Iranian Journal of Science & Technology, Transaction A, Vol. 30, No. A1
Printed in The Islamic Republic of Iran, 2006

CYTOGENETIC STUDIES IN ELEVEN CANOLA (*BRASSICA NAPUS* L.) CULTIVARS*

M. SHEIDAI[1**] AND Z. NOORMOHAMADI[2]

[1] Biology Department, Shahid Beheshti University, Tehran, I. R. of Iran
[2] Genetic Department, Tarbeiyat Modarres University, Tehran, I. R. of Iran
Email: msheidai@cc.sbu.ac.ir or msheidai@yahoo.com

Abstract – Meiotic study was performed in 11 *Brassica napus* cultivars considering chiasma frequency and distribution, chromosome pairing, as well as the occurrence of B-chromosomes and their effects on chiasma frequency. Such cytogenetic pairing, along with agronomic characteristics, may be used in planning hybridization among the *B. napus* cultivars. All the cultivars studied possessed n=19 chromosome number (4x) and showed a deviant course of prophase-I meiosis with a synezetic knot and post pachytene diffuse stage. Chromosome stickiness occurred in most of the cultivars from early prophase to late telophase-II leading to the formation of laggard chromosomes and micronuclei. The cultivars studied differed significantly in chiasma frequency and distribution as well as bivalent formation, indicating their genomic differences. Cluster analysis and ordination based on principal components analysis grouped those cultivars showing meiotic similarities. Some of the cultivars showed the occurrence of B-chromosomes.

Keywords – B-chromosome, canola, chiasma frequency, cluster analysis

1. INTRODUCTION

Oilseed rape (canola) (*Brassica napus* L.) is an important oil producing species extensively cultivated in Europe, China, North America and Iran. *Brassica. napus* is an amphidiploid species with 19 pairs of chromosomes and has evolved from interspecific crosses between *B. rapa* (2n=2x=20) and *B. oleracea* (2n=2x=18). Both summer and winter annual forms are cultivated in Iran.

Winter oilseed rape could be cultivated in rotation with wheat in order to reduce diseases of wheat. Experiments have shown increases in wheat yields of up to 10% after rapeseed. High seed yield (over two tones per hectare) and high oil content (approximately 40%), in addition to low water needs in dry regions of Iran, have made it one of the most important crop plants that provide the oil needs of the country [1].

Due to the economic importance of oilseed rape, several *B. napus* cultivars have been introduced in Iran during the last decade and at present, germplasm evaluation as well as hybridization programs are in hand both by the authors and other researchers in the Seed and Seedling Breeding Research Center, Karaj, Iran. Therefore, a cytogenetic, electerophoretic and agro-morphological study of *B. napus* accessions/cultivars has been initiated (by the authors) in order to provide the basic information on the genetic variability of the cultivars available for planning future breeding and hybridization programs. Some basic cytogenetic and electerophoretic information characteristics have already been reported in a few *B. napus* cultivars available in Iran [1, 2], including chromosome pairing and the effects of B-chromosomes in a few cultivars, indicating cultivars genomic differences.

The frequency and distribution of chiasma is under genetic control [3-5] and the heritable adjustment

*Received by the editor October 23, 2004 and in final revised form January 3, 2006
**Corresponding author

in the frequency and distribution of chiasma and recombination, as well as their effects on the variability of progenies and populations is established in both experimental and natural populations [4].

B-Chromosomes may affect the frequency and distribution of chiasmata as well as chromosome association, either directly or by affecting the genes present on the A-chromosomes that control meiosis [6]. The earlier studies in some of the *B. napus* cultivars showed either a significant increase or decrease in chiasma frequency and chromosome pairing in the cells possessing B-chromosomes [2].

The objective of the present meiotic study was to investigate the chromosome pairing and segregation, chiasma frequency and distribution, meiotic abnormalities, and the occurrence of B-chromosomes and their effects on genetic recombination in eleven unreported *B. napus* cultivars available in Iran, which may be used for planning hybridization among the cultivars.

2. MATERIALS AND METHODS

Eleven *B. napus* L. cultivars were planted in plots in the experimental field of the Seed and Seedling Breeding Research Center, Karaj, Iran. Of the cultivars studied, their origin and annual forms are: 1-Goliath (summer type, Denmark), 2-Magnum (summer type, Canada), 3-Ceres (winter type, Germany), 4-Gazell (winter type, Germany), 5-DP948 (winter type, Italy), 6-SYN1 (winter type, Canada), 7-Eurol (winter type, France), 8-Consul (winter type, Denmark), 9-GWC (winter type, Germany), 10-Orkan (winter type, Germany) and 11-Hanson (winter type, Italy).

