PGRs applications also changed the oleic/linoleic acids (O/L) ratio and iodine value (Table 2). While O/L ratio decreased with 10 μgml^{-1} PBZ and 50 μgml^{-1} SA, it increased with all the remaining treatments. Compared to controls the iodine

value was slightly decreased with 10 μgml^{-1} PBZ and 10 & 50 μgml^{-1} SA but remained nearly unchanged with remaining treatments. Similar observation have been recorded by Bhathal (1994) in B. carinata and by Kaur (2000) in Brassica hydrid 'PGSH-51'. The increase in O/L ratio with PGRs treatment is indicative of decrease in degree of unsaturation of oil which contributes towards more stability of oil (Setia et al. 1992).

Table 1. Effect of paclobutrazol (PBZ), salicylic acid (SA) and triacontanol (TRIA) on fatty acid composition (% of total) of oil from mature seeds of Brassica napus (cv. GSL-1).

Treatment	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Eicoseonic (20:1)	Behenic (22:0)	Erucic (22:1)
Control	3.18	0.65	16.17	15.08	9.94	12.70	0.48	45.64
PBZ ($10\mu gml^{-1}$)	3.49	0.71	16.04	15.23	8.52	11.58	0.44	44.05
PBZ ($20\mu gml^{-1}$)	3.16	0.74	18.14	14.81	7.98	11.63	0.35	43.06
SA ($10\mu gml^{-1}$)	3.33	0.78	17.29	14.64	8.27	10.71	0.39	44.21
SA ($20\mu gml^{-1}$)	3.26	0.79	18.57	16.00	7.96	10.37	0.31	42.72
SA ($50\mu gml^{-1}$)	3.00	0.75	15.20	15.45	7.64	11.10	0.33	46.51
TRIA ($1\mu gml^{-1}$)	3.21	0.55	19.31	14.48	7.71	10.84	0.36	43.19

Table 2. Effect of paclobutrazol (PBZ), salicylic acid (SA) and triacontanol (TRIA) on oil content, oleic/linoleic (O/L) ratio and iodine value (IV) in mature seeds of Brassica napus (cv. GSL-1).

Treatment	Oil content (%)	O/L ratio	IV
Control	39.9	1.07	50.0
PBZ($10\mu gml^{-1}$)	41.6	1.05	49.3
PBZ($20\mu gml^{-1}$)	40.7	1.22	50.4
SA($10\mu gml^{-1}$)	40.3	1.18	48.6
SA($20\mu gml^{-1}$)	41.4	1.16	51.8
SA($50\mu gml^{-1}$)	40.3	0.98	48.6
TRIA($1\mu gml^{-1}$)	40.2	1.33	50.2

REFERENCES

Bhathal, G. 1994. M.Sc. Thesis, Punjab Agricultural University, Ludhiana.

Kaur, R. 2000. M.Sc. Thesis, Punjab Agricultural University, Ludhiana.

Kimber, D. and McGregor, D.I. 1995. Brassica Oilseeds : Production and Utilization. CAB International. Wallingford. UK.

Luddy, F.E., Banford, R.A., Herbs, S.F. and Magidmann, P. 1968. J. Amer. Oil. Chem. Soc. 15 : 1549-1552.

Malik, C.P. 1995. Sectional Presidential Address. 82nd Indian Science Congress, pp. 1-34; (Calcutta, India : Indian Sci. Cong. Assoc.)

Malik C.P., Singh, P., Setia, R.C., Setia, N. and Bhatia, D.S. 1986. In : Hormonal Regulation of Plant Growth and Development, Vol. III; (ed. Purohit, S.S.) pp. 371-415. Bikaner, India : Agrobotanical Publishers.

Setia, N., Vaid, N., Setia, R.C., Malik, C.P. and Ahuja, K.L. 1992. J. Pl. Sci. Res. 8 : 48-52.

EFFECT OF WATER AND SALT STRESS ON TOTAL CHLOROPHYLL CONTENT IN INDIAN MUSTARD (*BRASSICA JUNCEA (L.) CZERN & COSS.*

Vinod Goyal, I.S. Sheoran, & M.L.Chhabra

Department of Botany & Plant Physiology.

CCS HARYANA AGRICULTURAL UNIVERSITY, HISAR. (INDIA).

Among oilseed crops Indian mustard is the second most important oilseed crop after groundnut and holds 27% share in total oilseed production. The crop is mainly grown in arid and semi arid regions of the country, which are generally affected by soil salinity or saline irrigation water. Hence the crop suffers salt and water stress right from the beginning i.e. germination to various stages of crop growth leading to reduced yield or crop failure. As a result of salt stress the crop simultaneously faces the problem of water stress. Thus, the study was conducted to see the effect of water and salt stress on *Brassica* genotypes. The present paper is a part of the comprehensive research project. The experiment was conducted on two Brassica genotypes (RH-30 and CS-52) in earthen pots having yellow sand mixed with FYM. Thirty-five days after sowing (35 DAS) irrigation was withheld for 3 days to achieve mild water stress (MWS) and for 10 days to achieve severe water stress(SWS). Similarly NaCl solution of 8 and 16dSm^{-1} were given to potted plants 35 and 70 DAS. Total chlorophyll content was estimated 10 days after treatment adopting Hiscox and Israelstam (1979) method.

Chlorophyll content was higher at 70 DAS stage than at 35 DAS stage. Increase in chlorophyll content was observed when plants were exposed to mild water stress (MWS) or severe water stress (SWS) or salinity of 8 or

16dSm^{-1}. The quantum of increase in chlorophyll increased with increase in stress i.e. higher the water or salinity stress, higher was chlorophyll content. The studies are in accordance with Zoppo *et al.* (1999) in wild wheat and in contrast to the result of Goyal *et al.* (2001) in pearl millet.

Table:1 Effect of water and salt stress on chlorophyll content (μmol/g dw) in Brassica

Treatments	RH-30		CS-52	
	35DAS	70DAS	35DAS	70DAS
Control	727.7	1890.0	760.0	1253.0
Mild water stress	807.3	1978.0	984.0	1420.0
Severe water stress	1730.3	2445.0	1557.0	1846.0
8 dSm^{-1} NaCl	1189.6	1446.3	1243.1	1846.3
16 dSm^{-1} NaCl	1524.1	2061.1	1613.1	2769.7

References:

Goyal, V., Bansal, P.; Goel, A. and Bishnoi, N.R. (2001). Effect of terminal water stress on different pigments of various genotypes of pearl millet. *Crop Res.* 21 (3): 271-273.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* 57: 1332-1334.

Zoppo, M.D., Galleschi, L., Onnis, A., Pardossi, A and Saviozzi, F. (1999). Effect of salinity on water relations, sodium accumulation, chlorophyll content and proteolytic enzymes in a wild wheat. *Biol. Plant.* 42 (1): 97-104.

EFFECT OF SALINITY ON PLANT GROWTH AND LIPID COMPONENTS OF INDIAN MUSTARD *(Brassica juncea* L.)

R.P.S. Parti, S.K. Gupta and Naveen Chandra
Department of Plant Breeding,
CCS Haryana Agricultural University, Hisar-125 004, INDIA

INTRODUCTION

The detrimental effects of salinity are due to the influence of Ca, Mg, Na, Cl and SO_4 the major ions, on the water activity of the external solution which affects the status of the plant due to osmotic effect and also the direct effects of the ions on the physiological and biochemical functions of the cells (Greenway and Munns, 1980).

MATERIALS AND METHODS

Mustard crop (Cv. RH-30) was raised in earthen pots in green house. The pots were lined with polyethylene bags and filled with 5 kg of sandy soil (ECe 1.8 d Sm^{-1}). The salinity levels of 4, 8 and 12 dSm^{-1} were obtained by adding Cl and SO_4 salts of Na, Ca and Mg. The Cl and SO_4 in the ratio of 7:3 while Na, Ca and Mg in the ratio of 4:1:3 were taken (Sharma and Manchanda, 1997). The plant samples were taken at 35 days after sowing and siliquae samples were taken at maturity which were further separated into seeds and siliquae wall. These were dried at 60^0C in the hot air oven and dry weight was recorded. At maturity plant height and no. of siliquae per plant were also recorded. Total lipids of seeds were determined which were further separated into polar and non-polar lipids (Nichols, 1964). Phospholipids and glycolipids were determined as per the methods of Jackson (1973) and Joseph (1954) respectively.

RESULTS AND DISCUSSION

The salinity affected the plant growth considerably (Table 1). The dry weight of plant, seeds and siliquae wall was maximum at 4 dSm^{-1} thereafter a consistent decrease was observed with the increase in salinity levels. There was a appreciable reduction in the number of siliquae plant and also in plant height (Table 1) with increase in salinity level. Kurban *et al.* (1995) observed reduction in plant growth with salinity in leguminous plants.

Lipid composition was affected considerably by different salinity levels (Table 2). Total and non-polar lipids showed reduction with increased salinity. However, at 4 ECe and in control, the total lipids and non-polar lipids were almost at par. Polar lipids and its components viz. phospholipids and glycolipids showed the reverse trend, that is these increased with increasing levels of salinity. Among in polar lipids the amount of phospholipids remained higher as compared to glycolipids at all the salinity levels.

Table 1 : Effect of salinity on dry weights of plant, seeds, siliquae wall and on no. of siliquae and plant height.

Salinity levels (ECe dSm^{-1})	*Dry wt. of plant (g/plant)	Dry wt. of seeds (g/1000 seeds)	Dry wt. of siliquae wall* (g/100 siliquae)	No. of siliquae/ plant**	Plant height ** (cm)
Control	0.33±0.04	4.52±0.14	4.10±0.12	34.6±1.11	116.9±2.27
4	0.37±0.03	4.99±0.08	4.26±0.15	32.6±0.98	114.4±1.01
8	0.24±0.02	4.05±0.11	3.32±0.09	23.21±0.61	105.5±1.91
12	0.11±0.01	3.54±0.32	3.26±0.16	22.4±0.99	95.5±0.78

± Indicates standard deviation
* Plant dry weight was taken at 35 days after sowing.
** Each value is the average of fifteen plants

Table 2 : Effect of salinity levels on lipid composition of mustard seeds (mg/g) dry weight basis.

Salinity levels (ECe in dSm^{-1})	Total lipids	Polar lipids	Non-polar lipids	Phospholipid	Glycolipid
Control	420.1±6.31	45.3±0.11	377.2±4.38	29.2±0.17	13.8±0.03
4	420.0±4.78	48.2±0.17	380.2±3.78	29.6±0.81	14.6±0.17
8	347.3±2.11	52.8±0.09	319.2±6.43	34.6±0.08	17.2±0.10
12	328.2±5.36	59.8±0.14	264.2±5.32	38.2±0.14	20.4±0.19

± indicates standard deviation

REFERENCES

Greenway, H. and Munns, R. 1980. *Ann. Rev. Plant Physiol.* 31 : 149-190.

Kurban, H., Saneoska, H., Nehira, K., Adilla, R. and Fujita, K. 1998. *Soil Sci. and Plant Nutr.* 44 : 589-597.

Jackson, M.L. 1973. Chemical analysis, Prentice Hall of India Ltd., New Delhi. pp. 151-152.

Joseph, H.R. 1954. *J. Biol. Chem.* 208 : 889-896.

Nichols, B.W. 1964. Methods for the estimation of non-polar and polar lipid fraction. Van Nostrand Co., London, pp. 321.

Sharma, S.K. and Manchanda, H.R. 1997. *Indian J. Agric. Sci.* 67 : 1-5.

EFFECT OF SALINITY ON MINERAL COMPOSITION OF INDIAN MUSTARD (*Brassica juncea* L.)

R.P.S. Parti, S.K. Gupta and Naveen Chandra
Department of Plant Breeding,
CCS Haryana Agricultural University, Hisar-125 004, INDIA

INTRODUCTION

Changes in growth of plants under salinity stress appears to be associated with reduced absorption of certain essential nutrients or high electrolytes levels contributing towards osmotic adjustment and salt tolerance and/or ion toxicity. Salinity tended to affect the absorption, mineral composition, accumulation and balance of different major nutrients in the different organs of plant (Lal and Bhardwaj, 1987).

MATERIALS AND METHODS

Mustard crop (cv. RH-30) was raised in earthen pots in green house. The pots were lined with polyethylene bags and filled with 5 kg sandy soil (ECe 1.8 dSm^{-1}). The salinity levels of 4, 8, 12 dSm^{-1} were obtained by adding Cl and SO_4 salts of Na, Ca and Mg. The Cl and SO_4 in the ratio of 7:3 while Na, Ca and Mg in the ratio of 4:1:3 were taken (Sharma and Manchanda, 1997). The plant samples were taken at 35 days after sowing while silquae samples were taken at maturity which were further separated into seeds and siliquae wall. The samples were digested (Chapman and Pratt, 1960) and were analysed for phosphorus (Jackson, 1973). Sodium and potassium on flame photometer. Nitrogen was determined by conventional kjeldahl method.

RESULTS AND DISCUSSION

There was a distinct decrease in nitrogen (N) content of mustard plant, seed and siliquae wall (Table 1). Comparatively, phoshphorous (P) content registered a slight reduction with salinity. Earlier reports also indicate that salinity reduces N accumulation in crop plants (Garg *et al.*, 1993). As for as P content is concerned, salinity may decrease, have no effect or increase P accumulation in plants depending upon the crop and environmental conditions (Feigin, 1985). As the salinity increased, the sodium (Na) content increased appreciably with corresponding decrease in potassium (K) content in plant, seeds and siliquae wall. Earlier reports suggest reduced uptake of K as Na accumulate in plant cells (Garg and Garg, 1980).

Table 1 : Effect of different levels of salinity on mineral content of Indian mustard plant, seed and siliqua wall (mg/g dry weight).

Salinity levels (ECe in dSm^{-1})	Nitrogen	Phosphorus	Potassium	Sodium
	Plant (35 days after sowing)			
Control	39.11±1.30	9.31±0.42	67.15±1.31	0.91±0.03
4	37.73±0.34	8.89±0.09	64.88±0.41	1.57±0.01
8	33.57±0.91	8.36±0.17	60.19±0.52	2.92±0.06
12	31.71±1.54	7.22±0.23	55.21±0.71	3.85±0.05
	Seed (maturity)			
Control	37.11±0.30	9.69±0.15	20.13±0.40	0.23±0.01
4	35.89±0.27	9.48±0.08	19.21±0.38	0.41±0.05
8	34.13±0.43	8.85±0.10	18.45±0.47	0.61±0.03
12	33.21±0.53	8.32±0.16	17.49±0.55	0.86±0.08
	Siliqua wall (maturity)			
Control	18.33±0.41	4.79±0.15	31.63±0.20	0.12±0.01
4	17.91±0.36	4.62±0.09	30.88±0.37	0.25±0.01
8	15.24±0.18	4.32±0.11	24.17±0.21	0.30±0.03
12	12.41±0.22	3.65±0.06	22.61±0.41	0.38±0.02

REFERENCES

Chapman, H.D. and Pratt, P.F. 1960. Method of analysis for soils, plants and water. University of California, 309-322.

Feigin, A. 1985. *Plant and Soil*. 89 : 285-299.

Garg, B.K. and Garg, O.P. 1980. *Proc. Indian National Sci. Acad.* B46 : 694-698.

Garg, B.K., Vyas, S.P., Kathju, S., Lahiri, A.N., Mali, P.C. and Sharma, A.C. 1993. *J. Plant Nutr.* 16 : 1637-1650.

Jackson, M.L. 1973. Chemical analysis, Prentice Hall of India Ltd., New Delhi. pp. 151-152.

Lal, P.K. and Bhardwaj, S.N. 1987. *Indian J. Plant Physiol.* 30 : 165-169.

Sharma, S.K. and Manchanda, H.R. 1997. *Indian J. Agric. Sci.* 67: 1-5.

WATER STRESS INDUCED CHANGES IN GROWTH CHARACTERISTICS AND PROLINE CONTENT IN SEEDLINGS OF *B. JUNCEA* CULTIVARS DIFFERING IN DROUGHT TOLERANCE

Phutela Archana, Jain Veena, Dhawan Kamal and Nainawatee H.S.*
Department of Biochemistry, CCS H.A.U. Hisar 125 004, INDIA
*Indian Council Of Agricultural Research, New Delhi, 110 012, INDIA

Drought is one of the main environmental limitations responsible for plant growth and crop productivity in arid and semi-arid regions of land. Water stress results in cellular dehydration within a plant cell which invokes cellular responses like decreased shoot growth (Meyer and Boyer, 1981) and photosynthetic activity (Boyer and Bowen, 1970) and increased proline content (Voetberg and Sharp 1991). *B. juncea* is an important oil seed crop whose production is affected by drought conditions. *B. juncea* cultivars differing in drought tolerance *viz.* Varuna, PPMS, RH 819 and Prakash were obtained from the Department of Plant Breeding and SR-3 from Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar. Seeds were germinated in sterilized petri plates lined with Whatman #1 filter paper in an incubator at 25+2 ^{0}C. A stress of –0.3 MPa and -1.0 MPa using PEG 6000 was given at the time of sowing and after 48h of sowing (after germination) respectively. Control seedlings contained sterile water only. Fresh weight, epicotyl length and hypocotyl length were measured at 7 days after sowing (DAS) stage after washing the seedlings with chilled sterile water. Proline content was estimated by the method of Bates *et al* (1973).

Maximum reduction in fresh weight (59.3%) was observed in sowing time stress effected seedlings of PPMS and minimum was in SR-3 subjected to stress after germination (Table 1). Epicotyl length also decreased and it was maximum in cv PPMS and minimum in cv Varuna at both the levels of stress, however, the reduction was more when stress was created at the time of sowing.

The hypocotyl length was maximum in cv Varuna (5.19 cm) and minimum in PPMS (3.89 cm) in non-stressed seedlings. Its length increased under stress condition in all the cultivars with higher elevation in the seedlings given stress after germination (mean length 7.18 cm) as compared to those under stress from sowing time (mean length 6.05 cm).

Among cultivars, the proline content was highest in cv PPMS (9.69 umoles/g dry weight) and lowest in cv Varuna (5.61 umole/g dry weight). Under both the conditions of stress at 0 and 48h of seed germination, the proline content increased drastically and the increase was maximum in cv Varuna (18-23 fold) and minimum in PPMS (8-11 fold). However, the enhancement was higher in seedlings subjected to stress after germination than those stressed at the time of sowing. Increased level of proline has been reported to be due to increased activity of proline biosynthetic enzymes and reduced activity of proline catabolizing enzyme (Phutela *et al*, 2000). Higher accumulation of proline has been positively correlated with drought tolerance (Van Rosenburg *et al*, 1993).

Water stress is known to decrease epicotyl length and fresh weight (Kuhad *et al*, 1989). Higher level of reduction in growth parameters with less increase in proline level in stress effected plants during germination as compared to those stressed after germination indicated seedlings could survive better if subjected to drought after germination.

References

Bates LS, Waldren RP and Teare ID. 1973. *Plant Soil* **39**: 205-207.
Boyer JS and Bowen BL. 1970. *Plant Physiol.* **85**: 2261-267.
Kuhad MS, Sheoran IS and Bishnoi NR. 1989. *Haryana J. Agric. Res.* **36**: 220-222.
Meyer RF and Boyer JS. 1981. *Planta* **151**: 482-489.
Phutela A, Jain V, Dhawan K and Nainawatee HS. 2000. *J. Plant Biochem. Biotechnol.* **9**: 35-39.
Van Rosenburg, L, Kruger GH and Kruger H. 1993. *J.Plant Physiol.***141**:188-194.
Voetberg GS and Sharp RE. 1991. *Plant Physiol.* **96**: 1125-1130

Table 1.Growth parameters and proline content under water stress in seedlings of *B. juncea* cultivars.

Imposition of Stress (HAS)	Genotypes					Mean
	Prakash	SR-3	RH 819	PPMS	Varuna	
	Fresh weight (g/100seedlings)[a]					
Nil	3.70±0.04	2.86±0.06	4.88±0.10	2.70±0.02	4.10±0.12	3.60
0	1.74±0.10	1.42±0.06	2.34±0.04	1.10±0.10	1.72±0.04	1.88
48	2.08±0.05	1.78±0.07	2.12±0.10	1.46±0.20	1.84±0.16	1.72
	Epicotyl length (cm)[a]					
Nil	2.59±0.13	2.88±0.11	3.59±0.4	2.87±0.22	3.01±0.09	2.98
0	1.64±0.13	1.78±0.07	2.13±0.31	1.47±0.13	1.97±0.29	1.82
48	1.71±0.12	1.94±0.06	2.19±0.18	1.30±0.08	2.35±0.11	1.90
	Hypocotyl length (cm)[a]					
Nil	3.92±0.25	3.96±0.31	4.17±0.19	3.89±0.26	5.19±0.37	4.04
0	6.25±0.32	7.23±0.25	4.98±0.26	4.29±0.55	7.50±0.49	6.05
48	7.89±0.18	8.59±0.75	5.13±0.34	5.47±0.40	8.15±0.56	7.18
	Proline content (µmole/g dry weight)[b]					
Nil	9.01±1.28	8.91±0.69	8.84±0.84	9.69±1.10	5.61±0.70	8.41
0	73.86±1.04	87.29±6.91	88.95±4.41	85.48±1.17	102.40± 4.39	87.88
48	120.50±8.97	134.28±9.53	119.81±7.49	100.78±7.32	132.50±4.53	121.57

[a]Values are mean of 3 replicates each of 30 seedlings ±SD
[b]Values are mean of 4 replicates±SD.
HAS refers to hours after sowing.

Diurnal changes in photosynthesis and non-structural carbohydrates of Indian mustard under elevated CO_2 conditions

A. C. Srivastava & Y. P. Khanna
Sher-e-Kashmir University of Agricultural Sciences & Technology
RS Pura, Jammu, India

Abstract

The diurnal changes in leaf net photosynthetic rate (Pn) and non-structural carbohydrates of Indian mustard (*Brassica juncea* L. Czern & Coss cv. Pusa Bahar) were analyzed at vegetative and flowering stages under ambient (350±25 ppm) and elevated (600±50 ppm) CO_2 conditions. Pn was higher under elevated CO_2 than ambient CO_2 but the decline in the increase was observed during afternoon. This down regulation in Pn was found to be associated with the accumulation of large amount of sugars under elevated CO_2.

Introduction

Leaf level studies have shown that photosynthetic responses to CO_2 partial pressure vary greatly with crops and plant developmental stages (Sage, 1994). Enhanced photosynthesis may not remain high for longer duration under high CO_2 and it may decrease (Ghildiyal *et al.*, 1998). The Pn can vary with species, source-sink relations, phenological age, reproductive status or combinations of these factors (Griffin & Luo, 1999). The present study makes an attempt examining some of these aspects in order to analyse the physiological reasons for the diurnal changes in Pn of Indian mustard.

Material and Methods

Seeds of Indian mustard (*Brassica juncea* L. Czern & Coss cv Pusa Bahar) were sown during 1999-2000 rabi (winter) at Plant Physiology Division, I.A.R.I., New Delhi (India). Cultural practices as recommended for the area were followed. In the experiment, a modified open top chamber (1.8X1.6 m) as described by Rogers *et al.* (1983) was developed to study crop responses to elevated CO_2. Pn of top most leaf was measured at hourly intervals from 8 hr to 17 hr by using IRGA (LiCOR-6200). Leaves for analysing sugars were sampled during forenoon and afternoon at 25 and 55 days after germination. Reducing sugar content were estimated by Nelson's arsenmolybdate method (Nelson, 1944). Dried samples of the residue leaf after extraction for reducing sugars were used for starch content (Pucher *et al.*, 1948).