For cytogenetical studies, 50-100 flower buds were collected randomly from 10 plants from each cultivar between 9:00 to 12:00 in the morning, fixed in glacial acetic acid: ethanol 70% (1:3) for 24 hrs, washed thoroughly with water and transferred to 85% ethanol until used [1].

Chromosome pairing and chiasma frequency were determined in a minimum of 100 meiocytes showing diakinesis/ metaphase-I stages, while chromosome segregation was studied in a minimum of 500 anaphase-I and II stages.

Pollen stainablity was used as a measure of pollen fertility by staining m 1000 pollen grains with a 1:1 solution of 2 % acetocarmine: 50 % glycerin for about ½ hr. Round and fully developed, completely stained pollen were recorded as fertile, while incompletely developed shrunken pollen with no stain were considered non-fertile pollen [2]. Analysis of variance (ANOVA) followed by least significant difference test (LSD) was performed on cytogenetic characteristics including chromosome pairing and chiasma frequency, as well as distribution to indicate any significant difference among the cultivars studied [1].

Different methods of cluster analysis were applied including UPGMA (unweighted paired group mean average) and WARD (minimum variance spherical clusters), as well as ordination based on principal components analysis (PCA) to identify cultivars showing similarities in their meiotic characteristics [7]. For cluster and principal components analysis, standard values (mean = 0, variance=1) were used. Squared Euclidean distance was used as a measure of similarity in cluster analysis [1]. The statistical analyses used SPSS ver.9 software.

3. RESULTS

a) Diffuse stage in meiosis-I prophase

The meiotic analysis of the eleven *B. napus* cultivars studied showed a deviant course of meiosis-I prophase sub-stages, i.e. the occurrence of synezetic knot stage instead of leptotene and zygotene. In the early synezetic knot stage, thin chromatin strands surround the nucleolus until it is totally covered (Fig. 1). In the pachytene stage paired chromosomes (now thick strands) unraveled from the knot. End to end attachment of chromosomes in pachytene is a feature reported in those taxa showing a synezetic knot stage

[4]. However despiralization of chromosomes occurred after pachytene towards the diffuse stage (Fig. 2). After diffuse, the diplotene stage commences showing secondary contraction followed by diakinesis and metaphase stages.

b) Chromosome pairing and chiasma frequency

The *B. napus* cultivars studied and their meiotic characteristics are presented in Tables 1 and 2 (Figs. 3-6). All cultivars possessed n=19 (2n=4x=38) chromosome number. The highest value of total chiasma occurred in the Goliath cultivar (34.59), while the lowest value occurred in the Gazell cultivar (31.67).

The highest value of terminal chiasma occurred in the Consul cultivar (33.40) and the lowest value occurred in the Gazell cultivar (30.54). In the case of intercalary chiasma, the highest value was observed in the Goliath cultivar (4.10) and the lowest values occurred in the Hanson cultivar (0.52).

With regard to chromosomal association, the highest value of ring bivalents occurred in the Consol cultivar (15.54), while the lowest value occurred in the Ceres cultivar (11.09). The highest value of rod bivalents was observed in the Gazell cultivar (5.59) and the lowest value occurred in the Goliath cultivar (2.98). Quadrivalents were formed in all the cultivars studied (Figs. 3 & 4) ranging from 0.02 in the Gazell cultivar to 0.35 in DP948 (Table 1). The quadrivalents formed were of ring shape, open chain and frying pan type. In general, a low frequency of univalents were observed in all the cultivars (Table 1). The highest value of univalents was observed in Syn1 cultivar (0.16), while the lowest value occurred in three cultivars of Ceres, DP948 and GWC (0.02).

B-chromosomes (0-2) were observed in Ceres and Magnum cultivars (Fig. 5). These were smaller than the A-chromosomes and did not form any meiotic association with them**,** although they could arrange themselves along with the A-chromosomes on the equatorial plane of the spindle and move to the poles during anaphase. In some cases they occurred as laggard chromosomes. The cultivars studied showed >0.95 % pollen fertility (Table 1, Fig. 6).

The ANOVA test revealed the presence of a significant difference ($p < 0.01$) for chiasma frequency and distribution, as well as bivalent formation among the cultivars studied (Table 2). Therefore, at least two cultivars differ significantly on their meiotic characteristics. The LSD test showed such a significance among most of the cultivars (Figs. 7 & 8), particularly those cultivars, which are placed in different clusters/groups in cluster analysis and ordination based on PCA (explained in the following paragraphs). No significant difference was observed for quadrivalents and univalents.

Table 1. Meiotic characteristics and pollen fertility in *B. napus* cultivars studied

Cultivar	Terminal Chiasmata	Intercalary chiasma	Ring bivalant	Rod bivalant	quadrivalant	univalant	Total chiasma	Pollen fertility	n
Goliath	30.85 ± 0.2 (25-35)	4.10 ± 0.26 (0-9)	12.04 ± 0.15 (9-15)	2.98 ± 0.15 (1-7)	0.17 ± 0.04 (0-1)	0.05 ± 0.02 (0-1)	34.95 ± 0.26 (31-42)	99.5	19
Magnum	31.06 ± 0.21 (28-35)	1.88 ± 0.16 (0-5)	12.37 ± 0.18 (10-16)	4.7 ± 0.17 (2-7)	0.05 ± 0.02 (0-1)	0.05 ± 0.02 (0-1)	32.94 ± 0.16 (30-36)	99.7	19
Ceres	30.60 ± 0.27 (26-33)	3.90 ± 0.24 (1-7)	11.09 ± 0.21 (8-14)	4.17 ± 0.21 (2-6)	0.12 ± 0.05 (0-1)	0.02 ± 0.02 (0-1)	34.51 ± 0.35 (29-40)	98.5	19
Gazell	30.54 ± 0.36 (25-35)	1.13 ± 0.16 (0-3)	12.05 ± 0.29 (7-16)	5.59 ± 0.35 (1-10)	0.02 ± 0.02 (0-1)	0.1 ± 0.05 (0-1)	31.67 ± 0.39 (26-37)	98.7	19

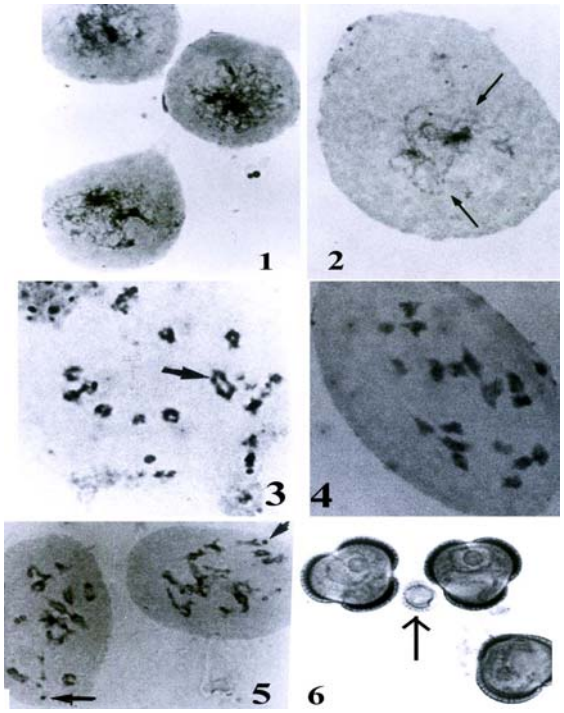
Table 1. (Continued)

DP948	32.05 ± 0.22 (29-35)	1.86 ± 0.18 (11-15)	12.94 ± 0.14 (11-15)	3.51 ± 0.18 (1-6)	0.35 ± 0.07 (0-1)	0.02 ± 0.02 (0-1)	33.91 ± 0.2 (32-36)	99.5	19
SYN1	31.07 ± 0.19 (27-36)	1.92 ± 0.15 (0-6)	12.41 ± 0.15 (9-17)	4.14 ± 0.11 (2-6)	0.2 ± 0.05 (0-2)	0.16 ± 0.04 (0-2)	33 ± 0.17 (8-29)	99.7	19
Eurol	31.98 ± 0.23 (28-35)	1.82 ± 0.21 (0-6)	14.85 ± 0.21 (13-18)	3.94 ± 0.22 (1-6)	0.08 ± 0.04 (0-1)	0.11 ± 0.05 (0-1)	33.79 ± 0.29 (30-38)	99.7	19
Consul	33.4 ± 0.25 (29-36)	0.85 ± 0.15 (0-3)	15.54 ± 0.18 (13-17)	3.4 ± 0.17 (2-6)	0.05 ± 0.03 (0-1)	0.05 ± 0.03 (0-1)	34.25 ± 0.25 (30-36)	99.8	19
GWC	32.1 ± 0.32 (27-35)	1.41 ± 0.21 (0-5)	14.92 ± 0.20 (12-18)	3.94 ± 0.20 (1-7)	0.1 ± 0.04 (0-1)	0.02 ± 0.02 (0-1)	33.53 ± 0.30 (29-37)	99.8	19
Orkan	32.87 ± 0.27 (28-36)	1.30 ± 0.19 (0-5)	15.15 ± 0.19 (13-18)	3.69 ± 0.18 (1-5)	0.06 ± 0.04 (0-1)	0.09 ± 0.05 (0-1)	34.18 ± 0.28 (30-36)	99.8	19
Hanson	32.38 ± 0.29 (28-35)	0.52 ± 0.09 (0-2)	14.52 ± 0.19 (12-17)	4.30 ± 0.20 (1-9)	0.11 ± 0.05 (0-1)	0.05 ± 0.04 (0-1)	32.91 ± 0.28 (28-35)	99.7	19