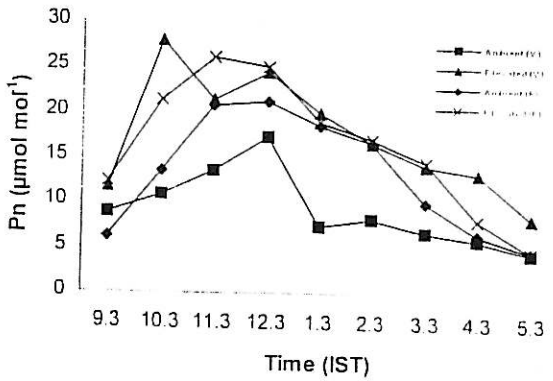


Fig 1. Diurnal Pn in Indian mustard at vegetative and flowering stage

Table 1. Non-structural carbohydrates in the leaves of Indian mustard at vegetative and flowering stage. The contents of sugars were measured at 10.00 and 15.00 IST

Crop Stage	Session	Parameters	Ambient	Elevated
Vegetative	Forenoon	RS	9.76±1.0	12.25±0.10*
		NRS	32.7±1.25	32.97±0.90^{ns}
		TS	42.46±0.10	46.26±7.83^{ns}
		Starch	47.52±0.10	50.40±0.04^{ns}
	Afternoon	RS	24.35±0.99	36.10±0.72*
		NRS	56.12±0.09	78.54±1.57*
		TS	80.47±1.08	114.64±1.38*
		Starch	84.38±1.30	118.40±3.19*
Flowering	Forenoon	RS	8.04±0.02	10.71±0.26^{ns}
		NRS	41.55±1.50	44.12±3.24^{ns}
		TS	49.59±1.48	54.83±3.50^{ns}
		Starch	56.12±0.09	60.03±1.40^{ns}
	Afternoon	RS	21.84±1.54	34.55±2.37*
		NRS	63.70±1.00	80.21±1.28*
		TS	85.54±2.54	114.76±2.89*
		Starch	77.35±0.57	103.20±1.91*

RS- Reducing sugar; NRS- Non-reducing sugar; TS- Total sugar

Values are mean ±sd of five replications.

- P< 0.05, ns= non significant

Results and Discussion

CO_2 enrichment enhanced the Pn, which was observed through out the day but the trend of photosynthetic enhancement under CO_2 enrichment differed entirely with the diurnal changes in photosynthesis. As the rate of photosynthesis increased, high CO_2 mediated photosynthetic enhancement decreased (Fig 1). Thus, the high CO_2 mediated enhancement in photosynthesis is more when the rate of leaf photosynthesis low. Such variations in photosynthetic enhancement and diurnal change may be due to the carbohydrates. We recorded higher sugar (reducing, non-reducing and starch) under elevated CO_2 in the afternoon which may inhibit Pn by mixing up Pi resulting in Pi limitation of RuBP generation capacity (Socias *et al.*, 1993). The other reason for the variation in diurnal changes could be declined export of carbohydrates from the leaves in the afternoon (Azcon-Bieto, 1983).

References

Azcon- Bieto J (1983) *Plant Physiol* **73**, 681-686.

Ghildiyal MC, Sharma-Natu P, Khan FA (1998) *Indian J Exp Biol* **36**, 217-220.

Griffin KL & Luo Y (1999) *Environ Exp Bot* **42**, 141-144.

Nelson N (1904) *J Biol Chem* **153**, 375-380.

Pucher GW, Leavenworth CS & Vickery HB (1948) *Ann Chem* **20**, 850-853.

Rogers HH, Heck WW & Heagle AS (1983) *Air Polution Control Assoc J* **33**, 42.

Sage RF (1994) *Photosynth Res* **39**, 351-368.

Socias FX, Medrano H & Sharkey TD (1993) *Plant Cell Environ* **16**, 81-85.

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

D. L. Woods, Agriculture and Agri-Food Canada, Research Farm, Box 29, Beaverlodge, Alberta, Canada, T0H 0C0.

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

---

[1] Crucifer Genetics Cooperative, Dept of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706, USA

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 $2P-P^2$, 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 $1/3(2P-1P^2)$.

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

Kelly, A., Fray, M., Arthur, E.A., Lydiate, D.J., and **Evans, E.** 1995. The genetic control of petalless flowers and upright pods. Proc 9th Int. Rapeseed Congr. Cambridge, UK, July 4-7. pages 732-734.

Strickenburg, M.W. 1985. Genetics, third edition. Macmillan Publishing Company, New York. pages 285-286.

Woods, D.L., and **Séguin-Swartz, G.** 1997. An investigation on linkage between white flower colour and erucic acid in summer rape. Can. J. Plant Sci. **78:** 109-111.

Preliminary studies on inheritance of the ecotypical male sterility-fertility in *Brassica napus* L.

Mei YUAN, Guang-sheng YANG*, Ting-dong FU and Hong-yan YAN
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, P. R. China

Introduction

It is a common fact that the expression of cytoplasmic male sterility in many plant species has been shown to be influenced by environmental conditions, and pol CMS is the same case. Breeding practices had proved that the pol CMS lines could be divided into three groups, i.e., high temperature male sterile lines(Group I), low temperature male sterile lines (Group II) and relatively stable male sterile lines(GroupIII) (Fu *et al*, 1989). An ecotypical male sterile line, AB1, which is Group I of pol CMS line , was male fertile when it was sown at Wuhan in Autumn, but it became sterile when it was sown at Kunming or Xining in Summer(Yang *et al*, 1999). Yang *et al* (1997) suggested temperature could be the main factor which could influence the fertility of AB1. The object of this study was to preliminarily study the inheritance of AB1.

Materials and methods

AB1 described by Yang (1999) and 1141A (a relatively stable pol CMS line) were developed by rapeseed laboratory in Huazhong Agricultural University. F_1 and BC_1 were generated at Wuhan by crossing 1141A as the female parent with fertile AB1 and fertile F_1, respectively. The F_1 plant was self-pollinated by bagging to produce F_2. In 2000, all plants described above were grown in a field at Wuhan and Lanzhou. Fertility was assessed by observing the ratio of stamen to pistil, at least twice during the anthesis. Seven-scale fertility index used in this study was described by Yang and Fu (1991).

Results and discussion

The fertility of the F_1 at Wuhan and Lanzhou was listed in Table 1. It can be seen that AB1 and F_1 were partial fertile at Wuhan by March 25, and male sterile both at Wuhan after March 25 and at Lanzhou during the whole flowering period. This suggested that F_1 and AB1 showed the similar trend of fertility change. Therefore, it can be concluded that the temperature-sensitivity of AB1 is incompletely dominant to temperature-insensitivity of 1141A on the basis of male sterile stablility of 1141A.

In table 2, it was shown that according to the data of March 19 fertility types of plants in BC_1 and F_2 populations in Wuhan ranged from F(0) to F(5) and there both existed a successive distribution partial to partial fertility. All these plants became male sterile according to the data of April 6 in 2001. It was also showed that all plants in BC_1 and

*To whom correspondence should be addressed

F_2 populations were male sterile when they were sowed in Summer at Lanzhou. These results indicated that relatively high-temperature contributes to the expression of male sterility. If temperature-sensitivity of AB1 was a kind of quality trait controlled by major genes, plants in BC_1 and F_2 populations should have only exhibited the fertility type of parents. Since there existed the fertility type of non-parent in BC_1 and F_2 populations on the basis of the data of March 19 and F_1 showed similar fertility to AB1 during the whole anthesis (seen in table 1), we may draw a conclusion that the temperature-sensitivity of AB1 might be controlled by several pairs of minor genes.

Table 1. The fertility of AB1 and F_1 between 1141A and AB1 (in 2000)

Location	Material	Fertility and Date of investigation									
Wuhan	F_1	F(4)	3/15	F(4)	3/20	F(0)	3/25	F(0)	3/30	F(0)	4/5
	AB1	F(4)	3/15	F(4)-F(3)	3/20	F(3)	3/25	F(0)	3/30	F(0)	4/5
Lanzhou	F_1	—	—	F(0)	6/27	F(0)	7/15	—	—	—	—
	AB1	F(1)	6/15	F(0)	6/27	F(0)	7/15	—	—	—	—

Table 2. The fertility segregation of plants in BC_1 and F_2 populations (in 2001)

Segregation progenies	Location	Date	No. of plants with the different fertility					
			F(5)	F(4)	F(3)	F(2)	F(1)	F(0)
BC_1	Wuhan	3/19	—	104(23.1)	16(25.8)	97(21.6)	70(15.6)	63(14)
		4/6	—	—	—	—	—	450(100)
	Lanzhou	7/27	—	—	—	—	1(0.82)	121(99.18)
F_2	Wuhan	3/19	11(2.1)	173(33.8)	115(22.5)	90(17.6)	60(11.7)	63(12.3)
		4/6	—	—	—	—	—	512(100)
	Lanzhou	8/2	—	—	—	—	13(10.3)	113(89.7)

* the number in the bracket were the percentage of plants with different fertility in the populations investigated

References

Fu T D, Yang X N and Yang G S. (1989) Development and studies on polima cytoplasmic male sterile three lines in *Brassica napus* L. *J Huazhong Agricultural University*, 8(3): 201~207

Yang G S and Fu T D. (1991) A prelimilary study on the restoring-maintaining relationship in rapeseed(*Brassica napus* and *Brassica campestris*). *Acta Agronomica Sinica*, 17(2): 151~156

Yang G S, Fu T D, Ma C Z *et al.* (1997) Studies on the ecotypical male sterile line of *Brassica napus* L. II. the influence of environment on the ecotypical male sterility. *J Huazhong Agricultural University*, 16(5): 330~334.

Yang G S, Duan Z H, Fu T D, *et al.* (1999) A promising alternative way of utilizing pol CMS for hybrid breeding in *Brassica napus* L. *Proc. of the 10th Intern. Rapeseed Congress*, Canberra, Australia.

Acknowledgement

This study is supported by the National 973 Project of China(Grand No. 2001CB10807)

A Preliminary Study on The Restoring-maintaining Relationship in Radish (*Raphanus Sativus* L.)

Zhang Li[1], Wei Yutang[2], Li Xiaoyan

[1]Beijing Vegetable Research Center, Beijing 100089;[2]Horticulter Department of Shengyang Agriculture University, Shengyang 110161,P.R.China

INTRODUCTION

Male sterility provides a simple method for producing radish hybrid. So far some male sterile cytoplasmisms have been found besides Ogu cms. But the relationship between these cytoplasmisms was not studied. In this paper, the restoring-maintaining relationship between 3 male sterile cytoplasmisms was tested. It was found that the relationship was different, which indicated that the three cytoplasmism belonged to different types.

MATERIALS AND METHODS

The materials used are two male sterile lines of Chinese radish-DA and JA, one of European radish-SA, and 18 varieties coded for Q_1-Q_7, B_1-B_{12}.

Crosses have been made between the 3 male sterile lines and 18 varieties.

RESULTS

The results obtained from the experiments are presented in table 1.

Table 1 Fertility segregation of F_1 between three male sterile lines and male fertile lines in radish

Male fertile lines	DA		JA		SA		Male fertile lines	DA		JA		SA	
	fertile plants	sterile plants	fertile plants	sterile plants	fertile plants	sterile plants		Fertile sterile Plants	plants	fertile plants	sterile plants	fertile plants	sterile plants
Q_1—2	18	0	18	6	11	5	B_3—2	22	0	29	0	32	29
Q_2—1	19	0	32	0	43	0	B_4—3	29	0	0	18	46	39
Q_3—4	4	10	23	11	40	0	B_5—3	11	0	17	0	30	0
Q_4—6	15	0	21	0	28	0	B_7—1	29	0	25	0	32	0
Q_5—2	18	0	28	0	32	0	B_8—4	18	8	0	24	33	30
Q_6—3	16	6	46	15	26	10	B_9—3	11	12	24	23	30	29
Q_7—1	0	15	0	13	9	3	B_{10}—1	0	38	0	29	0	36
B_1—1	0	23	0	24	0	38	B_{12}—3	0	38	0	31	0	42
B_2—1	9	10	19	0	24	21	B_{12}—4	0	46	15	14	0	34

To elaborated clearly, Table 2 was made according to Table 1.

Table 2 Fertility expression of F_1 from crosses between three male sterile lines and male fertile lines in radish

Male fertile lines	DA	JA	SA	Male fertile lines	DA	JA	SA
Q_1—2	F	FS	FS	B_3—2	F	F	FS
Q_2—1	F	F	F	B_4—3	F	S	FS
Q_3—4	FS	FS	F	B_5—3	F	F	F
Q_4—6	F	F	F	B_7—1	F	F	F
Q_5—2	F	F	F	B_8—4	FS	S	FS
Q_6—3	FS	FS	FS	B_9—3	FS	FS	FS
Q_7—1	S	S	FS	B_{10}—1	S	S	S
B_1—1	S	S	S	B_{12}—3	S	S	S
B_2—1	FS	F	FS	B_{12}—4	S	FS	S

Note: F--fertile; FS—fertility segregation; S--sterile.

Table 1 and Table 2 showed that the restoring-maintaining relationship between the 3 sterile cytoplasmisms was different when crossed with 18 varieties. Q_2,Q_4,Q_5,B_5 and B_7 could restore the fertility of 3 cytoplasmisms . B_{10} and B_{12-3} could maintain the sterility. But some lines have different restoring-maintaining on the 3 cytoplasmisms. For instance $Q_7$is maintainer of DA and JA, but the part-maintainer of SA. B_4 is restorer of DA, but maintainer of JA. The different restoring-maintaining relationship indicated that the 3 male sterile cytoplasmisms belonged to different types.

More and more male sterile cytoplasmisms of radish have been found, so it is important to classify these cytoplasmisms. Classification is the basis of utilizing them. The method used in this paper is an important means classifying sterile cytoplasmisms.

REFERENCES

Huang Qingyang et al.2000. Comparison of Fertility Inheritance of Three Kinds of Cytoplasmic Male Sterile Lines in Rice(Oryza sativa L.). Scientia Agriculture Sinica 33(3):8-13

Zhang Changxian.1996. Classification of Male Sterile Cytoplasmisms in Crops. Acta Agriculturae Boreali-occidentalis Sinica.5(3):83-86

Bonnet,A.,1977.Breeding in France of a Radish F1 Hybrid Obtained by Use of Cytoplasmic Male Sterility. Eucarpia Cruciferae Newslett.2:5

Heterosis studies in Indian mustard (*Brassica juncea* L.)

C. Kumar, S. Muralia and Deepak Gupta
Agricultural Research Station, Navgaon (Alwar)

Introduction

The exploitation of hybrid vigour in Indian mustard depends on the magnitude of heterosis and feasibility of hybrid seed production at commercial scale. Hence an attempt was made to evaluate the extent of heterosis for seed yield, yield attributing components and identification of high heterotic crosses over better parent and mid parent for utilizing them in heterosis breeding programme.

Materials and Methods

Three lines (RN-505, RN-393 and RN-514) and twelve testers (RN-490 RN-481, RN-453, RB-9901, Bio-902, PBR-181, PCR-43, Red leaf, RN-536, RN-539, Vabhav and NQR-9301) were crossed in a line x tester model suggested by Kemptorne(1957). The resultant thirty six F_1s and fifteen parents were grown in a randomized block design with 2 replications at Agricultural Research station, Navgaon (Alwar) during Rabi 2000-01. Each entry was accommodated in a single row of 3 meter length spaced 30 cm apart with a plant to plant distance of 10 cm. Data on 5 randomly selected plants were recorded and heterosis over better parent and mid parent were estimated.

Results and Discussion

The range of heterosis over better and mid parent are presented in Table 1. A negative heterosis is always desired for the development of characters viz. days to flowering and maturity. Highest heterosis for days to flowering was recorded in RN-505 x PCR-43 over better parent (-16.67%) and mid parent (-17.91%) and out of 36 crosses, 12 and 17 crosses showed desired heterosis over BP and MP. For days to maturity, cross RN-393 x RN-453 (-8.39% and -11.25%) showed highest heterosis over BP and MP.

Out of 36 crosses, 6 and 9 crosses showed significant heterosis over BP and MP for plant height and hybrid RN-393 x RN-539 showed highest heterosis over BP(31.58%) and MP (32.45%).

The highest heterosis recorded for siliqua bearing height was 60.71% and 62.16% and for siliqua on main stem 85.71% and 106.4% over BP and MP, respectively. The best hybrid for siliqua bearing height was RN-505 x RN-490 and siliqua on main stem was RN-393 X NQR-9301.

In addition, the crosses RN-505 x Bio-902 for siliqua size, RN-514 x RN-490 for seeds/siliqua and RN-505 x RB-9901 for siliqua /plant showed highest heterosis over BP and MP.

For the complex characters, seed yield the range of heterosis was -52.38% to 73.33% and -60.78 to 106.2% over BP and MP, respectively. The highest heterosis were recorded in the crosses RN-505 x RN-490 over BP and RN-505 x PCR-43 over MP. Similar magnitude of heterosis have also been reported by Chauhan *et al.*, 2000 and Verma *et al.*, 1998.

The comparative study of most promising 5 hybrids for seed yield/plant with useful and component characters showing desired heterosis is presented in Table 2. The high heterotic effects of seed yield in these cases were mainly with significant heterotic effects of days to flowering, maturity, siliqua/plant, siliqua bearing height, siliqua length and siliqua on main stem.

The hybrids RN-505 x RN-490, RN-505 x PCR-43, RN-393 x RN-481, RN-393 x RN-453 and RN-505 x RN-481 were found high heterotic hybrids for seed yield. They also had high heterosis for many yield attributing characters in desired direction. These hybrids offers best possibilities of further exploitation for development of high yielding varieties.

References

Kempthorne, O. 1957. An introduction of genetic statistics. *John Willey and Sons. Inc.*, New York p. 150.

Chauhan, S.S., Srivastava, R.K. and Kumar K. 2000. Heterosis in single and three way crosses in Indian mustard (Brassica juncea (L.) Czern and Coss). Indian J. Genet. 60(1): 131-132.

Verma, O.P., Singh, H.P. and Singh P.V.1998. Heterosis and inbreeding depression in Indian mustard. Cruciferae Newsletter. 20:75-76.

Table 1 : Range of heterosis over better parent and Mid parent for different characters along with best hybrid in Indian mustard.

Character	Range of heterosis(%) over BP & Best hybrids		Range of heterosis over BP & Best hybrids	
Days to flowering	-16.67 to 6.35	RN-505 x PCR-43	-17.91 to 7.94	RN-505 x PCR-43
Days to maturity	-8.39 to 1.92	RN-505 x RN-453	-11.25 to 2.56	RN-505 x RN-453
Plant height	-35.7 to 31.58	RN-393 x RN-539	-35.71 to 32.45	RN-393 x RN-539
Siliqua bearing height	-28.57 to 60.71	RN-505 x RN-490	-23.06 to 62.16	RN-505 x RN-490
Siliqua on main stem	-53.19 to 85.71	RN-393 x NQR-9301	-41.18 to 106.4	RN-393 x NQR-9301
Siliqua length	-23.64 to 33.33	RN-505 x Bio-902	-18.87 to 41.18	RN-505 x Bio-902
Seeds/siliqua	-9.09 to 41.67	RN-514 x RN-490	-13.04 to 47.83	RN-514 x RN-490
Siliqua/plant	-56.61 to 72.08	RN-505 x RB-9901	-57.4 to 83.49	RN-505 x RB-9901
Seed yield/plant	-52.38 to 73.33	RN-505 x RN-490	-60.78 to 106.2	RN-505 x PCR-43

Table 2: Comparative study of most promising heterotic hybrids for seed yield with useful and component characters showing desired heterosis.

Hybrids	Heterosis for seed yield over		Useful and significant heterosis over BP for component traits	Useful and significant heterosis over BP for component traits
	BP	MP		
RN-505 x RN-490	73.33**	92.59**	DF, DM, PH, SBH, SMS, SZ & SP	DF, DM, PH, SBH, SMS, SL, SS & SP
RN-505 x PCR-43	66.67**	106.2**	DF, DM & SP	DF, DM, SL, SS & SP
RN-393 x RN-481	70.00**	77.08**	DM, SBH & SMS	DF, PH, SBH, SMS & SP
RN-393 x RN-453	70.00**	88.89**	DF, DM, SMS, SZ & SP	DF, DM, SBH, SMS, SL & SP
RN-505 x RN-481	60.00**	81.13**	DF, DM, SBH & SP	DF, DM, SBH, SZ & SP

** Significant at 1% level

Where,

DF = Days to flowering
DM = Days to maturity
PH = Plant height
SBH = Siliqua bearing height
SMS = Siliqua on main stem
SL = Siliqua length
SS = Seeds/siliqua
SP = Siliqua /plant

Germplasm release of self-fertile summer turnip rape (*Brassica rapa* L.)

L. J. Lewis and D. L. Woods, Research Farm, Agriculture and Agri-Food Canada, Box 29, Beaverlodge, Alberta, Canada T0H 0C0.

Self-fertile *Brassica rapa* was identified and backcrossed into cultivar AC Sunshine. This character may be of use to plant breeders or geneticists in situations where self-pollination is advantageous.

Breeding

Seed from an S_2 plant exhibiting self-fertility (89-23-1, courtesy of Gary Stringam, University of Alberta) was crossed as the female parent with *Brassica rapa* summer turnip rape line CB 8719 (later released as cultivar AC Sunshine). F_1 plants were interpollinated. F_2 plants were both selfed and crossed to the recurrent parent AC Sunshine. BC_1F_1 seed was bulked. F_2 plants and succeeding generations were scored as self-fertile if 100 or more pollen tubes were observed penetrating the stigmatic papillae using aniline blue UV fluorescence microscopy (Martin 1959) and if seed set exceeded an average of 10 seeds per silique.

The F_3 plant ratio from selfed F_2 plants did not significantly differ from dominant monohybrid inheritance for self-fertility (SF). Subsequent test crosses, reciprocal crosses (ruling out cytoplasmic inheritance), and diallel crosses were consistent with nuclear monohybrid dominant inheritance. SF proved dominant in crosses with over 50 randomly selected AC Sunshine plants. We have no data to show whether or not SF is a dominant allele at the S locus. If SF is, in fact, a dominant S-allele, the possibility of other S-alleles being dominant in expression of self-incompatibility cannot be excluded.

F_2 and BC_1F_1 were backcrossed using AC Sunshine as the male parent followed by 2 cycles of backcrossing using the recurrent parent as the female. BC_4F_1 was selfed. Test crossing identified a number of self-fertile homozygotes from which one BC_4F_2 plant was again selfed and test crossed to confirm homozygosity of the SF character. CB 0109 descends directly from isolated seed increases of this single BC_4F_2 plant.

Availability

The SF *B. rapa* line CB 0109 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 designation CN 19060.

Reference

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain. Techn. **34**: 125-128.

Germplasm release of long podded summer turnip rape (*Brassica rapa* L.)

L. J. Lewis and D. L. Woods, Research Farm, Agriculture and Agri-Food Canada, Box 29, Beaverlodge, Alberta, Canada T0H 0C0.

Breeding

As described by Lewis et al. (2001), long pod summer turnip rape (*B. rapa*) was developed by introgressing the trait from oilseed summer rape (*B. napus*) (Line 91-2-4 University of Alberta, courtesy of Gary Stringam) and backcrossing to cultivar AC Sunshine producing 10 BC_3F_7 elite long podded lines. Pod (silique) length of plants, excluding pedicel and beak, of the 10 long podded lines and AC Sunshine were evaluated in replicated field trials at the Beaverlodge Research Farm (55° 12' N, 119° 23' W) in 1998 and 1999. Pod length data is summarized in Table 1.

Table 1. Mean pod length of 10 long podded *B. rapa* lines and AC Sunshine grown in 1998 and 1999 in Beaverlodge, Alberta, Canada.

Lines	Pod[z] Length (mm)
29367[y]	76.93
29369	74.65
29370	74.58
29371	73.56
29375	73.30
29381	71.60
29387	77.38
29388	69.27
29390	74.03
29392	70.44
AC Sunshine	47.06
$LSD_{0.05}$	4.62

[z]Pod (silique) length excluding the pedicel and beak.
[y]Long pod *B. rapa* lines.

Availability

Equal portions from each of the 10 elite lines were bulked to form CB 0110. The long podded *B. rapa* germplasm CB 0110 may be obtained from the primary author or from Plant Gene Resources of Canada, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2 under the designation CN 19061.

Reference

Lewis, L., J., Woods, D. L., and Cheng, B. F. 2001. Introgression of long pod genotype from spring rape (*Brassica napus* L.) into summer turnip rape (*Brassica rapa* L.). Can. J. Plant Sci. **81**: 59-60.

GENETIC COMPONENTS OF DIFFERENT YIELD ATTRIBUTES IN INDIAN MUSTARD UNDER SIX ENVIRONMENTS IN ACIDIC SOIL OF JHARKHAND

JAY LAL MAHTO (DEPARTMENT OF PLANT BREEDING AND GENETICS, BIRSA AGRICULTURAL UNIVERSITY, RANCHI 834 006, JHARKHAND, INDIA.)