First row=Mean, second row=Standard error, third row=range

Table 2. ANOVA of meiotic characteristics among *B. napus* cultivars studied

		Sum of		**Mean**		
		Squares	df	Square	F	Sig.
terminal chiasmata	Between Groups	95.309	10	9.531	3.048	.005
	Within Groups	137.600	44	3.127		
	Total	232.909	54			
intercalary chiasmata	Between Groups	155.309	10	15.531	9.707	.001
	Within Groups	70.400	44	1.600		
	Total	225.709	54			
ring bivalent	Between Groups	80.400	10	8.040	6.228	.001
	Within Groups	56.800	44	1.291		
	Total	137.200	54			
rod bivalent	Between Groups	88.036	10	8.804	5.663	.001
	Within Groups	68.400	44	1.555		
	Total	156.436	54			
quadrivalent	Between Groups	1.345	10	.135	1.480	.179
	Within Groups	4.000	44	9.091E-02		
	Total	5.345	54			
univalent	Between Groups	.327	10	3.273E-02	.900	.541
	Within Groups	1.600	44	3.636E-02		
	Total	1.927	54			
total chiasmata	Between Groups	130.036	10	13.004	4.321	.010
	Within Groups	132.400	44	3.009		
	Total	262.436	54			



Figs. (1-6). 1=Synezetic knot stage in Orkan cultivar, 2=A post pachytene diffuse stage in Magnum cultivar (arrows indicate decondensed chromatin regions), 3= Metahase I cell showing quadrivalents (arrow) in Gazell cultivar, 4=Metahase I cell showing 19 bivalents in DP948, 5=B-chromosomes (arrow) in Magnum cultivar, 6=Fertile and infertile (arrow) pollen grain in Goliath cultivar

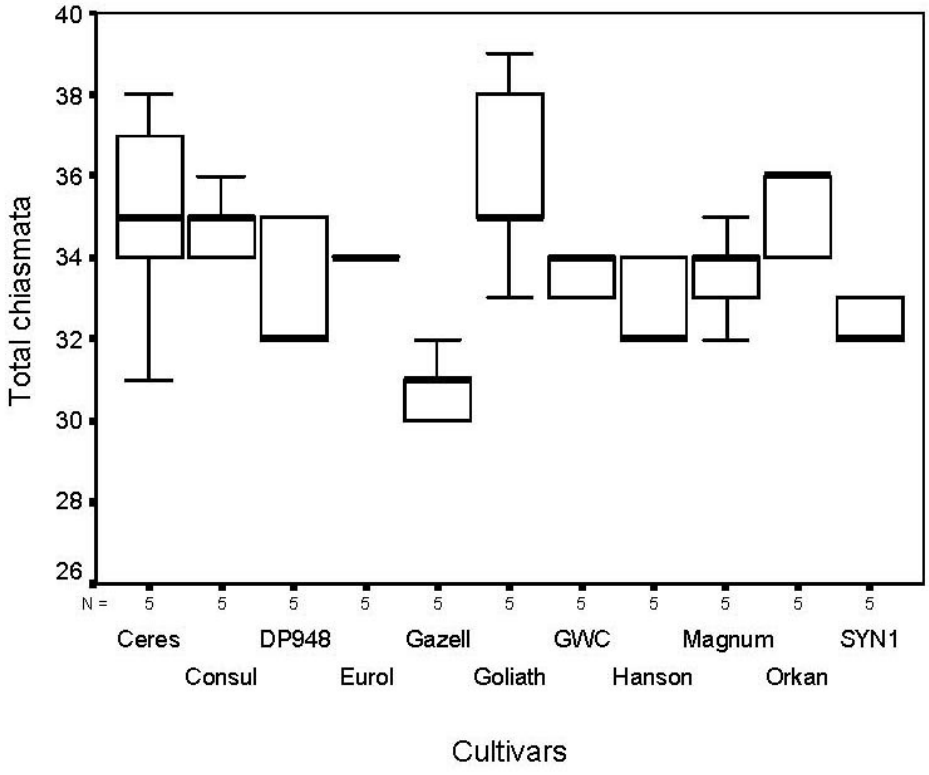


Fig. 7. Box plot showing the mean comparison of total chiasmata among *B. napus* cultivars studied (cultivars 1-11 as in Fig. 11)

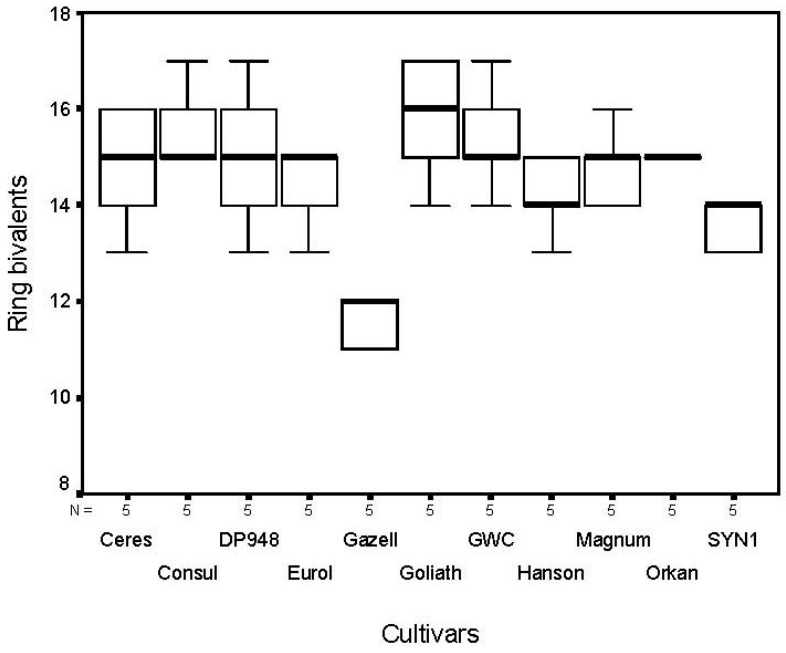


Fig. 8. Box plot showing the mean comparison of ring bivalents among *B. napus* cultivars studied (cultivars 1-11 as in Fig. 11)

Data with regard to chromosome segregation is provided in Figs. 9 & 10. Although in most of the cases, normal chromosome segregation occurred during anaphase and telophase stages, some amount of irregularities did occur which were mainly of chromosome stickiness, formation of laggard chromosomes and micronuclei. Sticky chromosomes were observed from early stages of prophase and continued to the final stages of meiosis in most of the cultivars studied. Such a phenomenon has also been reported in some of the *B. napus* and *B. campestris* cultivars in Brazil [8]. Chromosome bridges resulting from stickiness were observed in anaphase-I and II, as well as telophase-I and II stages. The percentage of cells showing stickiness differed among the cultivars studied. The thickness of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes and the cultivars studied.

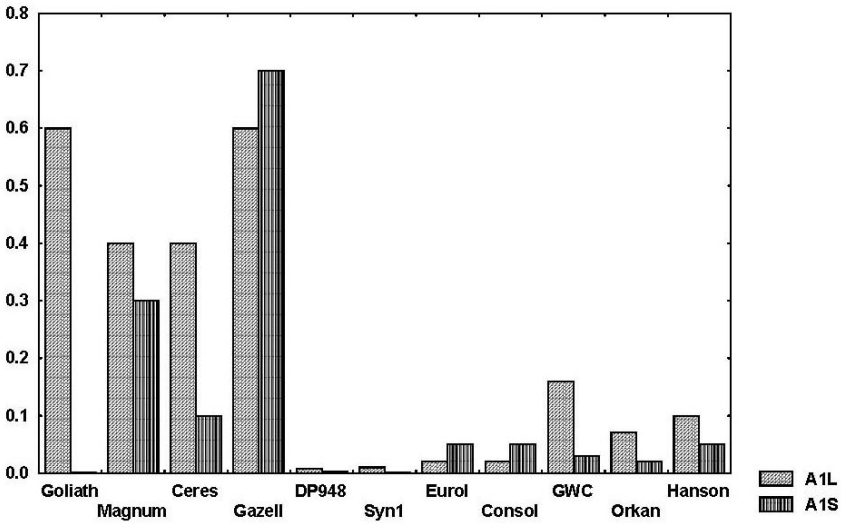


Fig. 9. Percentage of meiotic abnormalities in *B. napus* cultivars studied. Abbreviations as in Table 3