ABSTRACT: **In a 9*9 diallel cross analysis in six environments, both additive and non-additive genetic components were significant for seed yield and its attributes under environments. Breeding Methods selection (S_2 selection and reciprocal recurrent selection), which exploit both additive and non-additive components would be appropriate for the improvement of yield and yield traits.**

KEY WORDS: Mustard, environment, sowing direction (North-South and East-West).

INTRODUCTION: Indian mustard having the high yield potential is suitable for sole or inter-cropping under both irrigated and rainfed condition. In the present study an effort has been made to find out the genetic components of seed yield and its attributes and to formulate effective breeding strategy to improve seed yield along with other traits in Indian mustard.

MATERIALS AND METHODS: Nine parents (Rw873, Pr830, Kranti, Rw29-6-3, Pr18, Rh843, Rh851, Vardan and Br40) were involved in a diallel mating design (excluding reciprocals) in 1995-96. These 45 genotypes were grown on three dates (E_1 and E_4 on 27th September, E_2 and E_5 on 4th October and E_3 and E_6 on 11th October 1997) at North-South (E_1, E_2 and E_3) and East-West (E_4, E_5 and E_6) sowing directions with two replications on each date during winter at Birsa Agricultural University experimental area, Ranchi. The area is located between 23°17'' latitude and 85''19'E longitude and altitude is 625 meters above the sea level. The p^H of the soil being 5.9. The distance between rows and plants were maintained at 30cm and 10cm, respectively. Cultural practices as recommended for the area were followed. Ten competitive plants were randomly selected from each lines, replication and six environments to record the observations on 11 characters. The genetic components of variation were worked out as per Hayman (1954).

RESULTS AND DISCUSSION: There were significant differences among the parental lines as well as among the crosses for most of yield attributes in all most all environments. In 3rd environment number of primary branches/plant, plant height, number of seeds/siliqua and seed yield/plant were controlled by additive genetic variation (D). Whereas 6th environment showed additive genetic variation for number of seeds/siliqua, days to maturity, harvest index, 1000-seed weight and seed yield/plant. Second environment favoured the expression of non-additive(dominance) for days to 50% flowering, number of primary branches/plant, number of secondary branches/plant, plant height, number of siliquae/plant, number of seeds/siliqua, days to maturity, 1000-seed weight, seed yield/plant and oil content in percent. Third environment had non-additive(dominant) for days to 50% flowering, number of primary branches/plant, number of secondary branches/plant, plant height, number of seeds/siliqua, days to maturity, harvest index, 1000-seed weight, seed yield/plant and oil content. Non-additive (dominant) factor H_2 of 2nd environment favoured the expression of 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 content. Whereas 6th environment favoured the expression of non-additive dominant component (H_2) for days to 50% flowering, plant height, number of siliquae/plant, number of seeds/siliqua, days to maturity, harvest index, 1000-seed weight, seed yield/plant and oil content in percent. Value of H_2 was smaller than H_1. Magnitude of mean degree of dominance was higher than one in 3rd environment for days to 50% flowering, number of siliquae/plant, number of seeds/siliqua, harvest index, seed yield/plant and oil content, indicating the presence of over-dominance for these characters. In 6th environment there was a presence of over-dominance for days to 50% flowering, number of siliquae/plant, number of seeds/siliqua, days to maturity, harvest index, 1000-seed weight, seed yield/plant and percent of oil content. The ratio $H_2/4H_1$ indicated symmetrical distribution of genes as value was about to 0.25 for seed yield/plant and oil content under the 1st environment. The magnitude of ratio was symmetrical distribution of genes for number of siliquae/plant, seed yield/plant and oil content in 2nd environment. A symmetrical distribution of genes was observed for number of secondary branches, number of siliquae/plant, seed yield/plant and oil content in 3rd environment. In 4th environment number of secondary branches/plant, harvest index, seed yield and oil content was expressed the symmetrical distribution of genes. Seed yield/plant and oil content showed the symmetrical distribution of genes in most of environments. The dominant and recessive genes in parents showed the preponderance of dominant genes for plant height, days to maturity and oil content in per cent and the preponderance of recessive genes for days to 50% flowering, number of primary branches, harvest index and seed yield/plant under 1st environment. In 6th environment days to 50% flowering, number of siliquae/plant and oil content expressed the preponderance of dominance genes and number of seeds/siliqua, days to maturity, harvest index, 1000-seed weight and seed yield/plant expressed the preponderance recessive genes. Oil content showed preponderance of dominant genes in all environments, whereas seed yield/plant showed preponderance of recessive genes in most of all the environments. Selection (S_2 selection and reciprocal recurrent selection), which exploit both additive and non-additive components, would be appropriate for the improvement of yield and yield attributes in Indian mustard.

REFERENCES

Hayman, B.I. 1954. The theory and analysis of diallel crosses. *Genetics*,39:789-809.

Table 1. Genetic parameters in six different environments in Indian mustard for yield attributes.

PARAMETER		CHARACTERS										
		1	2	3	4	5	6	7	8	9	10	11
D	(N-S) E1	2.331 ±0.074	0.530 ±0.453	-8.802 ±6.360	62.32 ±80.21	-4341 ±1686	-1.623 ±4.895	40.377 ±22.78	0.001 ±0.002	-0.057 ±0.224	2.342 ±3.41	5.525* ±2.169
	(N-S) E2	17.31* ±3.119	0.342* ±0.268	-1.349 ±5.999	205.1* ±142.7	348.9 ±1718	1.583* ±0.619	-0.012 ±10.05	-0.000 ±0.005	-0.047 ±0.468	2.233 ±3.05	2.805 ±1.739
	(N-S) E3	3.352 ±41.43	2.329* ±0.455	15.44 ±6.041	623.6* ±110.6	4725 ±6602	5.569* ±2.607	-4.264 ±66.93	0.002 ±0.002	-0.025 ±0.156	5.72* ±1.57	1.185 ±1.722
	(E-W) E4	6.961 ±14.38	-0.144 ±0.470	1.860 ±9.528	249.2* ±93.24	-379.693 ±7245.6	-0410 ±3.565	28.176 ±15.19	0.009 ±0.003	-0.027 ±0.103	0.081 ±2.47	1.021 ±2.226
	(E-W) E5	-4.419 ±11.59	0.569 ±0.538	29.05* ±12.80	413.4* ±114.5	3322.2 ±4238.2	2.308 ±2.634	16.353 ±15.48	0.002* ±0.001	0.000 ±0.157	-1.15 ±19.3	1.046 ±1.081
	(E-W) E6	30.102 ±48.75	-0.599 ±1.472	-55.02 ±33.68	-31.03 ±214.7	266.58 ±18945	5.134* ±5.773	5.083* ±4.166	0.004* ±0.002	0.300* ±0.102	25.09 ±18.6	7.353 ±11.77
F	(N-S) E1	-65.47 ±0.893	-0.565 ±1.056	-17.15 ±14.84	-17.63 ±187.1	-7737 ±3934	-2.026 ±11.42	39.31 ±53.15	-0.001 ±0.004	-0.187 ±0.523	2.881 ±7.94	6.016 ±5.060
	(N-S) E2	-47.62 ±7.275	0.578* ±0.626	1.550 ±13.99	426.1* ±332.8	3818.5 ±4009.1	1.256* ±1.443	-11.25 ±23.46	-0.000 ±0.012	-0.172 ±1.093	2.672 ±7.12	4.314 ±4.058
	(N-S) E3	22.714 ±96.66	4.539* ±1.061	23.075 ±14.10	606.8* ±257.9	10655.4 ±15402	11.07* ±6.08	27.65 ±156.1	0.003 ±0.003	-0.081 ±0.364	9.92* ±3.66	3.959 ±4.018
	(E-W) E4	-21.72 ±33.55	-0.240 ±1.096	-6.931 ±22.23	172.3* ±217.5	-2210.6 ±16902	-0.559 ±8.316	44.921 ±35.44	0.015* ±0.007	-0.057 ±0.240	1.073 ±5.76	2.856 ±5.193
	(E-W) E5	-39.08 ±27.04	-0.370 ±1.254	17.58* ±29.90	312.36 ±267.1	1494.6 ±9886.9	1.393 ±6.144	16.439 ±36.11	0.001* ±0.003	-0.040 ±0.366	-8.53 ±45.1	2.208 ±2.522
	(E-W) E6	41.969 ±113.7	-2.668 ±3.435	-118.9 ±78.57	-244.3 ±501.0	-19207 ±44195	7.812* ±13.47	-32.23 ±9.718	0.006* ±0.004	0.003 ±0.013	45.2* ±43.5	14.396 ±27.45
H_2	(N-S) E1	158.15 ±2.281	2.990 ±0.999	39.150 ±12.07	8442* ±152.2	10156 ±3722.2	21.719 ±10.80	261.3 ±50.29	0.030* ±0.003	1.531* ±0.495	33.1* ±6.46	16.63* ±4.116
	(N-S) E2	-7.862 ±6.884	2.070* ±0.592	87.64* ±11.38	10138* ±270.6	39302* ±3793	4.638* ±1.365	67.55* ±22.19	0.016 ±1.011	6.806* ±0.889	25.1* ±6.73	13.53* 3.300
	(N-S) E3	334.4* ±91.45	5.462* ±1.004	43.45* ±11.46	7472* ±209.8	41299 ±14573	26.99* ±5.753	306.88 ±147.7	0.012 ±0.004	1.012* ±0.345	16.1* ±5.41	14.26* ±3.268
	(E-W) E4	115.5* ±31.74	4.529 ±1.037	51.45 ±18.08	6651* 176.9	24699.6 ±15999	24.272 ±7.868	150.5* ±33.53	0.028* ±0.006	5.680* ±0.195	26.9* ±5.45	24.43* ±4.224
	(E-W) E5	42.23 ±25.59	1.235 ±1.254	84.77* ±24.36	9989* ±217.2	22047 ±9354	6.687 ±5.813	79.972 ±34.16	0.004* 0.003	1.037 0.346	120.2 36.69	10.32* ±2.051
	(E-W) E6	460.5* ±107.6	5.286 ±3.25	288.83 ±63.90	9588* ±407.5	66706* ±41814	27.67* ±12.74	125.2* ±9.195	0.011* ±0.004	0.666* ±0.225	171* ±35.4	70.89* ±22.32
H_1	(N-S) E1	1960* ±32.89	11.89* ±0.859	17.481 ±14.04	663.4* ±177	20374 ±3199	78.37 ±9.288	7231.6 ±43.23	0.015* ±0.004	6.23** ±0.425	29.67 ±7.52	19.89* ±4.788
	(N-S)E2	1471* ±5.917	11.4** ±0.509	65.12* ±13.24	1252* ±314.9	54921* ±3260.7	77.53* ±1.174	9235* ±19.08	0.039 ±0.01	6.87* ±0.89	32.4* ±5.78	17.76* ±3.839
	(N-S)E3	2795* ±78.62	10.42* ±0.883	42.90* ±13.33	872.1* ±244.1	42049 ±12528	80.39* ±4.946	8253* ±126.9	0.029* ±0.003	5.99** ±0.296	16.1* ±2.98	18.93* ±3.802
	(E-W) E4	2262* ±27.28	12.312 +0.891	36.539 +21.03	649.9* +205.8	29442 +13747	89.15* +6.764	7517.2 +28.83	0.04** +0.006	1.153 +0.227	27.7* +4.68	27.41* +4.914
	(E-W) E5	1588* ±21.99	12.58* ±1.02	34.22* ±28.34	534.29 ±252.7	40864* ±8041	85.73* ±4.997	6415** ±218	0.024* ±0.002	6.409* ±0.298	114.6 ±42.6	12.56* ±2.386
	(E-W) E6	3095* ±92.56	19.06* ±2.79	135.12 ±74.34	807.70 ±474	14375 ±35945	82.00* ±10.95	8423* ±7.90	023** ±0.00	6.143* ±0.193	173* ±41.1	80.18* ±25.96
h^2	(N-S) E1	-2.642 ±-0.06	1.353 ±0.57	4.63 ±8.084	-54.63 ±101.9	702.816 ±2143	2.470 ±6.222	-8.374 ±28.9	-0.001 ±0.002	0.001 ±0.285	7.453 ±4.32	-0.162 ±2.757
	(N-S) E2	29.75* ±3.964	2.237* ±0.341	28.74* ±7.625	518.1* ±181.3	29508* ±2184.4	4.000* ±0.786	-5.390 ±12.78	-0.003 ±0.006	0.026 ±0.595	12.33 ±3.88	-0.962 ±2.211
	(N-S) E3	30.28* ±52.66	2.994* ±0.578	58.87* ±7.679	2.156 ±140.6	7277.5 ±8392.4	-0.559 ±3.313	-20.52 ±85.07	-0.001 ±0.002	0.088 ±0.198	4.39*±1. 99	2.306 ±2.189
	(E-W) E4	-11.42 ±18.28	0.343 ±0.597	1.760 ±12.11	-41.97 ±118.5	1367.2 ±9209.6	-0.766 ±4.531	11.332 ±19.31	-0.000 ±0.004	0.742 ±0.131	6.72* ±3.14	6.06* ±2.830
	(E-W) E5	48.03 ±14.74	-0.452 ±0.683	-9.855 ±16.32	-41.63 ±145.5	-1064.88 ±5387.0	-1.081 ±3.348	-9.986 ±19.67	0.003* ±0.002	0.142 ±0.200	26.01 ±24.6	-0.977 ±1.374
	(E-W) E6	-0.106 ±61.97	-0.461 ±1.872	28.836 ±42.81	-98.89 ±272.9	-4692.9 ±24080	-1.234 ±7.34	-5.788 ±5.295	-0.001 ±0.002	-0.073 ±0.129	-0.89 ±23.7	11.70 ±14.95
E	(N-S) E1	42.343 ±4.262	1.415* ±0.143	9.768 ±2.011	138.7* ±25.36	5058.99 ±533.28	2.057 ±1.548	21.755 ±7.205	0.002 ±0.001	0.120 ±0.071	0.764 ±1.08	0.993 ±0.086
	(N-S) E2	33.88* ±0.986	0.658* ±0.085	11.04* ±1.897	137.5* ±45.12	3428.99* ±542.45	1.497* ±0.196	19.58* ±3.180	-0.002 ±0.002	0.460* ±0.148	0.957 ±0.96	2.280* ±0.550
	(N-S) E3	46.09* ±13.10	0.421* ±0.144	9.001* ±1.910	243.9* ±34.97	2877.06 ±2087.9	2.086* ±0.824	68.951 ±21.16	0.002 ±0.001	0.159* ±0.049	0.396 ±0.49	1.345* ±0.515
	(E-W) E4	29.40* ±4.547	0.713 ±0.149	4.460 ±3.013	147.2* ±29.49	1489.47 ±2291.3	2.597 ±1.127	10.33 ±4.80	0.001* ±0.001	0.071 ±0.032	0.323 ±0.78	0.423 ±0.704
	(E-W) E5	26.732 ±3.066	1.244* ±7.317	25.76* ±4.060	186.3* ±36.21	5975.98* ±1340.2	2.739 ±0.833	25.279 ±4.89	0.001* ±0.000	0.231* ±0.050	5.433 ±6.12	1.986* ±0.342
	(E-W) E6	30.09* ±15.42	2.210* ±0.466	82.08* ±10.65	255.87 ±67.91	24821.4* ±5990.9	3.144* ±1.826	42.36* ±1.317	0.001* ±0.001	0.21** ±0.032	2.89* ±5.89	2.368 ±3.721
$(H_1 / D)^{0.5}$	(N-S) E1	8.237	2.376	NS	3.263	NS	NS	2.544	5.336	NS	NS	3.559	1.897
Mean degree of	(N-S) E2	NS	2.461	NS	2.470	10.613	1.712	NS	NS	NS	3.369	2.516
dominance	(N-S) E3	9.988	1.532	1.667	1.183	2.956	2.202	NS	2.511	NS	1.744	3.997
	(E-W) E4	4.074	1.474	4.433	1.615	NS	NS	2.311	1.726	NS	18.23	5.181
	(E-W)E5	NS	NS	1.085	1.137	2.572	1.702	2.211	1.596	243.03	NS	3.465
	(E-W) E6	3.911	NS	NS	NS	15.819	2.321	4.963	1.776	1.491	2.632	3.302
$H_2 / 4 H_1$	(N-S) E1	3.099	0.994	NS	3.182	NS	NS	0.919	0.492	NS	0.279	0.209
Genes with positive and	(N-S) E2	NS	1.371	NS	2.025	0.349	4.179	NS	NS	NS	0.319	0.190
negative effect in parents	(N-S) E3	2.089	0.477	0.253	2.142	0.255	0.745	NS	0.612	NS	0.232	0.188
	(E-W) E4	4.896	2.547	0.352	2.559	NS	NS	12.491	0.335	NS	0.257	0.223
	(E-W) E5	NS	NS	0.619	4.674	0.463	3.205	20.054	1.414	1.544	NS	0.205
	(E-W) E6	1.681	NS	NS	NS	0.429	0.741	16.822	0.512	2.306	0.247	0.221
KD / KR	(N-S) E1	-3.284	-1.681	NS	2.139	NS	NS	2.641	-1.016	NS	-3.296	1.042
Ratio of dominant and	(N-S) E2	-1.545	-1.513	NS	2.025	1.362	-2.567	NS	NS	NS	-3.183	1.087
recessive genes in	(N-S) E3	NS	-3.281	6.372	1.259	1.087	-10.37	NS	-1.031	NS	-11.12	1.235
parents	(E-W) E4	-24.41	-1.715	-2.818	1.443	NS	NS	3.343	-1.069	NS	-1.221	1.147
	(E-W) E5	NS	NS	3.267	1.328	1.104	-3.259	5.854	-1.029	-1.003	NS	1.221
	(E-W) E6	3.184	NS	NS	NS	1.425	-8.580	-8.401	-1.043	-1.473	-3.099	1.042

N=North, S=South, E=East, W=West and En=Environments. Character 1. Days to 50% flowering, 2. Number of primary branches / plant, 3. Number of secondary branches / plant, 4. Plant height, 5. Number of siliquae / plant, 6. Number of seeds / siliqua, 7. Days to maturity, 8. Harvest index 9. 1000-Seeds weight, 10. Seed yield / plant 11. Oil content in percent. * Significant 5% probability level.

MULTIPLE REGRESSION ANALYSIS IN INDIAN MUSTARD OVER ENVIRONMENTS IN ACIDIC SOIL OF JHARKHAND

JAY LAL MAHTO (Department of Plant Breeding and Genetics, Birsa Agricultural University, Ranchi 834 006, Jharkhand, India.)

ABSTRACT: Association between days to 50% flowering and days to maturity; number of primary branches/plant with number of secondary branches/plant, plant height and number of siliquae/plant; number of secondary branches/ plant with plant height, number of siliquae/plant and seed yield/plant; and plant height and number of siliquae/plant showed importance over environments. 4th environment showed its important for contribution of characters to seed yield.

KEY WORDS: Correlation, regression, mustard, environment

INTRODUCTION: It is a continuous process in plant breeding to improve the existing varieties. The selection of the genotypes having high genetic variability is a basic requirement in any successful hybridization to produced desirable combination for selecting high yielding genotype. Present investigation was undertaken to find out the major components of yield in mustard through correlation and multivariate regression.

MATERIALS AND METHODS: Nine parents (1. RW873, 2.PR830, 3.KRANTI, 4. RW29-6-3, 5. PR18, 6. RH843, 7.RH851, 8. VARDAN AND 9. BR40) were involved in a diallel mating design (excluding reciprocals). These lines and their 36 F_1's cross progenies were grown in winter at Birsa Agricultural University experimental area, Ranchi, in a single row in randomized block design in two replications under five environments of North-South (E_1, E_2 and E_3) and East-West (E_4 and E_5) with three dates (E_1 and E_4 on 27th September, E_2 and E_5 on 4th October and E_3 on 11th October 1997). Distance was maintained between rows and plants at 30cm and 10cm, respectively. The area is located between 23°17'' latitude and 85''19'E longitude and altitude is 625 meters above the sea level. The p^H of the soil being 5.9. Ten plants were randomly selected in each genotypes of replication under each environment to record the observations on the 11 characters. Correlation and multiple regression analysis were done as per method described by Snedecore and Cockran (1968).

RESULTS AND DISCUSSION: Analysis of variance for most of the characters in all environments showed the significant differences in genotypes. Correlation between days to 50% flowering and days to maturity; number of primary branches / plant with number of secondary branches / plant, plant height and number of siliquae / plant; number of secondary branches / plant with plant height, number of siliquae / plant and seed yield / plant; and plant height and number of siliquae / plant had significant positive in all five environments. Association between days to 50% flowering and harvest index was significant and negative in most of the environment.

Contribution of equation based on all characters under five environments varies from 50.62% in 1st environment to 67.87% in 4th environment, in which partial regression was positive for number of secondary branches / plant, days to maturity, harvest index and 1000-seed weight. Equation based on nine characters under five environments varies from 50.62% in 1st environment to 67.87% in 4th environment. In 4th environment debarring the character plant height, contribution of equation was 67.87% and partial regression was positive for the days to 50% flowering, number of secondary branches / plant, number of siliquae / plant, number of seeds / siliqua, days to maturity, harvest index and 1000-seed weight. Contribution of equation based on eight characters under five environments varies from 50.58% in 1st environment to 67.79% in 4th environment. Equation based on two characters under five environments, contribution varies from 38.92% in 1st environment to 64.57% in 4th environment with all positive partial regressions. Contribution of equation based on number of siliquae / plant in 2nd environment was 50.36%. In same way all the equation based on different characters combination showed the lowest contribution percent in 1st environment and the highest in 4th environment. On the above results 4th environment showed its important to expression of characters / contribution of characters to yield.

REFERENCE

Snedecore, G. W. and Cochran, W. G. 1968. *Statistical Methods.* Oxford & IBH Pub. Co., New Delhi (Indian Edition, 1968) pp.331-400.

Table 1. Correlation between different traits of Indian mustard in five environments.

Characters		CHARACTERS									
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{11}
Days to 50% flowering X_1	E_1	0.206*	0.072	0.011	-0.014	-0.316**	0.600**	-0.435**	-0.069	-0.048	-0.080
	E_2	-0.020	0.008	0.164	0.072	-0.061	0.520**	-0.279**	-0.25*	-0.150	-0.086
	E_3	-0.235*	-0.306**	-0.315**	-0.355**	-0.233*	0.605**	-0.345**	-0.032	-0.144	-0.204
	E_4	0.204	0.168	0.205	0.091	0.066	0.275**	-0.169	-0.033	0.025	-0.035
	E_5	0.223*	0.109	0.219*	0.024	-0.077	0.627**	-0.430**	-0.201	-0.145	-0.124
Number of primary branches/ Plant X_2	E_1		0.384**	0.468**	0.347**	0.030	0.210*	-0.070	-0.183	-0.212*	0.145
	E_2		0.476**	0.385**	0.407**	0.167	-0.020	-0.003	0.053	-0.039	0.211*
	E_3		0.574**	0.391**	0.555**	0.101	-0.157	0.067	0.251*	0.018	0.152
	E_4		0.532**	0.396**	0.551**	0.178	0.063	-0.025	-0.258*	0.211*	0.188
	E_5		0.559**	0.433**	0.334**	0.043	0.220*	-0.029	0.052	-0.099	0.29**
Number of secondary Branches/ Plant X_3	E_1			0.450**	0.717**	0.027	0.064	0.045	0.026	-0.175	0.231*
	E_2			0.424**	0.793**	0.215*	0.204	0.008	0.197	-0.038	0.44**
	E_3			0.348**	0.579**	0.333**	0.255*	0.233*	0.227*	0.192	0.28**
	E_4			0.475**	0.705**	0.161	0.115	0.035	0.102	0.048	0.41**
	E_5			0.263*	0.588**	0.058	0.280**	0.098	0.151	-0.031	0.42**
Plant height (cm) X_4	E_1				0.429**	0.129	0.113	-0.020	-0.118	-0.280*	0.127
	E_2				0.583**	0.184	0.255*	-0.093	0.208*	-0.058	0.34**
	E_3				0.409**	0.177	0.488**	0.067	0.133	0.043	0.066
	E_4				0.586**	0.203	0.178	-0.031	0.122	0.005	0.32**
	E_5				0.446**	-0.097	0.136	-0.184	0.196	-0.017	0.248*
Number of siliquae/ Plant X_5	E_1					0.091	-0.098	-0.018	-0.129	-0.208*	0.195
	E_2					0.252*	0.307**	0.036	0.169	0.132	0.50**
	E_3					0.325**	-0.235*	0.176	0.133	0.175	0.46**
	E_4					0.303**	0.098	0.162	0.054	0.124	0.59**
	E_5					0.038	0.027	0.306**	0.222*	0.084	0.55**
Number of seeds/ Plant X_6	E_1						-0.261*	0.499**	-0.156	0.208*	0.023
	E_2						0.012	0.145	0.078	-0.084	0.227*
	E_3						-0.154	0.529**	0.116	0.060	0.166
	E_4						-0.095	0.470**	0.247*	0.079	0.29**
	E_5						-0.119	0.160	-0.011	0.226*	-0.036
Days to Maturity X_7	E_1							-0.457**	-0.136	-0.038	-0.236*
	E_2							-0.212*	0.061	0.017	0.144
	E_3							-0.162	0.136	-0.109	-0.093
	E_4							-0.167	0.005	0.189	0.098
	E_5							-0.319**	-0.145	-0.226*	0.005
Harvest Index X_8	E_1								0.164	0.209*	0.28**
	E_2								0.012	0.143	0.122
	E_3								0.415	0.015	0.33**
	E_4								0.266*	0.069	0.35**
	E_5								0.176	0.264*	0.30**
1000-seed weight X_9	E_1									0.024	-0.082
	E_2									0.077	0.191
	E_3									-0.087	0.120
	E_4									-0.230*	0.231*
	E_5									0.098	0.39**
Per cent oil Oil content X_{10}	E_1										-0.208*
	E_2										0.044
	E_3										-0.003
	E_4										-0.003
	E_5										-0.080

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

Table 2. Multiple regression and contribution of different traits in Indian mustard under five environments.