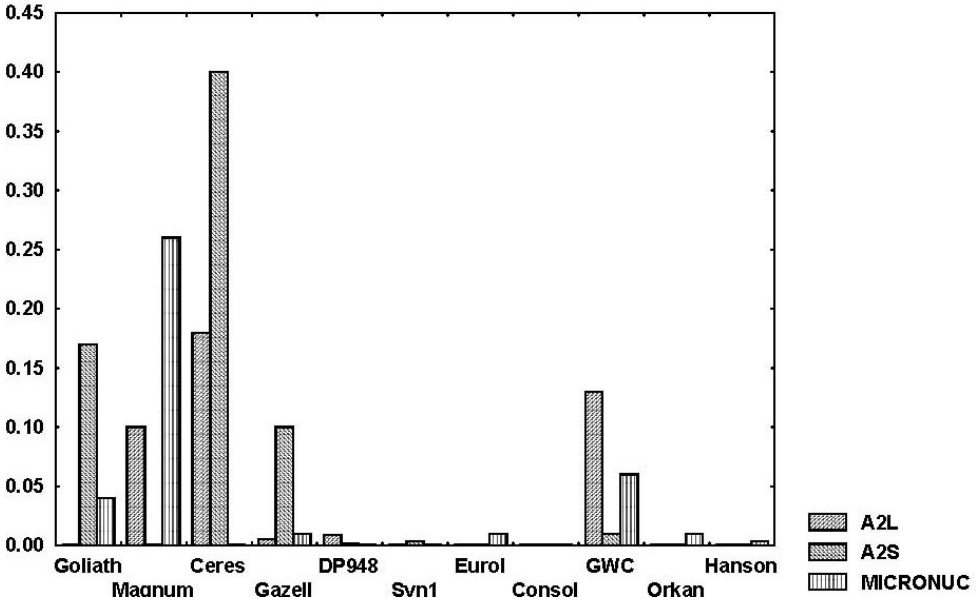


Fig. 10. Percentage of meiotic abnormalities in *B. napus* cultivars studied. Abbreviations as in Table 3

4. DISCUSSION

The occurrence of a diffuse stage has been reported in several plant species [9], which may be of the complete type (the whole chromosomes decondense) or it may be partial (some parts of the genome show decondensation). The present study showed the occurrence of partial diffuse in *B. napus* cultivars studied.

Various reasons have been suggested for the occurrence of the diffuse stage. These are: high synthetic activity analogous to the lampbrush stage in amphibian oocyte, shedding of the lateral elements in the synaptonemal complex, the post pachytene elimination or modification of histone proteins and meiotic arrest to withstand the adverse environmental conditions [10].

Domesticated *B. napus* exhibits predominantly bivalent chromosome pairing, while resynthesized *B. napus*, formed from interspecific crosses between *B. campestris* and *B. oleraceae*, exhibits multivalent formation that probably reflects pairing between homoeologous chromosomes [11]. Therefore it may be suggested that residual homoeologous recombination in the cultivars studied may be the reason for quadrivalent formation in those cultivars, as also suggested by Sharpe et al. [12] in other *B. napus* domesticated cultivars.

The presence of a significant difference in chiasma frequency and distribution as well as ring and rod bivalents among the cultivars studied may partly indicate their genomic differences as these plants were grown under uniform conditions in the experimental field. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness [13]. The difference observed in the percentage of the stickiness, and also its complete absence in some of the cultivars studied may also indicate the effect of genomic background on this phenomenon, however genomic-environmental interaction can not be ruled out as also suggested for the similar situation in *B. napus* [13] and *Avena sativa* cultivars [14].

The Pearson coefficient of correlation, determined among meiotic characters, revealed a positive significant correlation ($p < 0.05$) between the percentage of laggard cells in anaphase-I with stickiness in anaphase-I. On the other hand, pollen fertility showed a negative significant correlation with the percentage of stickiness and laggards in Anaphase I (Table 3). These meiotic abnormalities may have some effect on reducing the pollen fertility, however the cultivars studied showed a high pollen fertility (> 98 %, Table 1) indicating that meiotic irregularities mentioned do not have a major effect on pollen

fertility reduction. A similar situation has been reported in some polyploid species including hexaploid oat and wheat [14, 15].

Table 3. Correlation test between pollen fertility and meiotic abnormalities, r >0.60 indicates significant at 0.05 level

	A1L	**A1S**	**A2L**	**A2S**	**U**	**MIC**	**PF**
A1L	1.000	.621	.284	.589	-.205	.326	-.627
A1S	.621	1.000	.042	.110	-.155	.254	-.561
A2L	.284	.042	1.000	.578	-.195	.370	-.409
A2S	.589	.110	.578	1.000	-.165	-.168	-.827
U	-.205	-.155	-.195	-.165	1.000	-.111	.225
MIC	.326	.254	.370	-.168	-.111	1.000	.204
PF	-.627	-.561	-.409	-.827	.225	.204	1.000

A1L=Laggard chromosomes in anaphase I, A1S=Chromosomes stickiness in anaphase I, A2L=Laggard chromosomes in anaphase II, A2S=Chromosomes stickiness in anaphase II, U=Univalent, MIC=Micronucleus, PF=Pollen fertility

It has been suggested that infertility in polyploids is not solely due to the production of aneuploid gametes formed by improper segregation of chromosomes during anaphase/telophase stages, the genetic factors may also bring about pollen sterility as evidenced in different tetraploid strains of rye [5], as well as *Avena sativa* cultivars [13]. Therefore reduction in pollen fertility in *B. napus* cultivars may also be affected by genetic factors and not only by meiotic irregularities reported.