Multiple regression equation	Contribution (%)
$\hat{Y}_{E1}$ = 18.927** +0.033x_1 +0.089x_2 +0.121x_3 +0.000x_4 –0.001x_5 -0.188x_6 -0.069x_7 +12.997x_8** -0.707x_9 -0.230x_{10}	50.62
$\hat{Y}_{E2}$ = -0.835 -0.042x_1 -0.046x_2 +0.034x_3 +0.010x_4 +0.008x_5* +0.109x_6 +0.030x_7 +2.957x_8 +0.154x_9 –0.027x_{10}	54.56
$\hat{Y}_{E3}$ = 6.632 +0.002x_1 -0.250x_2 +0.041x_3 -0.010x_4 +0.010x_5** -0.128x_6 +0.000x_7 +9.431x_8** -0.167x_9 -0.091x_{10}	57.51
$\hat{Y}_{E4}$ = -1.929 -0.016x_1 -0.217x_2 +0.063x_3 -0.001x_4 +0.015x_5** +0.010x_6 +0.031x_7 +6.578x_8* +0.322x_9 -0.035x_{10}	67.87
$\hat{Y}_{E5}$ = 7.109 -0.077x_1 -0.042x_2 +0.109x_3 +0.010x_4 +0.018x_5** -0.095x_6 +0.049x_7 +15.859x_8 +1.942x_9**-0.405x_{10}	66.21
$\hat{Y}_{E1}$ = 18.988** +0.032x_1 +0.090x_2 +0.121x_3 -0.001x_5 -0.187x_6 -0.069x_7* +12.993x_8** –0.707x_9* –0.230x_{10}*	50.62
$\hat{Y}_{E2}$ = -1.037 -0.042x_1 +0.029x_3 +0.009x_4 +0.008x_5* +0.107x_6 +0.031x_7 +2.997x_8 +0.158x_9 –0.027x_{10}	54.53
$\hat{Y}_{E3}$ = 6.663 +0.003x_1 -0.249x_2 +0.041x_3 -0.010x_4 +0.010x_5** -0.128x_6 +9.427x_8* -0.166x_9 -0.091x_{10}	57.51
$\hat{Y}_{E4}$ = -1.966 -0.016x_1 -0.218x_2 +0.063x_3 +0.015x_5** +0.010x_6 +0.031x_7 +6.599x_8* +0.319x_9 -0.035x_{10}	67.87
$\hat{Y}_{E5}$ = 6.993 -0.078x_1 +0.106x_3 +0.010x_4 +0.018x_5** -0.096x_6 +0.049x_7 +15.903x_8 +1.948x_9**-0.403x_{10}*	66.20
$\hat{Y}_{E1}$ = 18.641** +0.032x_1 +0.087x_2 +0.108x_3 -0.190x_6 -0.067x_7* +13.151x_8** –0.693x_9* –0.228x_{10}*	50.58
$\hat{Y}_{E2}$ = -2.148 -0.040x_1 +0.035x_3 +0.010x_4 +0.008x_5* +0.112x_6 +0.030x_7 +2.874x_8 +0.153x_9	54.48
$\hat{Y}_{E3}$ = 6.912 -0.250x_2 +0.041x_3 -0.010x_4 +0.010x_5** -0.127x_6 +9.254x_8** -0.156x_9 -0.092x_{10}	57.49
$\hat{Y}_{E4}$ = -1.923 -0.015x_1 -0.214x_2 +0.062x_3 +0.015x_5** +0.030x_7 +6.746x_8* +0.330x_9 -0.034x_{10}	67.86
$\hat{Y}_{E5}$ = 8.007 -0.075x_1 +0.104x_3 +0.019x_5**–0.102x_6 +0.049x_7 +14.754x_8 +1.991x_9** -0.399x_{10}	66.14
$\hat{Y}_{E1}$ = 19.012** +0.034x_1 +0.121x_2* -0.185x_6 -0.065x_7 +13.254x_8** –0.728x_9* –0.239x_{10}*	50.32
$\hat{Y}_{E2}$ = -2.049 -0.040x_1 +0.009x_4 +0.009x_5** +0.113x_6 +0.029x_7 +2.936x_8 +0.167x_9	54.29
$\hat{Y}_{E3}$ = 6.427 -0.267x_2 +0.039x_3 -0.010x_4 +0.010x_5** -0.120x_6 +8.557x_8** -0.087x_{10}	57.30
$\hat{Y}_{E4}$ = -3.396 -0.016x_1 -0.222x_2 +0.064x_3 +0.015x_5** +0.032x_7 +6.658x_8* +0.356x_9	67.79
$\hat{Y}_{E5}$ = 7.300 –0.076x_1 +0.100x_3 +0.019x_5**+0.053x_7 +14.269x_8 +2.010x_9**–0.420x_{10}*	66.02
$\hat{Y}_{E1}$ = 18.906** +0.126x_2 -0.201x_6 -0.047x_7* +12.502x_8** –0.725x_9* –0.233x_{10}*	49.23
$\hat{Y}_{E2}$ = -1.825 -0.049x_1 +0.011x_4 +0.009x_5** +0.115x_6 +0.034x_7 +2.846x_8	53.99
$\hat{Y}_{E3}$ = 5.767 -0.209x_2 -0.010x_4 +0.010x_5** -0.110x_6 +8.745x_8** -0.077x_{10}	56.88
$\hat{Y}_{E4}$ = -3.775 -0.240x_2 +0.060x_3 +0.015x_5** +0.027x_7 +6.845x_8* +0.352x_9	67.58
$\hat{Y}_{E5}$ = 12.113 –0.047x_1 +0.118x_3 +0.019x_5**+14.076x_8 +1.993x_9**–0.438x_{10}*	65.84
$\hat{Y}_{E1}$ = 13.681* +0.115x_2 -0.187x_6 +14.918x_8** –0.674x_9 –0.250x_{10}*	47.01
$\hat{Y}_{E2}$ = -0.840 –0.046x_1 +0.011x_5** +0.122x_6 +0.035x_7 +2.554x_8	53.52
$\hat{Y}_{E3}$ = 2.744* -0.193x_2 –0.010x_4 +0.010x_5** -0.110x_6 +8.801x_8*	56.12
$\hat{Y}_{E4}$ = –4.006 –0.196x_2 +0.016x_5** +0.027x_7 +6.612x_8* +0.411x_9	67.24
$\hat{Y}_{E5}$ = 8.854 +0.113x_3 +0.018x_5** +16.876x_8 +2.073x_9** –0.435x_{10}*	65.62
$\hat{Y}_{E1}$ = 12.687* +0.111x_2 +11.742x_8** –0.509x_9 –0.272 x_{10}*	44.58
$\hat{Y}_{E2}$ = 0.161 -0.051x_1 +0.011x_5** +0.134x_6 +0.032x_7	53.07
$\hat{Y}_{E3}$ = 1.914 -0.235x_2 +0.009x_5** -0.117x_6 +8.925x_8*	55.25
$\hat{Y}_{E4}$ = -0.939 -0.193x_2 +0.017x_5** +6.155x_8* +0.446x_9	66.82
$\hat{Y}_{E5}$ = 10.084 +0.022x_5** +15.816x_8 +2.110x_9** -0.457x_{10}	64.71
$\hat{Y}_{E1}$ = 11.288 +0.110x_2 +10.970x_8* –0.271x_{10}*	42.47
$\hat{Y}_{E2}$ = 2.729 -0.038x_1 +0.011x_5** +0.131x_6	52.66
$\hat{Y}_{E3}$ = 1.169 +0.008x_5** -0.107x_6 +8.862x_8*	53.81
$\hat{Y}_{E4}$ = -1.840 +0.015x_5** +6.335x_8* +0.565x_9	66.21
$\hat{Y}_{E5}$ = 8.878 +0.024x_5** +2.226x_9* –0.366x_{10}	63.09
$\hat{Y}_{E1}$ = 13.235* +11.472x_8* –0.308x_{10}*	38.92
$\hat{Y}_{E2}$ = 4.245 -0.040x_1 +0.012x_5**	51.82
$\hat{Y}_{E3}$ = 0.523 +0.007x_5** +6.838x_8*	52.27
$\hat{Y}_{E4}$ = -0.434 +0.015x_5** +7.283x_9*	64.57
$\hat{Y}_{E5}$ = –4.791 +0.024x_5** +2.143x_{10}*	61.37
$\hat{Y}_{E2}$ = 2.213 **+0.012x_5**	50.36

$\hat{Y}$=Expected seed yield, E_n=Environments. *, Significant at 5 and 1% probability levels, respectively. Character 1. Days to 50% flowering, 2. Number of primary branches / plant, 3. Number of secondary branches / plant, 4. Plant height, 5. Number of siliquae / plant, 6. Number of seeds / siliqua, 7. Days to maturity, 8. Harvest index 9. 1000-Seeds weight, 10. Seed yield / plant 11. Oil content in percent**

Study of specific combining ability and reciprocal effects for earliness in toria

D.P. Pant, Pankaj Sharma, S. P. Singh and R. A. Khan

Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture & Technology Pantnagar-263 145, Distt.-U.S. Nagar (Uttaranchal)

Earliness in toria is a highly desirable trait to fit this crop in multiple cropping system, as it is usually taken as a catch crop between *Kharif* and *Rabi* crops. The combining ability and reciprocal effects for days to flowering and days to maturity were worked out in a 8 × 8 diallel mating system, following **Griffing (1956)** Method1 and Model I. Mean squares due to general combining ability (gca), specific combining ability (sca) and reciprocal effects were significant for both the traits, indicating importance of additive, non-additive and reciprocal effects. However, there was preponderance of non-additive gene-effects.

Parent PT 9600 had the maximum negative estimates of gca for both the traits. For days to flowering, out of nine significant F_1s, four were with negative sca effects. The cross PT 9600 × T 9 emerged as the best combination for earliness in flowering followed by PT 9700 × PT 9701 and PT 9700 × PT 507 whereas, out of 8 significant reciprocal F_1s (RF_1s) five were with negative estimates. The best RF_1 was PT 30 × PT 9700, followed by MSP 9212 × PT 9600 and PT 9701 × PT 9600.

For days to maturity out of 12 significant F_1s four expressed negative sca effects. The best F_1 was PT 9600 × PT 507, followed by MSP 9212 × PT 30 and PT 9600 × PT 30. In case of reciprocal crosses four out of six significant RF_1s were with negative estimates. The RF_1 PT 30 × PT 9700 was the best, followed by PT 30 × MSP 9212 and PT 9700 × PT 9600.

The crosses, involving PT 9600 as one of the parents, is expected to throw transgressive segregants in the later generations. It was interesting to note that significant crosses for both the traits involving PT 9600 as female parent threw higher negative estimates as compared to their reciprocals. The reciprocal crosses involving PT 9600 as male parent also gave desired reciprocal effects but the estimates were low. Similarly the crosses, involving PT 30 as female parent also gave more negative estimates as compared to its involvement as male parent.

The overall results suggest that PT 9600 and PT 30 should be used as female parent on priority for induction of earliness in the crosses. PT 9700 and MSP 9212 must also be tested further for their contribution in earliness. The study also suggests that reciprocal crosses may also be given weightage while testing the contribution of genotypes for earliness.

Reference:

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Aust. J. Biol. Sci.* 9 : 463-493.

Table 1: Combining ability analysis for earliness in toria

Source	Degree of freedom	Mean sum of squares	
		Days to flowering	Days to maturity
gca	7	2.813**	2.294*
sca	28	3.090**	4.919**
reciprocal effect	28	2.494**	2.522**
error	126	0.574	1.106

*,** significant at 5% and 1% probability levels, respectively

Table 2: General combining ability (diagonal) of 8 parents and specific combining ability and reciprocal effects for days to flowering and days to maturity

Parents	PT 9600	MSP 9212	PT 9700	PT 9701	PT 9719	PT 507	PT 30	T 9
PT 9600	-0.843** (-0.635**)	0.844 (1.614**)	0.615 (0.802)	1.761** (3.865**)	-0.635 (-1.777**)	-0.448 (-2.593**)	-1.052* (-2.014**)	-1.989** (0.468)
MSP 9212	-1.500** (0.333)	0.052 (-0.281)	0552 (0343)	-0.635 (0.989)	-0.698 (-0.197)	-0.344 (0.385)	-0.948* (-2.198**)	1.115* (1.781**)
PT 9700	-1.000 (-1.500*)	0.500 (-0.667)	-0.219 (-0.031)	-1.531** (-1.073)	0.406 (0.781)	-1.406** (-0.364)	1.323** (1.552*)	0.385 (-0.343)
PT 9701	-1.333* (-0.667)	1.500** (1.500*)	-0.333 (1.001)	0.302 (-0.135)	0.552 (-0.282)	-0.760 (-0.698)	1.969** (0.719)	0.365 (-0.031)
PT 9719	-0.167 (1.500*)	-1.000 (0.500)	-0.500 (0.500)	-0.167 (-0.167)	0.531** (0.010)	3.177** (2.760**)	-.927 (0.489)	-0.698 (1.884**)
PT 507	-0.167 (0.333)	0.833 (-0.667)	-0.167 (0.167)	0.333 (-0.667)	-1.500** (-1.331)	-0.010 (0.593*)	0.927 (1.427*)	0.323 (05.73)
PT 30	-0.167 (-0.167)	2.500** (-1.667*)	-2.167** (-3.001**)	0.000 (0.667)	0.000 (1.000)	-1.333* (-1.167)	0.281 (-0.156)	0.719 (1.323*)
T 9	0.833 (-0.833)	-0.500 (1.500*)	-0.500 (1.333)	2.667** (-0.833)	-0.833 (0.833)	-0.333 (-1.150)	1.000 (-0.500)	-0.114 (0.365)
SE (s_{ij})	0.473 (0.658)							
SE (r_{ij})	0.539 (0.744)							
SE (s_{ij})	0.177 (0.246)							

! figures in parentheses are for days to maturity

!! specific combining ability for F_1s are above the diagonal and for RF_1s are below the diagonal

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

HETEROSIS VIS-A-VIS GCA OF PARENTS IN TORIA (*Brassica campestris* L.)

Pankaj Sharma, D. P. Pant, S. P. Singh, Chanchal Sah and Ritesh Sharma
Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture & Technology Pantnagar-263 145, Distt.-U. S. Nagar (Uttaranchal), India

Toria (*Brassica campestris* L.) is an important oilseed crop and usually grown as a catch crop in northern India, while in eastern part it is grown as winter crop. In past few decades, heterosis breeding has been proved its utility in boosting up the production and productivity of many crops. It is reported that heterosis in F_1 is related to general combining ability (gca) of parents. In the present study, heterosis in relation to gca of parents was studied for seed yield, oil content and protein content.

Eight genotypes of toria viz., PT 9600, MSP 9212, PT 9700, PT 9701, PT 9719, PT 507, PT 30 and T 9 were crossed in all possible combinations (including reciprocals) and 28 F_1s and 28 RF_1s (reciprocal F_1s) were obtained. These F_1s and RF_1s alongwith the parents were grown in randomized block design with three replications in one row plot of five metre length at spacing of 30 cm and 10 cm between and within rows, respectively. The data were recorded on five randomly selected plants for the traits studied. Combining ability analysis was done following **Griffing's (1956)** Method 1 and Model I. Heterosis was measured over mid parent (MP) and respective better parent (BP) as per **Fonseca and Patterson (1968)**.

For seed yield out of 28 F_1s and 28 RF_1s, 24 F_1s and 21 RF_1s showed significant positive heterosis over MP and 16 F_1s and 21 RF_1s over BP. Of these heterotic crosses 50% F_1s and 66.7% RF_1s belonged to low x low gca classes, followed by low x high (25.0%) for F_1s and high x low (23.8%) for RF_1s. For oil content 6F_1s and 7 RF_1s displayed heterosis over MP and 3 F_1s and 2 RF_1s showed heterosis over BP. Out of these heterotic combinations, all the F_1s fall in high x low, low x high and low x low gca classes equally (33.33 % each) for both heterosis over MP and over BP. The RF_1s mostly (57.1%) belonged to low x low gca class for heterosis over MP, followed by low x high gca class (28.6%), while for heterosis over BP, all the RF_1s (100%) fall into high x low gca class. It was interesting to note that neither for seed yield nor for oil content F_1s and RF_1s fetch high x high gca class except for one of the F_1 combinations i.e. PT 9701 x PT 30 for seed yield, showing heterosis over MP. The protein content showed positive significant heterosis over MP for 10 F_1s and 12 RF_1s, out of 10 F_1s 50% belonged to low x high gca class followed by low x low and high x high gca classes (20% each) and out of 12 RF_1s, 5 RF_1s (41.7%) fall in high x low gca class followed by low x low gca class (25.0%). The heterosis over BP for protein content consumed low x high gca class (75%), followed by high x high (25%) in case of F_1s, while for RF_1s low x low and low x high (50% each) were important. These findings suggest that parent with low gca effect may throw high heterosis for seed yield and oil content, while for protein content parents with differing gca status are more important in the choice of parents for heterosis breeding.

References:

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Aust. J. Biol. Sci.* 9: 463-493

Fonseca, S. and Patterson, F. (1968). Hybrid vigour in a seven parent diallel cross in common wheat. *Crop Science*. 8, 1:85-88

Table 1:Frequency of heterotic crosses in different parental gca classes in toria

Traits	Parental gca classes										Range of heterosis
	Number and percentage for heterotic F_1crosses					Number and percentage for heterotic RF_1crosses					
	HH	HL	LH	LL	Total	HH	HL	LH	LL	Total	
Seed yield per plant											
Heterosis over MP	1 (4.2)	5 (20.8)	6 (25.0)	12 (50.0)	24	-	5 (23.8)	2 (9.5)	14 (66.7)	21	-41.4 - 248.7
Heterosis over BP	-	1 (6.3)	5 (31.3)	10 (62.5)	16	-	6 (28.6)	2 (9.5)	13 (61.9)	21	-56.4 - 223.1
Best cross combination	PT 9600 x PT 30 (240.4%) - over MP PT 9600 x PT 30 (223.1%) - over BP					PT 507 x PT 9600 (248.7%) - over MP PT 507 x PT 9600 (217.2%) - over BP					
Oil Content											
Heterosis over MP	-	2 (33.3)	2 (33.3)	2 (33.3)	6	-	1 (14.3)	2 (28.6)	4 (57.1)	7	-4.4 - 3.9
Heterosis over BP	-	1 (33.3)	1 (33.3)	1 (33.3)	3	-	2 (100.0)	-	-	2	-5.2 - 3.4
Best cross combination	PT 9701 x PT 30(3.9%) - over MP PT 9701 x PT 30(3.4%) - over BP					T 9 x PT 9600 (3.5%) - over MP PT 9701 x MSP 9212 (2.1%) - over BP					
Protein content											
Heterosis over MP	2 (20.0)	1 (10.0)	5 (50.0)	2 (20.0)	10	2 (16.7)	5 (41.7)	2 (16.7)	2 (25.0)	12	-19.5 - 15.4
Heterosis over BP	1 (25.0)	-	3 (75.0)	-	4	-	2 (50.0)	-	2 (50.0)	4	-21.6 - 10.1
Best cross combination	PT 507 x PT 9719 (15.4%) - over MP PT 9719 x PT 507 (5.5%) - over BP					PT 9719 x PT 9600 (12.3%) - over MP PT 507 x PT 9719 (10.1%) - over BP					

* Figures in parentheses are for the percentage of the crosses in different parental gca classes

Predict Double Low Hybrid Crosses Heterosis by Genetic Method in *B.napus*

Zhang Shufen Tian Baoming Wang Jianping Wen Yancheng Zhu Jiacheng Ren Lejian
(Cotton and Oil Crops Institute, Henan Academy of Agricultural Sciences, Zhengzhou, 450002, China)

Introduction

There is significant positive heterosis for cytoplasmic male sterile (CMS) hybrids in rapeseed, but there are large differences among crosses. The key is how to make out strong heterosis crosses, and test them quickly, shorten the breeding period.Because agronomic traits are affected by environment. It is required several years for screening and testing a strong heterosis cross by traditional breeding method, the breeding period is long. On the other hand, results analysis by phenotype value can't express total genotype value of hybrid crosses. So, only when we analyse the genotype value directly, we can exclude the influence of environment. The results are correct.

ADM model was adopted according to mixed liner model (Zhu jun, 1993). We calculated the heterosis of F_1, predicted the heterosis of F_2 based on F_1 and their parents. We can judge crosses and their potential utilization directly by genotype value. It can increase the foresight of our breeding. It can not only save land and labor but also accelerate the pace of breeding.

Materials and Methods

Materials

Three double low CMS lines in B.napus A_1(252A), A_2(217A) and A_3(384A), five double low restorers of R_1(PD121), R_2(PD036), R_3(CP015), R_4(CP019)and R_5(PD250) all come from Henan Academy of Agricultural Sciences. The CMS lines were used as female, the restorers were used as male. Fifteen hybrid crosses were obtained from them. Cross 1, 2……15 mean $A_1 \times R_1$, $A_1 \times R_2 \cdots A_3 \times R_5$.

Methods

The field experiment was in random block design with 3 replications, 5 rows per plot. Ten plants were sampled for investigate agronomic traits per plot.

The predicted formulas of genotype value are in references 4:

Results and analysis

Predicted results of genotype value and heterosis in main traits

Table 1 Predicted results of genotype value and heterosis in main traits

Items	Plant height	1st branch numbers	2nd branch numbers	Total pods numbers	Seeds number per pod	Yield of per plant	1000-seed weight	Plot yield
Pre(F_1)	207.6	8.9	2.3	307.9	22.1	22.6	2.7	0.915
Pre(F_2)	193.4	8.4	2.4	332.3	20.0	18.2	2.8	0.735
Pre(F_2)-Pre(F_1)(%)	-6.9	-5.6	14.3	-19.7	-9.5	-24.2	-25.0	-18.0
Hpm(F_1)	14.7	11.1	33.1	37.3	11.2	46.0	5.3	45.4
Hpb(F_1)	10.7	2.0	14.0	28.5	10.3	37.2	-8.0	30.5
Hpb(F_2)	3.3	-3.5	-2.6	9.9	9.7	14.2	-3.5	7.7
Hpb(F_2)-Hpb(F_1)(%)	-7.4	-5.2	-16.6	-18.6	-6.2	-23.0	4.5	46.5

Note: Pre(F_1)、Pre(F_2) mean F_1、F_2 Predicted genotype value.

Table 1 indicated that, genotype values of F_1 were very high. On the opposite, the genotype values of F_2 decreased, they dropped down from 5.6% to 25.0%, in which 1st branches number and plant height decreased slightly, they were 5.6% and 6.9%, respectively; The genotype values of 1000-seed weight, yield per plant, total pods numbers per plant, plot yield, 2nd branches number etc. decreased sharply, they decreased from 25.0% to 14.3%.

The table 1 showed that, middle parent heterosis of F_1 were high, from positive 5.3% to 46.0%, in which the heterosis of yield was the highest, the heterosis of yield per plant accorded with plot yield, they were 46.0% and 54.4%, respectively, the Hpm(F_1) of total pods numbers per plant was higher (37.3%). Hpm(F_1) of 1000-seed weight was lowest (5.4%), the others were moderate.

Above better parent heterosis accorded with middle parent heterosis. Hpb(F_1) of 1000-

seed weight was the lowest (-8.0%), Hpb(F_1) of yield per plant, and plot yield were high, they were 37.2% and 30.5%, respectively.

Hpb(F_2) were sharply down, they dropped from 5.5% to 46.5%. This is the reason why we shouldn't use the seeds of F_2.

Heterosis of crosses in yield traits

Table 2 showed that, the heterosis of cross 13 ($A_3 \times R_3$) in yield per plant was the highest one, Hpm(F_1), Hpb(F_1) and Hpb(F_2) were 93.6% , 92.6%, and 61.6%, respectively. The heterosis of cross 9 ($A_2 \times R_4$) was the higher, its Hpm(F_1), Hpb(F_1) and Hpb(F_2) were 77.1%, 76.9%, and 38.3%, respectively. The heterosis of crosses in plot yield had the same tendency.

The heterosis of cross 13($A_3 \times R_3$) in total pods numbers was the highest, its Hpm(F_1), Hpb(F_1) and Hpb(F_2) were 76.2%, 73.9% and 35.7%, respectively. The cross 9 ($A_3 \times A_4$) was near to cross 13.

Table 2 Crosses heterosis in main yield traits

Crosses		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Total	Hpm(F_1)	-5.9	3.0	46.5	33.3	8.5	23.7	51.2	40.1	76.1	49.5	46.3	32.0	76.2	31.8	48.5
pods	Hpb(F_1)	-6.3	2.3	34.2	23.1	-0.3	3.1	33.4	34.6	72.7	41.6	27.2	17.8	73.9	28.3	42.0
number	Hpb(F_2)	-3.4	0.8	10.9	6.5	-4.5	-8.7	8.6	14.6	34.6	16.9	4.00	1.8	35.7	12.3	17.7
Yield	Hpm(F_1)	22.3	1.0	70.3	60.2	32.5	1.6	28.6	43.6	77.1	48.6	-1.80	13.7	93.6	15.6	41.9
per	Hpb(F_1)	5.8	-17.2	70.0	60.0	21.3	23.2	8.9	43.6	76.9	34.3	-9.9	-2.20	92.9	65.5	33.9
plant	Hpb(F_2)	-5.3	-17.7	4.9	29.9	5.0	2.4	-5.4	21.8	38.3	10.0	-9.0	-9.10	61.1	30.9	12.9
1000-	Hpm(F_1)	-9.0	-17.2	13.1	12.3	-5.0	8.3	9.8	15.6	25.1	18.9	-9.00	-15.6	13.8	14.7	3.60
Seed	Hpb(F_1)	-14.4	-35.5	12.9	6.1	-11.0	7.4	-11.4	15.4	21.8	16.6	-14.0	-21.1	3.40	12.5	-0.90
weight	Hpb(F_2)	-9.9	-26.9	6.5	-0.1	-8.5	3.2	-16.4	7.6	9.30	7.20	-9.50	-13.4	-3.40	5.00	-2.70
	Hpm(F_1)	18.7	20.5	71.5	48.3	55.9	11.9	13.1	73.1	79.3	73.6	-18.3	-18.4	79.6	67.7	55.8
Plot	Hpb(F_1)	-12.2	-10.6	69.5	43.6	54.9	-19.0	-17.6	70.3	74.3	65.4	-40.3	-40.2	75.5	57.5	42.3
yield	Hpb(F_2)	-22.2	-20.9	33.7	19.5	32.9	-25.1	-24.1	38.8	35.1	28.6	-31.1	31.0	29.2	23.9	14.4

The heterosis of 1000-seed weight was lower than others. The heterosis of cross 9 ($A_2 \times R_4$) was the highest, its Hpm(F_1), Hpb(F_1) and Hpb(F_2) were 25.1%, 21.8% and 9.3%, respectively. Then was cross 10($A_2 \times R_5$). The others was low. The heterosis of 7 crosses such as cross 1($A_1 \times R_1$), 2($A_1 \times R_2$) etc were negative.

Discussions

From predicted heterosis of main traits in the paper, we found that, the CMS hybrid F_1 had significant heterosis ,but the heterosis of F_2 was sharply down. So, in general, we shouldn't use F_2 in production. However,only a few crosses such as cross 13($A_3 \times R_3$) also had significant heterosis in F_2, if there are not enough seeds, we should consider to use the F_2 replace theF_1.

Heterosis analysis showed that the heterosis of total pods number per plant in three major yield components was the highest, seeds number per pod and 1000-seed weight were relatively stable, their heterosis were not high, the variation between crosses were small. So we should focus more attention on increase the number of total pods per plant, not increase 1000-seed weight and seeds per pod in rapeseed breeding.

References:

Fu Tingdong. The study advance on cytoplasmic male sterility of rapeseed. Study on Agricultural and Livestock Husbandry, 1989, (1):1-8

Fu Tingdong. The study survey on utilization of rapeseed heterosis in China. Crop Research, 1990, 4(3):1-4

Zhu Jun, Ji Daofan, Xu Fuhua. A genetic appproach for analyzing intra-cultivar heterosis in crops. Acta Genetica Sinica, 1993, 20 (3): 262-271

Zhu Jun. Methods of predicting genotype value and heterosis for offspring hybrids. J.Bio.Math, 1993, 8(1): 32-44.

A quick and efficient method for blackleg disease resistance selection at the seedling stage of greenhouse-grown doubled haploid lines of *Brassica napus* L.

V.K. Bansal, L. Corrigan, G.R. Stringam and M.R. Thiagarajah

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Abstract
Using the proposed greenhouse screening protocol several thousand doubled haploid lines could be screened and rated for blackleg disease at the seedling stage. The DH lines classified as resistant or resistant? were also rated as resistant in the field screening experiments. Depending on the disease resistance source used to improve blackleg resistance, cotyledon bioassay can be a quick and efficient tool in the disease resistance breeding against blackleg disease.

Introduction
During the course of our canola breeding program for developing blackleg resistant cultivars we produce thousands of doubled haploid (DH) lines every year. To test these DH lines for blackleg disease resistance we have developed and used a greenhouse screening protocol. The results are presented for the DH lines tested during the years of 1999 and 2000.

Materials and Methods
In each screening, a set 61 DH lines were planted together with Quantum and Profit as resistant and susceptible controls respectively. Sixteen plants of each DH line and resistant control, and thirty two plants of the susceptible control were grown in pairs in plastic pots (6x6 cm) containing soil free growth medium (Stringam, 1971).

Growing conditions in the greenhouse were maintained at approximately 21^0 C and 16 h photoperiod supplemented with 400W, high pressure sodium lamps. Five isolates of *Leptosphaeria maculans* were collected from Alberta, Saskatchewan, and Manitoba. Single spore cultures of these isolates were maintained on V8 agar media supplemented with rose-bengal (0.05 g/L) and $CaCO_3$ (3.0 g/L). The isolates were incubated at room temperature under a 12 h photoperiod.

Pycnidiospore suspension mixture was prepared in sterile distilled water from 12 to 14-day-old growing cultures and the spore concentration adjusted to $1x10^6$ spores per ml. Six days after sowing, seedlings were divided into groups of eight plants and were placed in a completely randomized design. Inoculations were made by placing 10 µl of the spore suspension dispensed from an Eppendorf-micropipette on a wound made on each cotyledon leaf by a No. 1 entomological needle. Inoculated seedlings were incubated in a dark mist chamber at 100% RH for 2 days. From the mist chamber, seedlings were transferred onto greenhouse benches during the months of September to March, and from April to August, seedlings were grown in a growth-cabinet where growing conditions similar to the greenhouse were maintained.

The disease reactions on cotyledonary leaves were recorded 10 days after inoculation on a 0 (resistant) to 4 (susceptible) scale (Bansal et al., 1994). The DH lines were rated resistant (r), resistant? (r?) or susceptible (s) based on the calculated mean disease severity (MDS) values. The MDS values were calculated using the formula: disease severity = Σ (no. of plants in a disease scale category x diseases scale category)/(total no. of plants). The DH lines were classified as r, r? or s based on the MDS rating of resistant control Quantum. DH lines with $\leq$ Quantum MDS value were classified as "r", DH lines with > 2.1 MDS were classified as "s", and the remaining DH lines were classified as "r?".

Results and Discussion

The % of DH lines tested and classified into different classes is given in Table 1. In general, lines classified as Resistant or Resistant? based on visual observation were also r or r? based on MDS, except for 12 lines during the year 1999. Susceptible and Susceptible? lines based on visual observations always turned out to be susceptible based on MDS. Using this system we were able to screen two sets per week during winter months when seedlings after inoculation were placed in the greenhouse, and one and a half sets during summer months when seedlings after inoculation were placed in the growth cabinets. The green house selected lines were also found to be resistant under artificial inoculated conditions in the field screening experiments.

References

Bansal, V.K., Kharbanda, P.D., Stringam, G.R., Thiagarajah, M.R., and Tewari, J.P. 1994. A comparison of greenhouse and field screening methods for blackleg resistance in doubled haploid lines of *Brassica napus*. Plant Dis. 78: 276-281.

Stringam, G.R. 1971. Genetics of four hypocotyl mutants in *Brassica campestris* L. J. Heredity 62: 248-250.

Table: 1. The % DH lines screened for blackleg disease in the greenhouse over two years

Mean disease severity	**Year 1999** % DH lines screened	**Year 2000** % DH lines screened
Resistant (r)	42.5	63.1
Resistant? (r?)	24.4	25.2
Susceptible (s)	30.0	11.0
NG[1]	03.2	00.01
Total	100	100

[1]NG = no germination.

Distribution of A and B-group isolates of *Leptosphaeria maculans* in oilseed rape plants

Brun H.[1], Eber F.[2] and Jaouen S.[1]
Unité Mixte de Recherche INRA/ENSAR [1][BiO3P], [2]APBV, BP 35327, F-35653 Le Rheu Cedex, France.

Introduction

Leptosphaeria maculans is responsible for Blackleg of oilseed rape worldwide. *L. maculans* was divided at least in two main groups of isolates named A-group (responsible for crown canker) and B-group (responsible for weak symptoms and pith degradation) by Johnson & Lewis (1990, 1994). This latter group was identified as a new species : *Leptosphaeria biglobosa* (Shoemaker & Brun, 2001)
Both groups induce leaf lesions (Johnson & Lewis, 1994) and can coexist in a single leaf lesion (Brun *et al.*, 1997). In some situations varieties seem to differ at the leaf level for their susceptibility to each group (Brun *et al.*, 1997). This work aimed to assess which groups of isolates were present at the crown and stem base levels in the internal symptoms of winter oilseed rape cultivars at the end of the cropping season, in France.

Materials and methods

Plants were sampled 3 weeks before harvesting in June 1999 in two geographic locations for cv. Vivol (Le Rheu, Brittany) and La Minière (South of Paris). Cultivar Darmor was only sampled from the latter location. Fifty plants of 'Vivol' and 20 plants of 'Darmor' were analyzed. Plants were cut longitudinally and small pieces of damaged tissues were taken (1) directly within the crown, (2) on the stem until 3 cm upper the crown and (3) on the stem between 3 and 6 cm upper the crown. Plant pieces were sterilized in alcohol (90%) during 15 sec., rinsed 3 times in sterile water, dried between 2 sheets of sterile filter paper and deposited on malt (2%) agar (2%) medium supplemented with streptomycin sulfate (0.01%). A- and B- group isolates were differentiated by Phospho Glucose Isomerase markers and pigment production (Brun *et al.*, 1997).

Results

In total 147 isolates were obtained and they were divided into 111 A-group isolates and 36 B-group isolates. A-group isolates were always present in crown canker and B-group isolates were never found alone at this site (Table 1).
The frequency of A-group isolates decreased with the distance from the crown to the top of the plant when B-group isolates increased. It was possible to detect both groups of isolates in mixture from single damaged tissues whatever the site.
Independently of the site, the relative frequency of A and B-group isolates was similar on both cultivars (Table 2) and not statistically different (P=0.08). Nevertheless, the sample size being different, latter results should be taken with caution.

Discussion

Our results demonstrated that A-group isolates were always found in damaged tissues of the plant at the crown level for both varieties 'Vivol' and 'Darmor'and B-group isolates were not found alone at this site. Thürwächter *et al.* (1999) found more A-group isolates within single ascospores obtained from the crown region of stubble than those obtained from the upper parts of the stem. In the same way, we recovered from five distant regions in France more than one thousand single ascospore isolates from pseudothecia strictly

present at the crown level or just below of cv. 'Bristol'. All the isolates only belonged to A group isolates (Brun, unpublished data).
These results obtained from damaged tissues at the harvesting fit to the findings of Johnson & Lewis (1994) that A-group isolates were mainly responsible for crown canker.

Table 1. Frequency (%) of plants in which A or B group isolates of *Leptosphaeria maculans* were detected on malt agar medium according to the localization of symptoms along the plant (crown, stem basis, stem) independently of cultivars ('Vivol' and 'Darmor').

	Frequency (%) of A and B group isolates localized at each site of plant			
Site of sampling disease tissues	A group isolates alone	B group isolates alone	A and B group isolates associated	Total number of plants taking in *L. maculans* isolates[1]
Crown	75.8	0	24.1	58
Stem basis (from crown until 3 cm upper crown)	75	8.3	16.7	48
Stem (from 3 to 6 cm upper crown)	37.5	43.7	18.7	16

[1] Isolations were performed from the same initial sample of plants. As all plants did not display disease symptoms at all levels, plants were less frequent with stem symptoms inside the stem.

Tableau 2. Relative frequency (%) of A and B group isolates of *Leptosphaeria maculans* detected alone or in mixture, independently of the site of symptoms on plant, according to the cvs. Vivol and Darmor.

Cultivar	A-group isolates	B-group isolates
'Vivol'	78.5 (84)*	21.5 (23)
'Darmor'	67.5 (27)	32.5 (13)

* ( ) Number of isolates

References

Brun, H., Levivier, S., Eber, F., Renard, M., and Chèvre, A. M. 1997. Electrophoretic analysis of natural populations of *Leptosphaeria maculans* directly from leaf lesions. Plant Pathol. **46**:147-154.

Johnson, R. D., and Lewis, B. G. 1990. DNA polymorphism in *Leptosphaeria maculans.* Physiol. Molec. Plant Pathol. **37**:417-424.

Johnson, R. D., and Lewis, B. G. 1994. Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans.* Physiol. Molec. Plant Pathol. **43**:269-277.

Shoemaker R.A. and Brun H. 2001 The teleomorph of the weakly aggressive isolates segregate of *Leptosphaeria maculans*. Canadian Journal of Botany**79**:412-419.

Thürwächter F., Garbe V. and H.H. Hoppe. 1999. Ascospore discharge, leaf infestation and variations in pathogenicity as criteria to predict impact of *Leptosphaeria maculans* on oilseed rape. J. Phytopathology **147:**215-222.

A source of white rust resistance in Indian mustard derived from *Brassica carinata*

S. K. Chauhan and R. N. Raut

Division of Genetics, Indian Agricultural Research Institute, New Delhi -110012

White rust caused by the fungus *Albugo candida* (Pers. ex Lev.) Kuntze, has recently become the most wide-spread and destructive disease of Indian mustard (*Brassica juncea* (L.) Czern. & Coss.). Saharan *et al* (1984) reported yield losses up to 50 percent depending upon the intensity of staghead formation in late sown condition in Indian mustard. Most of the available cultivars are susceptible and chemical control has not been much successful. Therefore, genetic resistance is the most practical, economical and environment friendly method to overcome this disease and reducing yield losses. The present study reports the nature and mode of inheritance of white rust resistance in strain S-III a stable resistant strain of *B. juncea* deriving resistance from *Brassica carinata* and its test of allelism with resistance derived from *Brassica napus*.

All the white rust resistant and susceptible strains used in the study, were developed at the Division of Genetics, IARI, New Delhi. The resistant strain S-III was developed from a cross EC 287711 x AB-5, where EC 287711 is an exotic introduction for low erucic acid and AB-5 is white rust resistant strain developed from interspecific hybridization between *B. juncea* and *B. carinata* and stabilized as *B. juncea*. Two other white rust resistant strains, S-II and S-IV(R) (deriving resistance from *B. napus* and stablized as *B. juncea*), and reported to carry monogenic dominant resistance (S.K. Chauhan, 1998) were developed from the crosses Varuna x WR 16-3-1 and EC 287711 x WR 16-3-6 respectively. Susceptible strain S-X(S) is a line from cross Varuna x PCR04.

Crosses were attempted between resistant strain S-III and susceptible strain S-X(S) and two *B. napus* derived resistant strains S-II and S-IV(R) at IARI, New Delhi. Backcrosses were given on F_1 of cross S-III x S-X(S) using both the parents. Parents, F_1, F_2, B_1 and B_2 generations of these crosses were tested at IARI, New Delhi under artificial epiphytotic conditions. F_2 of the three crosses were also tested under natural epiphytotics at two hotspots in off-season nurseries at Wellington (T.N.), South India and Kukumseri (H.P.), North India. Observations for white rust were recorded using 0-5 disease scoring scale at vegetative and full podding stage. Data were subjected to chi-square analysis.

The data on P_1, P_2, F_1, F_2, B_1 and B_2 of these crosses are reported in Table 1. In the cross S-III x S-X(S), the F_1 plants were susceptible indicating the recessive nature of resistance to white rust. The F_2 plants of the cross showed segregation in 1R: 3S ratio with high degree of confidence level at all the locations. Heterogeneity χ^2 for all the three locations was significant suggesting that there is no effect of location on expression of resistance gene. This monogenic recessive nature of the resistance gene to white rust in this strain was confirmed when backcross population were tested. All the B_2 [backcross with susceptible parent S-X(S)] plants were susceptible. The backcross generation with resistant parent (B_1) segregated in 1R: 1S ratio confirming the single recessive gene for resistance in strain S-III.

The F_2s of crosses of strain S-III with *B. napus* derived resistant strains S-II and S-IV(R) showed segregation in 13R: 3S ratio with high degree of confidence levels at all the locations. Significant heterogeneity χ^2 values showed no influence of location on expression of resistance gene. The digenic 13R : 3S ratio suggested the interaction of one dominant and a recessive gene. The monogenic recessive nature of resistance in strain S-III, deriving resistance from

B. carinata, was further corroborated by the fact that strains S-II & S-IV(R) carries single dominant gene for white rust resistance. It is for the first time that recessive control of resistance to white rust in Indian mustard was discovered. This recessive gene for resistance to *Albugo candida* is tentatively designated as "ac-carinata". Although there are several examples of recessive genes for resistance in other crops viz. Stem rust resistance gene *Sr 8b* in wheat Singh and Mc Intosh, 1986), Bacterial leaf blight resistance gene *Xa 13* in rice (Zhang *et al*,1996).

References:

1. .S. Saharan, C.D. Kaushik, P.P. Gupta and N.N. Tripathi. 1984. Assessment of losses and control of white rust of mustard. *Indian Phytopathol.*, **39:** 397.
1. S.K. Chauhan 1998. Genetic analysis of white rust (*Albugo candida)* resistance and isolation of resistant high yielding genotypes of mustard (*Brassica juncea)*. Ph.D. Tesis submitted to P.G.School, IARI, New Delhi. (unpublished)
1. D.R. Knott and R.A. Mc Intosh. 1978. The inheritance of stem rust resistance in common wheat cultivar 'Webster'. *Crop Sci.*, **18:** 365-369.
1. G.Zhang, E.R. Angeles, M.L.P. Abenes, G.S. Khush and N. Huang. 1996. RAPD and RFLP mapping the bacterial blight resistance gene Xa-13 in rice. *Thear. Appl. Genet.*, **93:** 65-70.

Table 1. Inheritance of white rust resistance in Indian mustard (*Brassica juncea)*

S. No.	Cross	Generation	Location	No. of plants			Ratio (R : S)	Chi-square	P-Value
				R	S	Total			
1.	S-III x S-X	P_1	Delhi	18	0	18			
		P_2	Delhi	0	16	16			
		F_1	Delhi	0	19	19			
		F_2	Wellington	22	48	70	1:3	1.210	0.20-0.30
			Kukumseri	9	32	41	1:3	0.070	0.70-0.80
			Delhi	45	117	162	1:3	0.530	0.30-0.50
		Heterogeneity						1.240	0.50-0.70
		B_1	Delhi	10	8	18	1:1	0.222	0.50-0.70
		B_2	Delhi	0	22	22	All S		
2.	S-III x S-II	P_1	Delhi	22	0	22			
		P_2	Delhi	24	0	24			
		F_1	Delhi	32	0	32			
		F_2	Wellington	70	22	92	13:3	2.577	0.10-0.20
			Kukumseri	49	7	56	13:3	0.682	0.30-0.50
			Delhi	190	31	221	13:3	5.858	0.01-0.02
		Heterogeneity						3.860	0.10-0.20
3.	S-III x S-IV(R)	P_1	Delhi	18	0	18			
		P_2	Delhi	21	0	21			
		F_1	Delhi	29	0	29			
		F_2	Wellington	66	24	90	13:3	3.210	0.05-0.10
			Kukumseri	41	12	53	13:3	0.302	0.50-0.70
			Delhi	227	49	276	13:3	0.320	0.50-0.70
		Heterogeneity						4.272	0.10-0.20

STRATEGIC APPLICATION OF LIME, FERTILISERS AND FUNGICIDES FOR IMPROVED CONTROL OF CLUBROOT

EC Donald[A], IJ Porter[A] and RA Lancaster[B]

[A] Agriculture Victoria, Institute for Horticultural Development, Private Bag 15, Ferntree Gully Delivery Centre VIC 3156. e-mail: caroline.donald@nre.vic.gov.au

[B] Agriculture Western Australia, Manjimup Horticultural Research Institute, Locked Bag No. 7, Manjimup WA 6258.

Introduction

Integrated strategies have been developed to control clubroot, caused by *Plasmodiophora brassicae* in Australia using lime, fertilisers and/or fungicides. The effectiveness of these products can be improved by better distribution during application into the soil. In heavy soils, poor infiltration, resulting in inadequate product distribution, has limited the effectiveness of fungicide drench applications (Porter *et. al.*, 1998). Broadcast application of these products followed by incorporation, whilst effective, is often not economic and can lead to a build up of residues in the soil which can be undesirable in subsequent crops. For instance, high residual soil pH can lead to increased incidence and severity of scab diseases when potatoes are grown in rotation with brassica crops. Preplant banded incorporation of lime, fertilisers and fungicides along the transplant rows was investigated as a means of improving the efficacy of fungicides, reducing treatment cost and minimising soil residues.

Methods

A fully adjustable machine was developed to incorporate liquids and/or solids to a depth of 15-20cm in two 23cm wide bands along transplant rows immediately before transplanting.

Fluazinam (as Shirlan; 50% a.i. s.c.; Crop Care Australasia) was band incorporated into the transplant rows at 3 L/ha in 2500 L/ha water. This method of application was compared with the recommended spot drench (3.0 L/ha in 5000 L/ha water, 100 ml solution poured around plant base) and the 'grower preferred' continuous spray (3.0 L/ha in 2500 L/ha water, applied as a continuous band over the plants). Calcium cyanamide (Perlka; SKW Trostberg Germany) and Ground Burnt Agricultural lime (GBA lime; David Mitchell Ltd, Lilydale) were band incorporated into the transplant row at 368 kg/ha (equivalent to 1 t/ha broadcast). This method of application was compared to broadcast application followed by incorporation of these products at 1t/ha. (The amount of product incorporated into the transplant row was calculated using a treated area 2x30cm wide and was approximately one third of the broadcast rate (bed width 1.63m)). Fluazinam was applied at transplanting, lime and Perlka treatments were applied 7 days before transplanting. There were 5 replicates of each treatment.