Different cluster analyses and ordination of cultivars based on PCA produced a similar result (Figs. 11 & 12), producing 3 major cluster/groups. The first major cluster is comprised of 3 sub-clusters. The cultivars Consol and Orkan show more similarity in meiotic characteristics and form the first sub-cluster, while cultivars Syn1 and Eurol form the second sub-cluster. The cultivars GWC, Hanson and Magnum comprise the third sub-cluster.

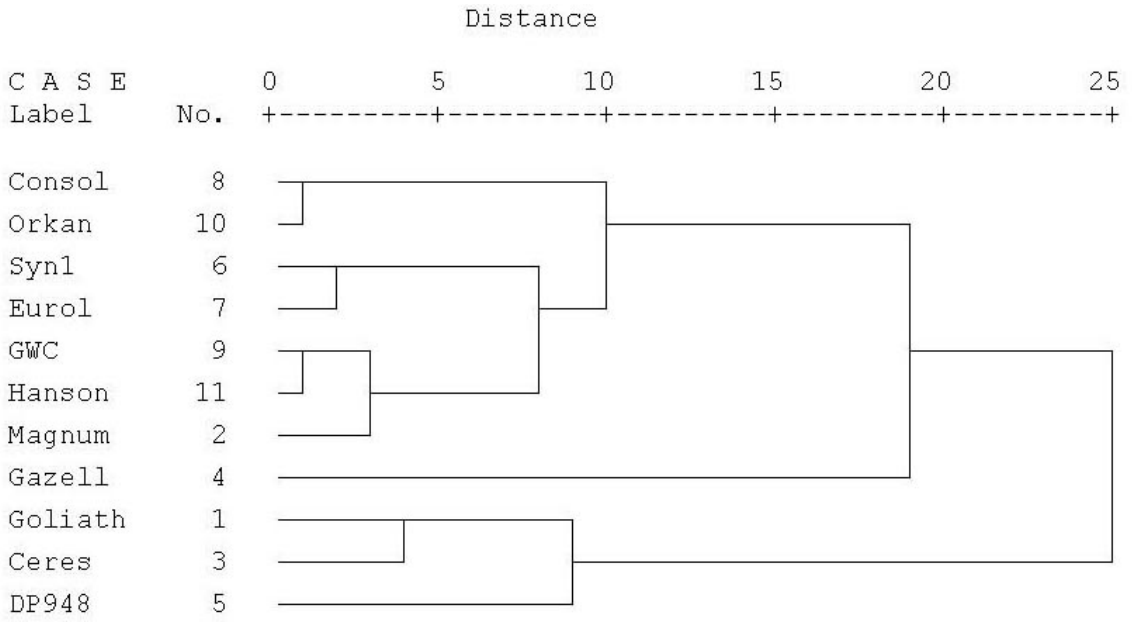


Fig. 11. WARD cluster analysis of *B. napus* cultivars

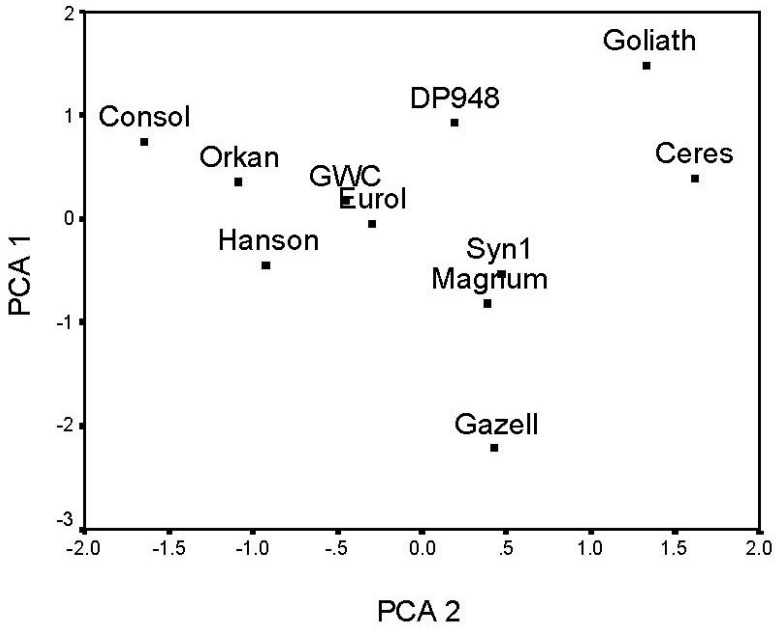


Fig. 12. PCA ordination of *B. napus* cultivars

The cultivar Gazell alone comprises the second major cluster, while the cultivars Goliath, Ceres and DP984 comprise the third major cluster in which the cultivars Goliath and Ceres show more similarity to one another. As stated earlier, the members of different clusters differ significantly in their meiotic characteristics from the others.