Treatment efficacy was measured in terms of soil pH, disease development and marketable yield of broccoli and/or cauliflower. Soil pH was measured in 0.01M calcium chloride. Disease assessment was conducted 6 or 8 weeks after transplanting. Four plants were removed from each plot and visually assessed for root galling due to clubroot on a scale 1-9 where 1 = no root galling, 9 = 100% roots galled. At maturity, marketable yields were measured from the centre (5-7 m) of each 10 m plot. At least two, but up to six cuts were made to allow for variation in the rate of maturity.

Data were subjected to analyses of variance (ANOVA) using the GENSTAT 5 release 4.1 statistical package [Lawes Agricultural Trust (Rothamsted Experimental Station)].

Results and Discussion

Preplant incorporation of fluazinam into the soil in bands was found to be the most effective method of applying this product. Plants grown in soil treated in this way developed significantly less clubroot than plants treated with fluazinam applied using either a spot drench (100 ml/plant) or a continuous spray over the plants immediately after transplanting (Fig. 1A). This method of application also increased the marketable yield of both broccoli and cauliflower by at least 80% compared with the yield obtained using either of the other more conventional methods of application (Fig. 1B).

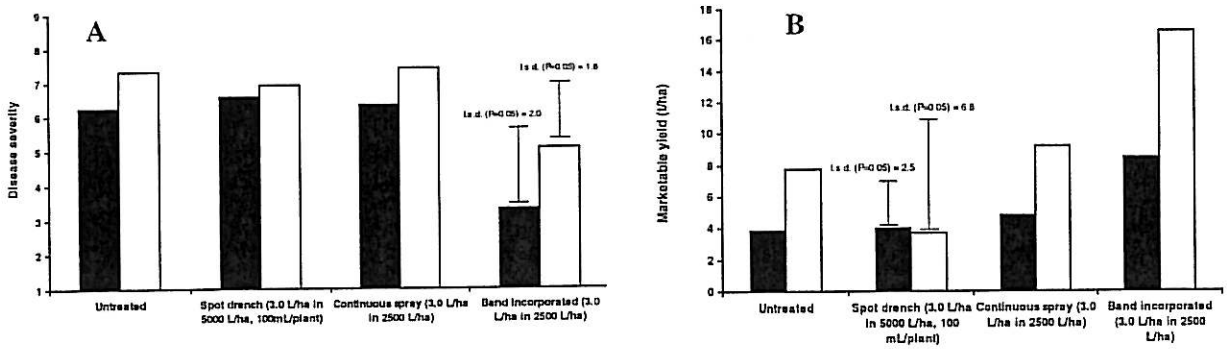


Figure 1. Effect of the method of application of fluazinam (as Shirlan) on disease severity (A) and marketable yield (B) of broccoli (■) and cauliflower (□) grown in field soil naturally infested with *P. brassicae*.

Band incorporation of GBA lime and calcium cyanamide was as effective as broadcast application (Table 1 and Fig. 2). There was no significant difference between soil pH within the GBA treated band and the corresponding broadcast area (Table 2), however, band incorporation reduced the amount of lime and fertiliser product used by approximately two thirds. This has important consequences for grower profit (Fig. 2) and soil residues, such as soil pH, which may affect the performance of rotation crops.

Table 1. Effect of application method on soil pH, disease development and marketable yield of broccoli from soil treated with Ground Burnt Agricultural lime.

Treatment	Soil pH ($CaCl_2$) 6WATr[B]	Clubroot score (1-9) 6WAT[A]	Marketable yield broccoli (t/ha)
GBA lime band incorporated	6.84	3.3	7.86
GBA lime (1t/ha) broadcast	6.77	3.1	7.95
Untreated	6.39	4.8	7.00
l.s.d. (P=0.05)	0.56	1.7	2.13

[A] WAT = weeks after transplanting [B] WATr = weeks after treatment

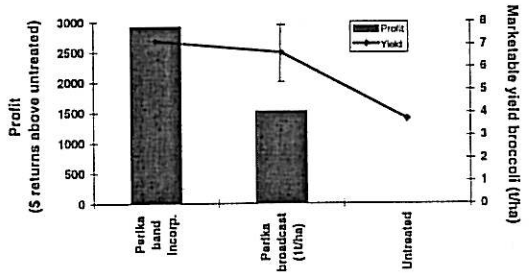


Figure 2. The effect of application method on the marketable yield (t/ha) and profitability ($/ha) of broccoli from calcium cyanamide (Perlka) treated soil (based on $1/kg for broccoli).

There is potential for this method of application to be used to apply a wide range of products to control *Plasmodiophora brassicae* and other root pathogens in row crops.

References

Porter, I.J., Donald, E.C. and Cross, S.J. (1998) Field evaluation of fluazinam against clubroot (*Plasmodiophora brassicae*) of cruciferous vegetable crops. *Tests of Agrochemicals and Cultivars* No. 19, (*Annals of Applied Biology* **132,** Supplement), pp. 12-13.

EFFECT OF CRUCIFEROUS PLANT EXTRACTS ON PARASITIC BEHAVIOR OF OOPARASITOIDS, *TRICHOGRAMMA* SPP. (HYM.: TRICHOGRAMMATIDAE)

K. S. Ganesh, M. A. Khan and S. Tiwari
Biological Control Laboratory, Department of Entomology,
G.B. Pant University of Agriculture and Technology,
Pantnagar – 263145, India

INTRODUCTION

Cruciferous are the most important vegetable and oil seed crops, which are grown throughout the world. Several insect pests attack these crops. Several insect pests and their natural enemies co-exist in the cruciferous crop ecosystem. Among those insect pests, Diamond back moth, *Plutella xylostella* Linn. has emerged as the serious problem throughout south and southeast Asia, and has also developed resistance to several classes of insecticides **(Dhaliwal and Arora, 2001)**. Hence, there has been upsurge in other methods of control measures and biological control is found to be the most effective for controlling this pest. Egg parasitoids of genus *Trichogramma* are known to be important natural enemies of *Plutella xylostella* L. **(Wukhrer and Hassan, 1993; He Yu Rong, 2000)**. The influence of host plants on parasitism by *Trichogramma* spp have been investigated by various workers. The di and tri- trophic interaction involving host plants, host insect and entomophagous are mediated predominantly by chemical cues or signaling chemicals which an organism can detect in its environment and affect the organism's behavior or physiology **(Ananthakrishann *et al.*, 1991)**. The present study was conducted with a view to understand the effect of different species of cruciferous plants on the parasitic behavior of *Trichogramma chilonis* Ishii, *T. japonicum* Ashmead and *T. poliae* Nagaraja at Biological Control Laboratory, Department of Entomology, GBPUA & T. Pantnagar, India.

MATERIALS AND METHODS

Corcyra cephalonica St. larvae were reared on broken maize grains mixed with 2% yeast at 28 ± 2 ^{0}C and 60 ± 5% RH. Fresh eggs of *C. cephalonica* (0-24 h old) were exposed to U V radiations to kill the host embryo and used for culturing the *T. chilonis*, *T. japonicum*, and *T. poliae* in a B.O.D. incubator at 25 ± 2 ^{0}C and 70 ± 5% RH. Clean, healthy, 0-24 h old eggs of *C. cephalonica*, sterilized under UV light were washed in hexane to remove any traces of scales and kairomones present on the surface of eggs. Then they were pasted equidistantly on 2x5 cm white sheet at the rate of 100 eggs per piece (here after referred to as Cards).

Eleven different plant species of the cruciferous family were collected from Pantnagar. About 50 gram of leaves of individual species was taken, shade dried and chopped into small pieces, chopped leaves were immersed into the known quantity of acetone for 72 hrs. The extracts were taken out by using Whatman No 41 filter paper. Then these extracts were used for treating the egg cards.

Each card was sprayed with plant extracts. Spraying was done by glass atomizer and 0.5 ml of spray liquid was used for each card. The sprayed cards were allowed to dry in shade and were introduced into glass vials of 15x2.5 cm. Each treatment was replicated three times and control was maintained by spraying the cards with Acetone. The sufficient number of healthy well fed, 0-24h old adults of the three species of *Trichogramma* were transferred to separate glass vials and anesthetized using etherized carbon dioxide as described by **Paul (1973)** for 15 seconds. Then the healthy fast reviving females of each species were transferred in each vial containing the card at the rate of 20 per card (with 100 eggs). They were allowed to parasitize the treated host eggs for 24 hrs and then egg cards (here after referred to as Trichocards) were shifted to fresh glass vials. On the 6^{th} day parasitization was recorded by observing the number of blackened eggs. Whole set up was maintained at 25±2C and 70±5% RH.

RESULTS AND DISCUSSION

Among the three *Trichogramma* species tested, *T. chilonis* recorded highest mean per cent parasitization of 70.00 in the cards treated with Indian mustard extract, while lowest mean per cent (23.66) parasitism by *T. chilonis* in the cards treated with the extracts of ornamental rai. *T. poliae* showed highest mean percent parasitism of 42.03 in the cards treated with cauliflower extracts and the lowest mean percent parasitism of 16.66 in the extracts of ornamental rai, Indian mustard and turnip. The lowest mean percent parasitism was recorded in all the extracts by *T. japonicum* in comparison with other two species under test.

The various amount of semiochemicals, which are present in the different tested plants, elicit differential responses from *Trichogramma* spp as revealed by differential mean percent parasitization (Table 1). The results are in conformity with the work of **Yadav *et al.* (2001)**, where parasitization by *T. chilonis* on the eggs of *Plutella xylostella* ranged from 77.06 to 94.87 per cent in stray plants of mustard, 42 per cent in cabbage and 4 per cent in cauliflower. Thus it could be said that among the different cruciferous plants, all the plants have differential effect in eliciting responses among the different species of *Trichogramma*.

REFERENCES

Ananthakrishnan, T.N., Senrayan, R., Murugesan, S. and Annadurai, R.S. (1991). Kairomones of *Heliothis armigera* and *Corcyra cephalonica* and their influence of the parasite potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera). *J. Biosci* **16**: 111-119.

Dhaliwal, G.S. and Ramesh Arora. (2001). Integrated pest management. Kalyani publishers. Ludhiana.

He, YuRong, Lu-li Hua, Pang-XiongFei, He-Y. R., Lu-LH., Pang-X. F. (2000). Simulation of Control effectiveness of several parasitoids against population of the diamond Back moth (*Plutella xylostella* L.) *Journal of South China Agricultural University* **21**: 2, 18-20.

Paul, A. V. N. (1973). Studies on the egg parasites *Trichogramma australicum* Girault and *T. japonicum* Ashmead (Trichogrammatidae: Hymenoptera) with special reference to host parasite relationship. M. Sc Thesis, TNAU, Tamil Nadu, India.

Wührer, B. G. and Hassan, S. A. (1993). Selection of effective species/strains of *Trichogramma* (Hym, Trichogrammatidae) to control the diamond back moth *Plutella xylostella* L. (Lep, Plutellidae). *Journal of Applied Entomology* **116**: 1, 80-89.

Yadav, D.N., Anand, Jha and Devi, P.K. (2001). *Trichogramma chilonis* (*Hym, Trichogrammatidae*) on *Plutella xylostella* (Lep, Plutellidae) in Gujarat. *Indian Journal* of *Agricultural Science* **71**(1): 69-70.

Table 1: Mean percent parasitism recorded by three species of *Trichogramma*

S. No.	Name of the species	Conc. of the extract	Mean % parasitization by *T. chilonis* Ishii	Mean % parasitization by *T. japonicum* Ashmead	Mean % parasitizati by *T. poliae* Nagara
1.	Ornamental Rai *Rhaphanobrassica* sp	0.03 g/ml	23.66 (29.10)	18.66 (25.58)	16.66 (24.09)
2.	Indian Rape *Brassica* campestris var *toria* Duth	0.01 g/ml	41.66 (40.20)	29.33 (32.78)	26.66 (31.08)
3.	Chinese cabbage *Brassica chinensis* Linnaeus	0.03 g/ml	30.00 (33.21)	40.00 (39.23)	28.33 (32.15)
4.	Indian mustard *Brassica juncea* Linnaeus	0.01 g/ml	70.00 (56.78)	12.33 (20.55)	16.66 (24.08)
5.	Rocket salad (Taramira) *Eruca sativa* Mill	0.02 g/ml	66.00 (54.73)	13.66 (21.68)	26.66 (31.08)
6.	Turnip *Brassica cumpestris* var rapa Linnaeus	0.03 g/ml	68.33 (55.75)	11.66 (19.96)	16.66 (24.08)
7.	Indian colza/rapeseed/yellow sarson *Brassica campestris* L. var *sarson* Prain	0.03 g/ml	43.33 (41.16)	21.66 (27.73)	28.33 (32.15)
8.	Cauliflower *Brassica oleracea* var *botrytis* Linnaeus	0.01 g/ml	45.00 (42.13)	28.66 (32.36)	42.33 (40.58)
9.	Cabbage *Brassica oleracea* var *capitata* Linnaeus	0.01 g/ml	46.00 (42.70)	23.33 (28.87)	30.00 (33.21)
10.	White mustard *Synopsis alba* Moench	0.01 g/ml	58.33 (49.80)	21.66 (27.71)	21.33 (27.50)
11.	Radish *Raphanus sativa* Linnaeus	0.02 g/ml	57.66 (49.41)	25.00 (29.98)	20.00 (26.56)
	C.D. at 1%		4.006 (2.35)	3.32 (2.31)	2.30 (1.591)
	Sem		1.005 (0.59)	0.834 (0.579)	0.577 (0.399)
	C.V.		3.47 (2.27)	6.46 (3.604)	4.019 (2.329)

Values in parenthesis are angular transformed values

METABOLIC CHANGES IN MUSTARD LEAF AND SILIQUA WALL DUE TO THE INFECTION OF ALTERNARIA BLIGHT (*ALTERNARIA BRASSICAE*)

S.K. Gupta and C.D. Kaushik
Department of Plant Breeding,
CCS Haryana Agricultural University, Hisar-125 004, INDIA

INTRODUCTION

Phenolic compounds are involved in disease resistance and are widely distributed in higher plants. The host proteins, especially the enzyme proteins, undergo marked changes on infection with various pathogen (Gupta *et al.*, 1992). Therefore, the present investigation was undertake to study the change in total phenols and specific activities of phenylalanineammonialyase (PAL) and tyrosine ammonialyase (TAL) in leaf and siliqua wall of mustard varieties as influence by Alternaria blight.

MATERIALS AND METHODS

The healthy (H) and diseased (D) leaf and sliquae samples of mustard varieties were collected from the CCS Haryana Agricultural University Research Farm. These were analysed for total phenol content (Swain and Hillis, 1959) and assayed for PAL and TAL specific activities (Biehn *et al.*, 1968).

RESULTS AND DISCUSSION

Total phenols (Table 1) were higher in diseased leaves and siliquae wall as compared to healthy ones in all the varieties. The amount of total phenols was higher in leaves in comparison to siliquae wall. The specific activities of enzymes PAL and TAL were also higher in diseased leaves and siliquae wall as compared to healthy ones.

The activities of PAL and TAL were higher in leaf in comparison to siliquae wall. Among the enzymes PAL had higher activity in comparison to TAL both in leaf as well as siliqua wall. These two enzymes are involved in the synthesis of phenolic compounds cinnamic and coumaric acids from phenylalanine and tyrosine. Their increased activity following infection and also

the increase in total phenols suggest their possible involvement in the protection of plant against the disease infection.

Table 1: Specific activity of phenylalanineammonia lyase (PAL) and tyrosineammonia lyase (TAL) and total phenols of leaf and siliqua wall of mustard varieties as influenced by Alternaria disease.

Mustard varieties	Total phenols (% dry wt.)		PAL*		TAL**	
	H	D	H	D	H	D
	Leaf					
Varuna	2.21	2.66	21.45	30.84	10.51	14.38
RH-30	2.16	2.48	19.36	34.16	9.45	13.18
RH-8113	2.24	2.54	22.83	28.46	12.17	15.62
RH-8112	2.05	2.40	19.43	26.41	9.82	13.93
	Silique wall					
Varuna	1.73	2.12	12.14	17.38	6.14	9.47
RH-30	1.66	2.01	11.67	18.45	5.11	8.19
RH-8113	1.91	2.22	14.14	19.32	4.36	8.74
RH-8112	1.76	1.98	12.48	18.14	5.42	9.12

H= healthy; D= diseased

* 1 unit = μ mole cinnamic acid accumulated min^{-1} mg^{-1} protein

** 1 unit = μ mole coumaric acid accumulated min^{-1} mg^{-1} protein

REFERENCES

Biehn, W.L., J. Kuc' and Williams, E.B. (1968). Accumulation of phenols in resistant plant-fungi interaction. *Phytopath. Z.* 58 : 1255-12-60.

Gupta, S.K., Kaushik, C.D. and Chawla, H.K.L. (1992). Metabolic changes in groundnut leaf due to infection by leaf spot pathogens. *Indian Phytopath.* 45 : 434-438.

Swain, T. and Hillis, W.E. (1959). The phenolic constitutents of *Prunus domestica* 1. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* 10 : 63-68.

Effect of sowing dates on infection & development of white rust in Mustard Cv. Brown Sarson.

* Sachin Gupta, Y. P. Khanna and Moni Gupta.
Division of Biochemistry & Plant physiology ,S.K.U.A.S.T., R. S. Pura, Jammu (J&K)- India.
* Deptt. of Agriculture, J&K Govt.

Introduction :-

Mustard is an important oilseed crop and occupy an important place in the economy of Indian agriculture. Among the various diseases, white rust (*Albugo candida*(Lev) Kuntze) is severe and widespread on mustard crop all over India. The disease has been found to cause about 17-34 per cent loss in yield. (Bains and Jhooty,1979). The following experiment was conducted to study the effect of planting date on occurence and development of white rust on mustard Cv. Brown Sarson.

Materials and Methods :-

Mustard cv. Brown Sarson was sown on different dates starting from first week of September to first week of December at fortnight interval and the experiment was repeated during two consecutive years. The observations on progress of white rust was recorded at weekly interval upto maturity of crop. The number of white rust pustules on 20 randomly selected tagged leaves were recorded.

Results and discussion -:

Data from Table 1 reveals that white rust infection and development increased with delay in date of sowing. The maximum disease development was recorded during the months of December and January while the crop sown in early September recorded least disease severity. Prevalence of high temperature during growth period of early sown crop did not favour the disease development while in late sown crop, low temperature made the condition congenial for white rust development. The results are in agreement with the earlier report of Kumar *et.al* (1986). It can be concluded from the present study that progression of white rust disease on mustard is affected by variation in sowing dates. Thus, the disease occurence in field can be lessened by altering the sowing dates.

Reference:-

Bains, S.S and Jhooty J.S. (1979) Mixed infection by *Albugo candida* and *Peronospora parasitica* on *Brassica juncea* inflorescence and their control. *Indian Phytopath.* **(32)** : 268-271.

Kumar, V, Kaushik, C.D., Saharan, G.S and Gupta, P.P (1986). Role of environment and cultivar interaction on the epidemiology of white rust of mustard. *Cruciferae Newsletter* **(11) :** 108-109.

Table 1: Effect of sowing dates on white rust development on mustard leaves (Cv. Brown sarson)

Disease recording (Week)	Increase in number of white rust pustules with respect to dates of sowing						
	September 7	September 23	October 14	October 30	November 17	December 8	Mean
1	0	0	0	0	0	1	0.16
2	0	0	2	2	3	10	2.83
3	0	0	6	7	5	11	4.83
4	0	0	10	17	18	25	11.66
5	0	3	15	27	32	52	21.50
6	0	7	22	40	62	68	33.16
7	0	9	30	45	64	70	36.33
8	2	10	40	54	69	70	40.83
9	11	19	42	56	70	70	44.66
10	16	19	42	59	70	70	46.00
Mean	2.9	6.7	20.9	30.7	39.3	45.0	

Infection of Oilseed Rape by *Verticillium dahliea* and *V. nigrescens*

Qiong Hu, Jan Fahleson and Christina Dixelius

Department of Plant Biology, Swedish University of Agricultural Sciences

Box 7080, S-750 07 Uppsala Sweden. E-mail: Qiong.Hu@vbiol.slu.se

Introduction

Verticillium wilt has become one of the major diseases of oilseed rape (*Brassica napus*) in Europe. This disease is caused by a fungal species which was recently designated as *V. longisporum* (previously reported as *V. dahliea*) (Karapapa *et al.*, 1997). In contrast to *V. dahliea*, which infects many plant genera, *V. longisporum* is more host-specific. All *V. longisporum* strains reported so far were obtained from infected cruciferous plants such as oilseed rape, cabbage, horseradish and cauliflower except one from sugar beet grown in a field previously planted with rape (Heale & Karapapa 1999, Karapapa & Typas 2001). Pathogenicity tests showed *V. longisporum* strains are virulent on oilseed rape seedling whereas *V. dahliea* from various hosts failed to cause any symptoms (Heale & Karapapa 1999). Because of the host specificity of *V. longisporum* in Crucifers, it is generally perceived that *V. longisporum* is the only causal agent of Verticillium wilt in oilseed rape. From oilseed rape plants collected in northern Germany, however, strains of *V. dahliea* and *V. nigrescens* were obtained. We report here the isolation of non-*longisporum* strains from oilseed rape and primary results of their pathogenicity on their host of origin.

Material and methods

Verticillium strains were isolated from infected oilseed rape stem samples collected from northern Germany. After surface sterilization, small pieces of stem tissue were cultured on PDA and incubated for 5-10 days. Verticillium identity was confirmed by observing verticillate conidiophores. Conidia length and nuclear DNA content were measured using spore suspension from two weeks old culture. DNA was extracted from lyophilised mycelia using DNeasy Plant Mini Kit (Qiagen). Extracellular polyphenol oxidase (PPO) activity was observed using Eckert s medium (Eckert 1962). AFLP (amplified fragment length polymorphism) was analysed using *Eco*RI and *Mse*I primers and polymorphism was detected using ABI Prism™ 377 DNA sequencer (Perkin Elmer/Applied Biosystems) after selective *Eco*RI primers were fluorescently labelled. Root dipping method was used for inoculation of two-week old seedlings for pathogenicity test.

Results and discussion

From 30 stem samples with symptoms such as vascular browning and microsclerotinia formation, 24 samples gave rise to colonies similar to *Verticillium* species. Single spore culture of these colonies resulted in 35 strains (2-3 distinct single spore colonies arose from 9 of the original colonies). Among these strains 26 were found with verticillate conidiophores. Twenty-two of them showed typical *V. longisporum* morphology, such as elongated irregular microsclerotia and long conidia (6-10 m), and thus were classified as *V. longisporum*, which was further confirmed by *longisporum*-specific PCR primers. Four other strains showed different morphology with conidia significantly

shorter (<5 m) and did not have the *longisporum*-specific fragment after PCR amplification excluding them from *V. longisporum* group. Three of the strains did not form any microsclerotia on PDA. Instead, chlamydospores were developed and caused melanian colony. These strains did not exhibit PPO activity. The other strain formed compact and spherical microsclerotia typical of *V. dahliea* and showed PPO activity. All four strains had nuclear DNA content less than 0.05 pg/cell indicating their haploid nature.

AFLP analysis grouped the three strains producing chlamydospores together with *V. nigrescens* strains CBS455.51 and CBS470.64, which were isolated from *Solanum tuberosum* and *Medicago sativa*, respectively and the other one with *V. dahliea* strains from various hosts. This result confirmed that the first three strains are *V. nigrescens* and the fourth belongs to *V. dahliea*. Recovery of haploid *V. dahliea* has been reported from *Brassica* vegetables (Zeise & Tiedemann 2001, Bhat & Subbarao 2001) but not oilseed rape. In another study we identified three *V. dahliea* strains from rape plants collected in France (Fahleson *et al.* in prep.) and one from Russian oilseed rape. To our knowledge, however, *V. nigrescens* has never been reported from any cruciferous plants. This species has been reported pathogenic mainly to cotton and potato (Kitazawa & Sato 1984, Wyllie & DeVay 1970).

Pathogenicity test using verticillium wilt susceptible winter oilseed variety Hanna revealed that all these four strains are pathogenic to oilseed rape though disease severity was not as high as the virulent *V. longisporum* control strain Vd1. Plants were stunted after inoculation and chlorosis was observed. Vascular browning was found from pre-mature plants and seeds from diseased plants were much smaller than controls. The *V. dahliea* strain was more virulent than *V. nigrescens* strains in terms of plant stuntness and negative effects on yield components such as number of siliques per plant, number of seeds per silique and thousand seeds weight.

Even though the majority of Verticillium strains obtained to date from oilseed rape belongs to *V. longisporum* (Hu et al. 2001, Karapapa & Typas 2001, Zeise & Tiedemann 2001), the occurrence of non-*longisporum* strains on oilseed rape plants in Germany may complicate disease control strategies of this pathogen on oilseed rape.

References

DeVay JE & Pullman (1984) Epidemiology and ecology of diseases caused by *Verticillium* species, with emphasis on verticillium wilt of cotton. Phytopathologia Mediterranea 23:95-108.

Eckert JW (1962) Fungistatic and phytotoxic properties of some derivatives of nitrobenzene. Phytopath. 52: 642-649.

Fahleson J, Lagercrantz U, Steventon LA, Dixelius C (2002) Characterisation of *Verticillium* isolates using AFLP analysis (In preparation).

Karapapa VK, Bainbridge BW, Heale JB (1997) Morphological and molecular characterization of *Verticillium longisporum* comb. Nov., pathogenic to oilseed rape. Mycol. Res. 101: 1281-1294.

Heale JB & Karapapa VK (1999) The verticillium threat to Canada s major oilseed crop: canola. J. Plant Pathol. 21:1-7.

Hu Q, Fahleson J, Dixelius C (2001) Molecular and morphological variation of *Verticillium* isolates from *Brassica napus*. In: Proc. of 8th Int Verticillium Symposium, C rdoba, Spain.

Zeise K & Tiedemann A von (2001) Morphological and physiological differentiation among vegetative compaptibility groups of *Verticillium dahliea* in relation to *V. longisporum*. Phytopath. 149: 469-475.

Alterations in sugars and phenols in *Brassica juncea* during interaction with white rust (*Albugo candida*)

Ramesh Kumar, N.K. Thakral, S.K. Gupta and C.D. Kaushik
Oilseeds Section, Deptt. of Plant Breeding,
CCS HAU, Hisar – 125 004 (India)

White rust is a major disease of Indian mustard (*Brassica Juncea*) and it causes severe losses to seed yield and quality. The present investigation was aimed to study the effect of this disease on sugars and total phenols in leaves. These characters have been reported to impart resistance/susceptibility against diseases.

Materials and Methods

Healthy and diseased leaves of four varieties of Indian mustard RH8113 (R), RC781(R), Varuna(S) and Sarita(S) were collected and analysed for total soluble sugars, reducing sugars, non-reducing sugars and total phenols.

Results and Discussion

Sugars and phenols were affected significantly by stages and environments and these decreased at later stages (siliqua formation stage) and under late sown conditions. Reduction of total soluble sugars were less in resistant varieties than susceptible ones after the attack of disease because sugar is consumed by pathogen during disease development. Similar trend was also observed for total phenols. Total phenols play important role in post-inflectional defence. After infection by pathogen an important host enzyme glucosidase is activated which converts non-toxic glucosides to toxic phenols which are inhibitory to pathogens. Similar results were reported by Gupta *et al.*, (1984b) for *Alternaria* and Dhawan *et al.*, (1981) for white rust in Indian mustard.

References

Dhawan, K.; Yadava, T.P.; Kaushik, C.D. and Thakral, S.K. 1981. Changes in phenolic compounds and sugars in relation to white rust of Indian mustard. *Crop Improv.* 8: 142-144.

Gupta, S.K.; Kumar, P.; Yadava, T.P. and Saharan, G.S. (1984b). changes in phenolic compounds, sugars and total nitrogen in relation to *Alternaria blight* in Indian mustard. *Haryana Agric. J. Res.* 14: 535-537.

Table 1: Total soluble sugars, reducing sugars, non-reducing sugars and total phenols in *Brassica juncea*.

Variety	Stage		Total soluble sugars (mg/g)	Reducing sugars (mg/g)	Non-reducing sugars (mg/g)	Total phenols (mg/g)
RH8113	Vegetative stage (35DAS)	E_1	68.99	12.90	56.38	37.65
		E_2	37.99	5.57	32.45	24.96
	Siliqua formation stage (65DAS)	E_1	52.17	9.71	43.67	19.37
		E_2 (H)	22.43	8.89	18.75	21.17
		E_2 (D)	17.90	5.62	12.29	18.34
RC781	Vegetative stage (35DAS)	E_1	84.67	23.46	60.86	56.57
		E_2	22.16	6.54	15.54	22.63
	Siliqua formation stage (65DAS)	E_1	53.03	8.49	44.67	27.78
		E_2 (H)	19.57	3.95	15.40	19.23
		E_2 (D)	16.87	5.69	15.17	18.32
Varuna	Vegetative stage (35DAS)	E_1	85.53	12.67	70.74	34.56
		E_2	49.19	7.57	38.43	28.26
	Siliqua formation stage (65DAS)	E_1	66.78	4.64	34.56	17.43
		E_2 (H)	43.29	4.17	22.19	20.65
		E_2 (D)	3.87	5.64	18.03	12.77
Sarita	Vegetative stage (35DAS)	E_1	54.45	14.55	39.75	29.63
		E_2	37.55	7.87	29.46	31.98
	Siliqua formation stage (65DAS)	E_1	48.07	5.01	29.63	21.88
		E_2 (H)	47.09	5.01	17.71	20.01
		E_2 (D)	27.58	7.84	10.68	13.00

DAS = Days after sowing, E_1 = Timely Sown, E_2 = Late sown, H = Healthy, D = Diseased

Efficacy of seed dressing fungicides in the management of stem rot disease of mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary.

A.K.Pathak and S. Godika
Agricultural Research Station, Navgaon(Alwar) Rajasthan 301025-India

Sclerotinia sclerotiorum is a polyphagous pathogen infecting 64 plant families, 225 genera, 361 species and 22 other cultivars of plant kingdom (Prudy, 1979). It is wide spread on rapeseed-mustard in China (liu *et al.*, 1990; Liu, 1991) causing losses upto 50 percent (average 10- 12%), England and Wales (Sansford, 1995), France, Germany (Kruger and Stoltenberg, 1983), and many other countries. In India,it has emerged in serious form in many parts of the country. It appears regularly in mild to severe form in Himachal Pradesh (Kumar and Thakur, 2000), causes loss upto 72 per cent in Uttat Pradesh (Chauhan *et al.*, 1992) and with 50 to 70 per cent incidence in Rajasthan (Lodha *et al.*, 1992).As the fungus prepetuates in soil through its resting bodies (sclerotia), dressing the seeds with fungitoxicants may protect initial infection and provide better germination and plant growth. The present studies were therefore, conducted during 1998-2000 with the same objectives

Mustard (Varuna) seeds were contaminated overnight with 15 days old culture of the fungus @ 10 conidia/ ml per 10 g seed and dressed with fungicides @ 2 g per kg seed. Healthy, untreated seeds and contaminated, untreated seeds were kept for comparison.The trial was conducted during 1998-99 and 1999-2000 in earthen clay pots (5 seeds per pot) having 30 cm diameter and soil mixed with 10 g inoculum per kg soil, except in healthy control.The experiment was conducted in CRD with 5 replications per treatment.

The results presented in table 1 reveal that all the seed dressers were superior over contaminated check for all the parameters under observations. Among seed dressers, Ridomil MZ gave maximum germination (44%) and the post emergence mortality was also nil. Carbendazim showed 40 per cent germination but also had post emergence mortality (6%).Plant height, dry plant weight and seed test weight was superior in Carbendazim (89.75 cm, 2.63 g, and 4.22 g, respectively). Seed dressing with Carbendazim was statistically at par with Ridomil MZ and Mancozeb.

References:

Chauhan, L.S., Singh, Jyoti and Chandra, D.R.1992. Assessment of losses due to stem rot to yellow sarson. *Proceedings of National symposium on management of microbes in service of mankind.* Nov. 19-21 at Univ. Of Allahabad, India : 65-66.

Kruger,W. and Stoltenberg, J. 1983. Bekmfung bon Rapskrankhieiten. II. Mabnahmenzur Befllsvrringprung Bon Sclerotinia sclerotiorum unter Berucksichtigug okonomischir Faktoren. *Phytopathologische Zeitschrift.* 108 : 114-126.

Kumar, A. and Thakur, K.S.2000. Effect of nitrogen levels on sclerotinia stem rot in canola and non canola type cultivars of Rapeseed-Mustard. *National seminar on*

Oilseeds and oil-Research and development needs in the millennium. Feb. 2-4, 2000 D.O.R., Hydrabad, India. 224-225.

Liu, C.Q. 1991. Study on tolerance to *Sclerotinia sclerotiorum* and the inheritance properties in *Brassica napus*. *Scientia Agriculture Sinica*, 24 (3): 43-49.

Liu, C.Q., Du,D.Z. , Zou, C.S. and Huang, Y.J. 1990. Initial studies on tolerance to *Sclerotinia sclerotiorum* (Lib.) de Bary in *Brassica napus* L. *In Proceedings of the Symposium, China International Rapeseed Science. Shanghai*, China: 70-71.

Lodha, B.C., Bhatnagar, M.K., Doshi, A. Mathur,S., Bairwa, L.N., Sharma, D. and Trivedi, A.1992. *Plant Pathological Thoughts and News*. No. 6 (3), Deptt. Plant Pathology, RCA, Udaipur (India).

Prudy, L.H. 1979. *Sclerotinia sclerotiorum* : History, disease, symptomotology, host range, geographical distribution and impact. *Phytopathology*. 69: 875-886.

Table-1: Efficacy of seed dressing fungicides against stem rot disease of mustard.

S. No.	Treatment	Germination (%)	Post emergence mortality (%)	Plant height (cm)	Dry plant weight (g)	Test weight (g)
1.	Carbendazim	40.0	6.00	89.75	2.63	4.22
2.	Mancozeb	32.0	6.25	85.00	2.67	4.04
3.	Thiram	32.0	6.25	86.00	2.57	3.24
4.	Ridomil MZ	44.0	0.00	84.50	2.60	3.68
5.	Apron SD	30.0	0.00	76.50	2.06	3.48
6.	Bayletan	32.0	12.50	87.00	1.93	3.24
7.	Inoculated, untreated seeds	18.0	12.50	64.25	0.50	2.58
8.	Healthy, untreated seeds	92.0	`0.00	83.00	2.40	4.00
	General Mean	39.81	3.69	77.31	2.09	3.38
	C . D. (5%)	5.58	1.15	6.49	0.35	0.28
	S. Em.+	1.90	0.39	2.21	0.12	0.096
	C.V. (%)	9.53	21.28	5.71	11.63	5.67

Multiple disease resistance in different genotypes of rapeseed-mustard

A.K.Pathak and S. Godika
Department of Plant Pathology, Agricultural Research Station, Navgaon (Alwar):301025-India

With the second largest area under oilseeds in the world, India ranks fourth in the world's production of oilseeds. Among the cultivated oilseed crops, India stands third in the production of rapeseed -mustard. The White rust, Alternaria blight, Powdery mildew and the stem rot are the most important diseases affecting the productivity of rapeseed-mustard. Yield losses by White rust on leaf and staghead are reported upto 27.4 and 62.17 per cent respectively in isolation and 89.8 per cent in combination (Lakhara and Saharan, 1989). Yield losses (10 to 75%) and reduction in oil content (1 to 10%) are reported to be caused by Alternaria blight on mustard (Verma and Saharan, 1994). Losses upto 72 per cent in rapeseed-mustard are reported (Chauhan *et al.*, 1992) due to stem rot. Powdery mildew generally do not cause much damage but under disease severity, considerable yield losses are reported (Kolte, 1985).

Trials were conducted (1996-2001) under artificial inoculation conditions for Alternaria blight and White rust diseases , in sick beds against stem rot disease and under natural conditions for Powdery mildew disease to evaluate multiple disease resistance in different disease resistance in different genotypes of rapeseed-mustard.

Results presented in table 1 reveal multiple disease resistance to Powdery mildew, stem rot, Alternaria blight and White rust diseases in five genotypes via. RN-490, RN-505, PBC-9221, PBN-9501 and PBN-9502. RN-490 is identified as a promising entry against Alternaria blight in multilocational All India Coordinated Research Trials (Rapeseed-mustard) consistently for the past three years (1996-99) and is showing better yield performance against National checks . Genotypes namely RH-9401, RW-8410 and Hyola-401 showed promise against White rust, Alternaria blight and stem rot diseases, but were susceptible to highly susceptible to Powdery mildew. Similarly, PHR-2 and PAB-9511 were susceptible to highly susceptible to stem rot, but were resistant to moderately resistant against the remaining diseases. Genotypes viz. BIO-902, RH-30 and YS-841 were highly susceptible to Alternaria blight, but showed promise against White rust, Powdery mildew and Stem rot diseases. RGN-8006 was highly susceptible to White rust but was moderately resistant to Alternaria blight, stem rot and Powdery mildew diseases. The source of resistance among these genotypes may be employed in breeding programmes for improvement in high yielding, commercial cultivars of Rapeseed-mustard. Varieties such as RN-490, having multiple disease resistance and better yield performance can directly be passed on to the cultivators.

References

Chauhan, L.S., Jyoti Singh and D.R. Chandra. 1992. Assessment of yield losses due to stem rot to yellow sarson. In: Proceedings of National Symposium on Management of Microbes in service of Mankind. Nov. 19-21 at University of Allahabad, India: 65-66.

Kolte, S.J. 1985. Diseases of Annual Edible Oilseed Crops, Volume II. CRC Press Inc. Boca Raton,Florida. 47 pp.

Lakhara, B.S and G.S. Saharan. 1989 Correlation of leaf and staghead infection of white rust and yield component of Indian mustard. Indian J. Mycol. & Pl. Path. 19: 279-281.

Verma, P.R. and G.S.Saharan. 1994. Monograph on Alternaria blight disease of crucifers. Tech. Bull. 6E. Res. Branch, Agri and Agri. Food, Canada Res. Centre, Saskatoon, Seacatechwan.

Table 1 : Multiple disease resistance in different genotypes of rapeseed-mustard.

S. No..	Genotype	Source	Species	Powdery mildew		Stem rot		Alternaria Blight (Disease index (0-5 scale)			White rust (Disease index 0-5 scale)		
				Disease index (0-5 scale)	Reaction	Per cent disease Incidence	Reaction	Leaf	Pod	Reaction	Leaf	stag head	Reaction
1.	RN-393	Navgaon (Rajasthan)	*B. juncea*	2.5	HS	18.57	MR	2.0	1.0	MR	1.0	------	MR
2.	RN-490	Navgaon (Rajasthan)	*B. juncea*	2.0	MR	13.33	MR	2.0	1.0	MR	1.0	------	MR
3.	RN-505	Navgaon (Rajasthan)	*B. juncea*	1.5	R	2.0	MR	2.0	--	MR	2.0	------	MR
4.	RN-510	Navgaon (Rajasthan)	*B. juncea*	2.5	HS	7.69	R	3.0	--	HS	2.0	------	MR
5.	Bio-902	NRCPB (New Delhi)	*B. juncea*	2.0	MR	16.66	MR	3.0	2.0	HS	2.0	------	MR
6.	PCR-10	Hissar (Haryana)	*B. juncea*	2.5	HS	4.28	R	2.0	2.0	MR	3.0	------	HS
7.	RH-9401	Hissar (Haryana)	*B. juncea*	2.5	HS	12.58	MR	1.0	2.0	MR	1.0	------	MR
8.	RW-8410	Barahampur (W. B.)	*B. juncea*	2.0	S	17.24	MR	2.0	2.0	MR	1.0	------	MR
9.	PAB-9511	Pantnagar (U.P.)	*B. juncea*	1.0	MR	34.28	HS	1.0	1.0	MR	--	------	R
10	RGN-8006	Ganganagar (Raj.)	*B. juncea*	1.0	MR	14.28	MR	1.0	1.0	MR	3.0	------	HS
11.	PHR-2	Hissar (Haryana)	*B. juncea*	1.0	MR	25.15	S	1.0	-	MR	1.0	------	MR
12.	PBC-9221	Pantnagar (U.P.)	*B. carinata*	0.0	R	11.11	MR	2.0	---	MR	-	-----	R
13	PWR-9538	Pantnagar (U.P.)	*B. juncea*	2.5	HS	16.21	MR	2.33	0.66	S	0.33	------	R
14	JMM-915	Morena (M.P.)	*B. juncea*	2.5	HS	19.67	MR	2.33	1.0	S	1.0	----	MR
15	PBN-9501	Pantnagar	*B. napus*	0.0	R	14.28	MR	1.0	----	MR	1.0	-----	MR
16	PBN-9502	Pantnagar	*B. napus*	0.0	R	11.42	MR	1.0	----	MR	1.0	-----	R
17	PWR-9541	Pantnagar	*B. juncea*	1.0	MR	6.25	R	3.0	----	HS	3.0	Traces	HS
18	RH-30	Hissar	*B. juncea*	1.5	MR	12.67	MR	3.0	2.0	HS	2.0	Traces	MR
19.	RL-1359	Ludhiana (Punjab)	*B. juncea*	2.5	HS	22.90	S	4.0	2.0	HS	2.0	Traces	MR
20.	Pusa Bold	IARI (New Delhi)	*B. juncea*	2.0	S	14.28	MR	4.0	3.0	HS	2.0	Traces	MR
21.	Kranti	Pantnagar	*B. juncea*	2.5	HS	11.42	MR	3.0	2.0	HS	2.0	----	MR
22.	Varuna	Varanasi (UP)	*B. juncea*	2.5	HS	36.02	HS	3.0	2.0	HS	2.0	Traces	MR
23	YS-841	P.C. Unit, Bharatpur	*B. carinata*	0.0	R	7.14	R	3.0	3.0	HS	----	-----	R
24	RGN-7	SriGanganagar(Raj.)	*B. juncea*	2.0	S	15.00	MR	3.0	2.0	HS	3.0	1.6	HS
25	RGN-9	SriGanganagar (Raj.)	*B. juncea*	2.0	S	19.67	MR	3.0	2.0	HS	2.0	2.4	MR
26	RGN-11	SriGanganagar (Raj.)	*B. juncea*	2.5	HS	12.5	MR	3.0	1.0	HS	2.0	2.6	MR
27	Hyola-401	Banglore (A.P.)	*B. napus*	2.5	HS	4.28	R	1.0	1.0	MR	---	------	R

Entomopathogenic fungi, *Verticillium Lecanii* (Zimm.) as a potential bio-control agent against mustard aphid, *Lipaphis Erysimi* (Kalt.) on Rapeseed -mustard

J.S.Rana and D. Singh
Oilseeds Section,
Department of plant Breeding
CCS Haryana Agricultural University
Hisar-125004 Haryana, India

Rapeseed (*Brassica rapa*) and Indian mustard (*Brassica juncea*) are major oilseed crops of India grown on a large area. The oil produced by these crops is used for different purposes like human food, cooking, animal feed etc. and therefore these crops accomplish significant part of the dietary needs of human being. The successful cultivation of this crop is hampered by attack of various insect-pests like mustard aphid, *Lipaphis erysimi* (kaltenbach), painted bug, *Bagrada hilaris* (Burm.), saw fly, *Athalia lugens* (Klug), larger moth, *Crocidolomia binotalis* Zellen and diamond back moth, *Plutella xylostella* (Linnaeus). Among all of the above mentioned insect-pest mustard aphid is the most serious and inflict heavy losses to the crop (Bakhetia and Sekhon, 1984; Brar *et al.*, 1987; Joshi *et al.*, 1989). Many chemical pesticides are being used to control this pest but due everrising awareness about environmental pollution and development of resistance in aphid species there is an immediate need to think about some alternative to chemical pesticides. Many Bio-control agents are available for the management of this pest but entomopathogenic fungi, *Verticillium lecanii* has been reported to be of immense importance for aphid control in the tropics (MC Clelland and Tucker, 1929; Viegas, 1939; Baird, 1958). Several reports about its efficacy has stimulated us to study its effect on the management of mustard aphid, *L. erysimi* under field conditions.

The Rapeseed, *B. rapa cv.*. BSH-1 was sown in field after following all the package of practices to grow a healthy crop of rapeseed- mustard. The fungal suspension was made following all the standard procedure and sprayed as tank mix after mixing the formulation with sticker (Triton) for uniform crop coverage. The fungi was sprayed at the rate of 10^6 conidial spores / ml. A plot size of 200 m^2 was kept for spraying. Spray was done after ensuring the enough population of mustard aphid in the field by calculating the economic threshold level (ETL) of 13-15 aphids per plant or 10 per cent plant infestation. Four observations were recorded i.e. one before spray of the solution and three after the spray at 4, 7 and 10 days after spray (DAS). The results on the efficacy of the fungal suspension are presented in Table 1.

The results presented here showed that the aphid infestation index (AII) before spray was 3.9 which was almost at par among all the treatment statistically. The population of mustard aphid in treated sets after spray of fungal spores showed that there was a significant reduction in AII after spray of this fungal spore solution particularly at 10 DAS when the AII reduced to 1.2 from 4.2 (AII before spray).

From the results presented above it can be concluded that these are consistant and encouraging so far as the control of mustard aphid is concerned. Only a single spray of *V. lecanii* could reduce aphid population well below ETL. Hence, it can be

used as an effective IPM tool for the management of aphid on oilseeds crops which may in turn may reduce overreliance on chemical pesticides to a certain extent.

Table 1. Biological control of mustard aphid, *Lipaphis erysimi* using *Verticillium lecanii*

Treatment	Aphid Infestation Index				
	Before spray	Days After Spray			
		4	7	10	Average
Unsprayed	3.9	3.8[a]	3.9[a]	3.8[a]	3.66
Sprayed	4.2	4.0[a]	3.1[b]	1.2[c]	2.76

Figures superscribed by similar letters are at par with one another

References:

Baird, R.B. 1958. The artificial control of insects by means of entomopathogenic fungi: a compilation of references with abstracts. Entomology laboratory, elleville, Ontario, Canada.

Bakhetia, D.R.C.; Sekhon, B.S. and Arora, R. 1994. Yield loses due to mustard aphid, *Lipaphis erysimi* in some varieties of four *Brassica species* the Punjab Souvenir and abstract, 5th National Symposium on aphidology Jan. 27-30, 1994 held at Banglore.

Brar, K.S.; Bakhetia, D.R.C. and Sekhon, B.S. 1987. Estimation of losses in yield of rapeseed -mustard due to mustard aphid. J. oilseeds Res. **4**. 261-264.

Joshi, M.L.; Ahuja, D.B. and Mathur, B.N. 1989. Loss in yield by insect-pest and their ocurrence on different dates of sowing in Indian mustard (*Brassica juncea* sub sp. *Juncea*) Indian J. Agric. Sc. **59**. 116-168.

McClelland, T. B. and Tucker, C.M. 1929. Green scale, *Coccus viridis*, a new pepst in coffee and citrus. Agricultural notes of the porto Rico Agricultural Experimental sation, Mayaguez, San Juan **48**. 1-2.

Viegas, A.B. 1939. Um amigo do fazendeiro *Verticillium lecanii* (Zimm.) on year ound chrysanthemums. I. Summer season. Annals of applied biology **56**, 439-59.

Sustainable enhancement of yield potential of Mustard (*Brassica juncea* L. Czern. Coss.) through Integrated Nutrient Management (INM) in a legume based cropping system for the inceptisols

Abraham T[1] and Lal R.B[2]

1. Asst. Professor, Department of Agronomy, 2. Vice Chancellor,

Allahabad Agricultural Institute (Deemed University), Allahabad-211007

Uttar Pradesh, India

INTRODUCTION

Organic manures seem to be marginally risk reducing inputs in both irrigated and dryland farming systems, indicating that the increased use of which would largely decrease level of yield uncertainty faced by farmers (Babu and Subramanian, 1990). The application of biological and organic manures not only supply a balanced amount of micronutrients but also improves the physico-chemical and biological properties of soil, thus creating a conducive environment for crop production. It is pertinent that for sustainability in cropping systems, combining manure application with a crop rotation involving legumes could be an effective crop management practice.