PCA analysis of meiotic data revealed that the first 2 factors comprise about 75% of total variance. In the first factor, which comprises about 50% of total variance, meiotic characters like ring bivalents and total chiasma possessed the highest positive correlation (>0.90), while intercalary chiasma possessed the highest negative correlation (>-0.90). In the second factor, which comprises about 24% of total variance, intercalary chiasma possessed the highest positive and negative correlation (>0.80). Therefore these are the most variable meiotic characteristics among the cultivars studied as also revealed by the ANOVA test discussed earlier. The first PCA factor separates mainly the Gazell cultivar from the others, while the second factor mainly separates the cultivars Goliath and Ceres from the other cultivars studied (Fig. 12).

B-chromosomes are accessory chromosomes reported in many plant and animal species. They show numerical polymorphism and when present in high number, they negatively affect the growth and vigor of the plants, while in low number they may benefit the plant possessing them [16]. In our earlier studies a significant change in the chiasma frequency and chromosome pairing was noticed in the cells possessing B-chromosomes in some of the *B. napus* cultivars [2], but t-test analysis did not show any significant difference in meiotic characteristics of the cells possessing B-chromosomes compared to the cells devoid of B-chromosomes in the cultivars studied. Therefore, it seems that B-chromosome have different effects on meiotic characteristics of different canola genotypes which should be determined.

In general, if the cytogenetic differences observed in the cultivars studied is accompanied by other agronomic differences, a better hybridization and selection program may be planned for *B. napus* cultivars.

Acknowledgements- This report is a part of a project supported by a Shahid Beheshti University grant.

REFERENCES

1. Sheidai, M., Mansoorniya, M. R. & Ahmadi, M. R. (2001a). Cytomorphological study of some rapeseed (*Brassica napus* L.) cultivars in Iran. *The nucleus*, *44*, 42-45.
2. Sheidai, M., Noormohamadi, Z. & Ahmadi, M. R. (2001b). Notes on B-chromosomes and SDS-PAGE proteins in some rapeseed (*Brassica napus*) cultivars. *The nucleus*, *44*, 165-170.
3. Quicke, D. L. J. (1993). *Principles and Techniques of Contemporary Taxonomy*. Glasgow, Blackie Academic & Professional.
4. Rees, H. & Jones. R. N. (1977). *Chromosome Genetics*. London, Edward Arnold.
5. Hazarika, M. H. & Rees, H. (1967). Genotypic control of chromosomes behavior in rye X. Chromosome pairing and fertility in autotetraploids. *Heredity*, *22*, 317-332.
6. Camacho, J. P. M., Sharbel, T. F. & Beukeboom, L. W. (2000). B-chromosome evolution. *Phil. Trans. R. Soc. Lond. B355*, 163-178.
7. Digby, P. G. N. & Kempton, R. A. (1994). *Multivariate analysis of ecological communities*. Chapman & Hall.
8. De Souza A. M. & Pagliarini, M. S. (1996). Spontaneous chromosome stickiness in canola. *The nucleus*, *39*, 85-89.
9. Sybenga, J. (1992). *Cytogenetics in Plant Breeding*. Berlin, Germany, Spring-Verlag.
10. Sheidai, M. & Inamdar, A. C. (1991). Variations in the meiotic chromosomes in *Asparagus* L. *Biovigyana*m, *17*, 45-47.
11. Attia, T. & Robbelen, G. (1986). Meiotic pairing in haploids and amphidiploids of spontaneous versus synthetic origin in rape. *B. napus L. Can. J. Genet. Cytol*., *28*, 330-334.
12. Sharpe, A. G., Parkin I. A. P., Keith, D. J. & Lydiate, D. J. (1995). Frequent nonreciprocal translocations in the amphidiploid genomes of oilseed rape (Brassica napus). *Genome*, *38*, 1112-1121.
13. Golubovskaya, I. N. (1989): Meiosis in maize: mei genes and conception of genetic control of meiosis. *Adv. Genet*., *26,* 149.
14. Baptista-Giacomelli F. R., Palgliarini, M. S. & Almeida, J. L. (2000). Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia*, *65*, 371-378.
15. Merker, A. (1971). Cytogenetic investigations in hexaploid triticale. I. Meiosis, aneuploidy and fertility. *Hereditas*, *68*, 281-290.
16. Clark, M. S. & Wall, W. J. (1996). *Chromosomes The Complex Code*. London, Chapman & Hall.