Key words: Mustard, Legume based cropping system, yield potential, INM,

MATERIALS AND METHODS

The field experiment was carried out in *kharif*, *rabi* and *zaid* seasons during 1997-1999 at the Crop Research Farm of the Allahabad Agricultural Institute (Deemed University) (25°.57' N, 81°.50' E and 98 m). The soil was sandy-loam alluvium and analysed low in organic carbon, nitrogen and phosphorus but high in potassium. The cropping system chosen was:

Kharif	*Rabi*	*Zaid*
Soybean (*Glycine max* L. Merr.)	Mustard (var. Pusa Bold) (*Brassica juncea* L. Czern. Coss.)	Fodder cowpea (*Vigna unguiculata* L. Walp.)

Treatment combinations were 27, replicated thrice. The design opted was Randomized complete block. Fertilizer (NPK) doses (F) were F_0-Nil application, F_1-33% recommended dose (RDF) 30, 20, 15 kg ha^{-1} and F_2-100% RDF 90, 60, 45 kg ha^{-1}. Forms of organic manures (C) were C_0-Nil application, C_1-Farm compost (FC) @ 5 t ha^{-1} + Vermicompost (VC) @ 1 t ha^{-1} and C_2-Farm compost (FC) @ 5 t ha^{-1} + Poultry manure (PM) @ 0.5 t ha^{-1}. Forms of biofertilizers and/or foliar application of organic manure (B) used were B_0-Nil application, B_1-Phosphate solubilizing bacteria (PSB) + *Rhizobium* (Rhz) or *Azospirillum* (Azsp) and B_2-Phosphate solubilizing bacteria (PSB) + 2 foliar application of 33% cow's urine (CU).

Soil analysis for organic carbon and crop analysis for test weight (g), seed and biological yield, protein and oil content in the seed were conducted. All results are presented in Table 1.

RESULTS AND DISCUSSION

Application of 33% RDF maintained the soil organic carbon which was on par with 100% RDF. Warren and Garry (1985) observed the same. During the 1st year the test weight was higher in 33% RDF which was on par with 100% RDF, but in the 2nd year the latter showed significantly increased values over that of 33% RDF and no fertilizer application. The seed yield was significantly increased with 100% RDF. The percentage of seed yield over 33% RDF and zero RDF and no fertilizer application rate was 38.3 and 303.9% in the 1st year and 53.6 and 223.3 in the 2nd year respectively. The same trend was noticed in biological yield also. The quality parameters like oil and protein content were significantly higher in 33% RDF as compared to 100% RDF.

The interaction effect between 33% RDF and poultry manure significantly built up the soil organic carbon which lead to increased seed yield and biological yield during 1st year.

However, during the 2nd year of experimentation, 33% RDF with vermicompost showed significant build up of soil organic carbon which lead to increased seed yield and biological. Dravid and Goswami in 1988 observed similar positive effects of integration of chemical fertilizers with organic manures. Further, 33% RDF in combination with poultry manure significantly increased seed protein content as compared to vermicompost. This is in concurrence with the findings of Sardana and Sidhu (1994).

In biofertilizers, 33% RDF in combination with Phosphate solubilizing bacteria + *Azospirillum* showed higher seed yield and biological yield though stataistically not significant over the other combination. However, Phosphate solubilizing bacteria + 2 foliar application of 33% cow's urine showed significant increase in seed oil content over the other combination. In contrast, the protein content of seed was found to be significantly higher in double inoculation (Phosphate solubilizing bacteria + *Azospirillum*) with 33% RDF. Ram *et al.*, 1992 reported similar results in sunflower.

REFERENCES

Babu, S.C. and Subramanian, S.R. (1990) Substitution between organic manures and chemical fertilzers, under yield uncertainity – policy comparisons for irrigated and dryland farming systems. Sustainable Agric. – Issues, Perspectives and Prospects in Semi-Arid Tropics. Vol. 1 Proc. Ist Int. Symp. on Nat. Resources Management, pp 1-16

Dravid, M.S. and Goswami, N.N. (1988) Relative efficiency of mustard and sunflower in utilizing soil phosphorus in presence of P, Zn and FYM under normal and saline soil conditions. *Journal of Nuclear Agric. Biol.* **17**: 18-22

Sardana, V. and Sidhu, M.S (1994) Effect of INM on the quality and yield of Indian rape (*B. campestris,* var. *toria*) + Swede rape (*B. napus*) intercropping system. *Crop Res.* Hissar **8** (3): 431-436

Ram, G.; Patel, J.K.; Chame, N.N. and Choudhary, K.K. (1992) Single and combined effect of biological, organic and inorganic fertilzers on yield of sunflower and soil properties under rainfed conditions., *Advances in Plant Sci.* **5** (1): 161-167

Warren, W.S and Garry, L, (1985) Crop rotaions and manure versus agricultural chemicals in dry land grain production. *J. Soil Water Cons.* 40 (4): 511-515

Table 1: Effect of INM on the soil organic matter content, certain yield attributes and oil & protein content in seed of mustard (var. Pusa Bold) crop in the cropping system

Factors	Soil Organic Carbon (%)		Test weight (g)		Seed yield (kg ha^{-1})		Biological yield (kg ha^{-1})		Oil content of seeds (%)	Protein content of seeds (%)
	1997-98	1998-99	1997-98	1998-99	1997-98	1998-99	1997-98	1998-99	1998-99	
Levels of fertilizers (F):										
F_0	0.275	0.565	4.96	5.04	746.90	1078.00	2864.00	4816.00	31.54	5.062
F_1	0.354	0.625	5.73	5.16	2181.00	2269.00	6967.00	8935.00	32.22	4.862
F_2	0.347	0.639	5.63	5.25	3017.00	3486.00	10830.0	13270.0	31.16	4.670
CD(0.05)	-	-	0.3264	0.0437	269.46	321.18	1051.20	976.81	0.0923	0.00497
Interaction between level of fertilizers & forms of manure (FxC):										
$F_1 \times C_1$	0.266	0.714	5.70	5.15	2010.00	2376.00	6422.00	9256.00	31.92	4.723
$F_1 \times C_2$	0.468	0.599	5.79	5.27	2382.00	2113.00	6941.00	8672.00	32.04	4.927
CD(0.05)	0.1450	0.1470	0.5655	0.0758	466.82	556.30	1820.95	1692.10	0.1599	0.0086
Interaction between level of fertilizers & biofertilizers and/or organic spray (FxB):										
$F_1 \times B_1$	0.398	0.572	5.87	5.26	2139.00	2308.00	7052.00	9084.00	32.67	5.147
$F_1 \times B_2$	0.363	0.657	6.06	5.04	2105.00	2238.00	6759.00	8919.00	33.06	4.853
CD(0.05)	0.1450	0.1470	0.5655	0.0758	466.82	556.30	1820.95	1692.10	0.1599	0.0086

Performance of mustard (*Brassica juncea L.*) in varying temperature regimes under rainfed condition.

M.K.Khushu and J.S.Jamwal

All India Coordinated Research Project On Agrometeorology
Dryland Research Station, SKUAST- J
Bari-Brahmana , Jammu - 181 133 (India)

Introduction

The day and night temperatures at which crops are grown have a decisive effect on the yield and quality of mustard. They influence the production and utilization of biochemical compounds and energy required for plant growth. Each phase of mustard growth has critical low and high temperature thresholds. Hence, an attempt was made to assess the performance of mustard in varying temperature regimes under rainfed conditions.

Materials and Methods

An investigation was carried out at research farm of Dryland Research Station, SKUAST, Dhiansar (32^0-39' N, 74^0-58' E and 332 meters amsl) during the *rabi* 1998-99. The climate of this area during *rabi* is influenced by western disturbances with average rainfall of 283 mm. Two mustard cultivars Varuna and Pusa Bahar were sown on three different dates (D_1- October 9, D_2- October 24, and D_3- November 8) with 15 days interval in a randomised block design with four replications to expose the crop to different set of environments. All recommended package of practices for mustard under rainfed conditions were followed. Total dry matter production and seed (q /ha) were recorded in each plot. Daily temperature data recorded at Agromet observatory Dhiansar were used for this study. The average daily temperature experienced by the crops from emerengence to flower bud initiation, flower bud initiation to pod formation and pod formation to maturity were denoted as T_1, T_2 and T_3, respectively.

Results and Discussion

The results presented in table:-1 show that date of sowing have significant effect on both seed yield and total drymatter production. Second date of sowing (D_2) gave significantly higher seed yield than first (D_1) and third (D_3) dates of sowings. The lower yield in first date of sowing as compared to second date of sowing could be because of relatively higher day and night temperatures exprienced by the crop during the period from emergence to flower bud initiation (T_1)

Table.1 Seed yield as affected by different temperature regimes

Treatment	Mean day/ night temperature (°C)			Seed yield (q/ha)	Total Drymatter (q/ha)
	T_1	T_2	T_3		
9- October (D_1)					
Varuna	29.1/14.6	25.6/9.0	20.1/8.1	5.40	28.60
Pusa Bahar	29.2/14.4	25.9/9.1	20.1/8.1	4.25	24.30
24- October (D_2)					-
Varuna	27.2/10.8	19.7/6.0	21.3/9.2	7.50	43.97
Pusa Bahar	27.3/11.0	20.1/6.2	21.6/9.3	7.35	38.90
8- November (D_3)					
Varuna	24.4/8.4	16.2/6.1	24.6/10.7	4.67	26.20
Pusa Bahar	24.5/8.4	16.2/6.1	24.3/10.5	3.92	18.50
			CD at 5 % for Date	0.97	3.80
			Variety	N.S	3.11
			Date x Variety	N.S	N.S

and from flower bud initiation to pod formation (T_2), whereas relatively higher temperature prevailed during the period from pod formation to maturity in third date of sowing have reduced the yield of mustard significantly. The higher temperature might be responsible for imblance between the production and utilization of phothosynthates thereby reducing the yield. Similar trend was observed in total drymatter production. The results are in close agreement with findings of Kar and Chakravarty (1999).

References:

Kar, Gouranga and Chakravarty N.V.K. (1999). Thermal growth rate, heat and radiation utilization efficiency of Brassica under semiarid environment. *Journal of Agrometeorology* 1(1) :41-49

INFLUENCE OF SOWING DATES ON TEST WEIGHT, SEED YIELD AND OIL CONTENT IN *Brassica juncea*.

Ramesh Kumar and N.K. Thakral
Oilseeds Section, Deptt. of Plant Breeding,
CCS HAU, Hisar – 125 004 (India)

INTRODUCTION

Indian mustard (*Brassica juncea* L.) is an important rabi oilseed crop, normally sown in mid October but due to late picking of cotton crop, sowing of mustard get delayed in northern India (Jain *et el.*, 1986). So, the present investigation was aimed to evaluate 10 Indian mustard genotypes under two environments.

MATERIALS AND METHODS

The material for this study comprised of 10 genotypes namely RH 8113, RC 781, UDN 69, RH 9617, RH 9624, Bio-902, RH 819, RL 1359, Varuna and Sarita were sown on Oct. 21st (E_1) and Nov. 23rd 2001 (E2). The data were recorded for 1000-seed weight, seed yield and analysed for oil content.

RESULTS AND DISCUSSION

1000 seed weight, seed yield as well as oil content were greatly influenced by sowing dates. Early sown crop had performed better for all the three characters as compared to late sown crop. The genotype Bio-902 under normal environment had boldest seed size whereas, Sarita had maximum test weight under late sown environment. Seed yield per plant was maximum in Bio-902 whereas, oil content was maximum in the genotype RC781 under late sown conditions. The higher oil content and seed yield of early sown crop might be due to the longer reproductive phase and cooler temperature at the time of seed developmental stage of the crop. Similar results were also reported by Shastry and Kumar (1981) and Jain *et al.*, 1986.

REFERENCE

Jain, V.K.; Chauhan, Y.S.; Khandekar, M.P; Jain, P.C and Rathore, D.R. 1986. Performance of raya varieties under late sown conditions. *Indian J. Agron.* 31 (4): 374-376.

Shastry, A.B. and Kumar, A. 1981. Variation in yield, its attributes and quality of Indian mustard in relation to planting time and level of plant population. *Indian J. Agri. Sci.* 51:27-32.

Table: Effect of date of sowing on 1000-seed weight, seed yield and oil content of Indian mustard.

Genotypes	1000-seed weight (g)		Seed yield/plant (g)		Oil content (%)	
	E_1	E_2	E_1	E_2	E_1	E_2
RH 8113	4.62	4.63	17.26	10.68	39.17	38.33
RC 781	3.11	2.77	14.93	10.88	41.83	40.75
UND 69	2.82	2.56	11.33	8.40	41.25	38.24
RH 9617	4.61	4.11	11.59	11.32	40.58	37.91
RH 9624	5.50	4.46	20.46	11.16	41.43	38.59
Bio-902	6.16	4.59	17.43	14.03	39.03	37.00
RH 819	4.14	3.01	16.46	9.30	40.37	37.42
RL 1359	4.53	4.00	15.37	12.24	41.65	37.52
Varuna	5.57	4.41	18.91	12.08	39.92	37.60
Sarita	5.60	4.76	17.91	11.86	40.40	38.52
Mean	4.76	3.93	16.16	11.19	40.56	38.18
CD (5%)	1.92	1.82	1.59	2.24	1.43	1.60

E_1 = Early sown (21st Oct, 2000)
E_2 = Late sown (23rd Nov., 2000)

INFLUENCE OF LEAF EXTRACTS ON CULTIVATED TURNIP VI. ROOT WEIGHT AND NO. OF SILIQUE/PLANT

Chandreshwar Prasad
University Department of Botany, Vinoba Bhave University, Hazaribag - 825 301, Jharkhand, India

ABSTRACT

Moderate higher doses of periwinkle (*Catharanthus roseus* Don.) leaf extract have enhancing effect on root weight and number of silique/plant in turnip (*Brassica rapa* L.). Carrot weed (*Parthenium hysterophorus* L.) extract demonstrates a deleterious effect, but neem (*Azadirachta indica* A. Juss.) leaf extract has been found stimulating on both the traits.

INTRODUCTION

The first and foremost goal of a plant breeder is to achieve higher yield. Being a root crop plant, any improvement in the root may be regarded as an achievement in the crop. Besides number of silique/plant is a quantitative character and is directly related to yield. The commonly used chemical mutagens are not only expersive but also injurious to health and environment. Hence search for safe and effective mutagens of botanical origin can be an important approach (1-5). Present work was carried out in order to see the effect of aqueous leaf extracts of periwinkle, carrot weed and neem on root weight and number of silique/plant in turnip.

MATERIALS AND METHODS

'Rose Red' variety of turnip coustituted the material for present investigation. The method of preparing solutions of different concentrations, mode of seed treatment and raising of the plants were the same as described earlier (3). The roots of 25 randomly selected plants in each concentration under all the three treatments were weighed separately to score mean root weight. To compute number of silique/plant, the silique attached on each of 25 plants in each case were counted. The results are presented in Table 1.

RESULTS AND DISCUSSION

The marked effects of the three leaf extracts were noted on root weight and number of silique/plant of turnip. Under the periwinkle extract, a decrease in the root weight was observed at 20% concentration, followed by a gradual increase from 40% to 80% and again a sudden fall at 100% concentration in M_1 generation. The plants under 80% treatment bore the largest and heaviest roots and a noticeable improvement took place in M_2 at all the doses. Carrot weed extract caused a gradual decrease in root weight from lower to higher doses in M_1 generation, with a considerable recovery in M_2 at all the doses but not to the extent of control. There was an enhancing effect of neem extract on the root weight of turnip at all the doses (except 100%) in M_1 generation which further improved in M_2. The plants under 40% treatment demonstrated maximum enhancement in root weight. So far as the effect of various leaf extracts on the number of silique/plant was concerned, more or less it followed the same pattern as seen in case of root weight.

Several alkaloids have been isolated, purified and determined chemically from periwinkle plant and two most active among them are vinblastine and vincristine which have antitumour effect (6). The activity of the extract is found to be entirely located in the alkaloid fraction and the prolonged exposure to relatively high concentrations of alkaloids do not produce gross morphological or cytological changes (6). Perhaps this is the reason why periwinkle extract at 100% concentration in unable to make any drastic charge in the yield and yield components of turnip. The alkaloids of periwinkle produce the effects similar to those observed after colchicine treatment at the cellular level, with a tendency to polyploidy (8). Vincristine is also known to interfere with some metabolic reactions related to DNA and RNA synthesis (9). Under the present work, there is stimulating effect of periwinkle extract on both the traits at the moderate higher doses.

Carrot weed yields a non-alkaloidal, non-glycosidic substance called parthenin (10). It has some soluble inhibitors like caffeic acid, P-coumeric acid, anedic acid, vanillic acid and P-hydroxy benzoic acid (11-12). The extract exhibits a retarding effect on both the traits. Similar effects have been reported on growth and yield of some other crops like wheat (12), cowpea (13) and ragi (11). There in a sincere suggestion not to use carrot weed as green manune because it exhibils allelopathic activity (11, 14).

Neem leaf extract has nimbin, nimbenene, 6 - desacetabimbinene, nimbadiol, nimbolide and querction (15). Besides appreciable amount of protein, minerals, carotenes and trace elements (except zinc) are also present (16). N, P and C balances are positive. Under present work an enhancing effect of neem extract was seen on both the traits in M_1 generation, which further improved in M_2. At present it may be presumed that all or a few of these constituents may be directly or indirectly related to the stimulating effect of the extract. However, only future biochemical investigations can give a correct answer in this regard.

The M_2 plants at 80% and 40% concentrations, under periwinkle and neem leaf extracts treatment respectively, had almost two times larger and heavier roots than their control. From these, superior line(s) can be developed through selection in successive generations.

Table 1 . Effect of botanical extracts on root weight and number of silique/plant in turnip.

Leaf extract	Dose	Generation	Root weitht (g)		No. of Silique/plant	
			Mean± SE	CV (%)	Mean± SE	CV (%)
Periwinkle	Control	M_1	170.4±2.08	6.09	78.2±1.02	6.50
		M_2	175.4±2.08	5.92	80.0±1.10	6.85
	20%	M_1	164.6±2.30	6.97	78.0±1.06	6.78
		M_2	168.8±2.90	5.43	77.8±1.10	7.07
	40%	M_1	172.0±2.12	6.15	77.6±1.02	6.60
		M_2	173.6±2.11	6.07	77.8±1.06	6.83
	60%	M_1	180.0±1.96**	5.44	77.6±0.94	6.08
		M_2	184.2±1.95**	5.30	77.2±1.06	6.88
	80%	M_1	220.0±2.19**	4.98	100.4±1.02**	5.06
		M_2	242.0±2.19**	4.52	103.0±1.20**	5.83
	100%	M_1	112.8±2.00**	8.87	72.2±1.13**	7.87
		M_2	130.4±2.08**	7.96	75.2±0.96**	6.37
Carrot weed	Control	M_1	650.4±1.06	0.81	200.4±0.98	2.44
		M_2	659.6±1.02	0.77	190.2±0.97	2.54
	20%	M_1	447.6±0.99**	1.11	186.6±1.06*	2.83
		M_2	640.6±0.81**	0.63	199.8±1.11**	2.78
	40%	M_1	499.6±1.07**	1.07	167.0±1.17**	3.49
		M_2	597.6±0.94**	0.79	185.4±1.01**	2.72
	60%	M_1	451.2±1.04**	1.15	131.4±1.06**	4.02
		M_2	547.4±0.90**	0.82	186.4±1.08*	2.89
	80%	M_1	350.6±0.91**	1.29	111.4±1.09**	4.90
		M_2	548.0±0.98**	0.89	185.4±0.93**	2.50
	100%	M_1	248.8±0.92**	1.86	39.41.00**	12.69
		M_2	450.2±0.92**	1.02	180.0±1.02**	2.83
Neem	Control	M_1	168.3±2.17	6.45	68.0±0.34	2.51
		M_2	172.0±2.02	5.88	68.7±0.52	3.83
	20%	M_1	185.7±2.32**	6.25	72.8±0.67**	3.67
		M_2	187.3±2.10**	5.61	77.4±0.50**	3.23
	40%	M_1	438.7±3.84**	4.38	84.8±0.55**	3.26
		M_2	445.4±3.74**	4.20	90.6±0.58**	3.20
	60%	M_1	281.2±2.97**	5.28	82.7±0.64**	3.88
		M_2	279.2±2.95**	5.28	89.3±2.24**	2.24
	80%	M_1	261.8±2.30**	4.40	79.8±0.63**	3.98
		M_2	262.6±2.01**	3.83	82.0±0.43**	2.61
	100%	M_1	160.8±1.52**	4.75	65.5±0.39**	2.96
		M_2	162.8±1.51**	4.63	66.9±0.47**	3.54

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

REFERENCES

1. Prasad, C. (1998). *Columban J. Life Sci.* **6** : 349-351.
2. Prasad, C. (2001a). *Bionature.* **21** : 31-35
3. Prasad, C. (2001b). *Eucarpia Cruciferae Newsletter.* **23** : 27-28.
4. Prasad, C. (2001c). *Eucarpia Cruciferae Newsletter.* **23** : 29-30.
5. Prasad, C. (2002). *Geobios.* **29** : 20-23.
6. Johnson, I. J., Wright, H. & Svobodo, G. (1959) *T. Lab. Clin. Med.*, **54** : 830.
7. George, P., Journey, L. & Goldstern, M. (1965). *J. Natt. Cancer Inst.* **35** : 355-361.
8. Degraeve, N. (1978). *Mutation Research.* **55** : 31-42.
9. Shrivastava, S., Rao, S. & Shah, V. (1973). *Cytologia.* **38** : 1-9.
10. Arny, H.V. (1897). *Ameri. J. Pharm.* **69** : 168.
11. Kanchan, S.D. (1975) *Curr. Sci.* **44** : 358-359.
12. Laxmi Ranjan (1973). *Curr. Sci.* **42** : 729-730.
13. Kanchan, S.D. & Jaychandra (1976). Proc. Seminar on Parthenium : a possitivedanger. Bangalore p.p. 11-13.
14. Whittaker, R.H. & Feeny, P.P. (1971). *Science*, 171 : 757.
15. Devakumar, C. & Mukherjee, S.K. (1983). *I.A.R.I. Res. Bull.* **40** : 1-17.
16 Singhal, K.K. & Mudgal, V.D. (1983).*J. Punjab Agricultural University*, Ludhiana, p. 165.

BOOK ANNOUNCEMENT

Field Guide to
PESTS, DISEASES AND DISORDERS *of* VEGETABLE BRASSICAS

By C. Donald, N. Endersby, P. Ridland, I. Porter and J. Lawrence
Agriculture Victoria, Institute for Horticultural Development, Knoxfield and
L. Ransom Australian Quarantine and Inspection Service (AQIS)

A full colour, hard wearing booklet covering pests (with built in scale to size drawings), beneficial insects, diseases and nutritional, genetic, physiological, environmental and chemical disorders of the vegetable brassicas.

85 pages, including 120 colour photographs
Laminated and conveniently sized for field or reference use.
Price: A$25.00 (plus postage A$9.00 for delivery outside Australia)

DEPARTMENT OF NATURAL RESOURCES AND ENVIRONMENT
Crop Health Services Bookshop
Private Bag 15
Ferntree Gully Delivery Centre
Victoria 3156 Australia
Fax: +61 3 9210 9356 Phone: +61 9887 3166

EUCARPIA CRUCIFERAE NEWSLETTER Nr. 25

Instructions to authors
2002

Deadlines : **december 31st 2002**

Next Cruciferae Newsletter Nr 25 will be produced and edited at the beginning of year 2003. 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.
If possible, the contribution should be sent by email in attached file (2 pages) to email
bernard@agrorennes.educagri.fr

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 – If sent by air or post, please do not fold the script and do not clip the pages together.
Please send two copies of each protected by card.
All invoices to :

The Editor
Cruciferae Newsletter
INRA - ENSAR
UMR Amélioration des plantes et biotechnologies végétales
(UMR APBV)
Domaine de la Motte
BP 29
35653 LE RHEU CEDEX
FRANCE

All contributions must be sent before :
January 31st 2003 to the above address for edition in the next issue **: Cruciferae Newsletter Nr. 25**