CYTOGENETICAL AND BIOCHEMICAL VARIATIONS BETWEEN SOME *BRASSICA* SPECIES AND THEIR HYBRIDS

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ABSTRACT

Cytogenetical and biochemical variations among six *Brassica* species and their F_1 hybrids were studied. The chromosomal behavior of the diploid *Brassica* species (*B. nigra*, *B. oleracea* and *B. campestris*) and the tetrapolid ones (*B. carinata*, *B. juncea* and *B. napus*) was proven to be normal. Irregular chromosomal configurations including univalents, trivalents and quadrivalents were detected in the six interspecific F_1 hybrids. The number of chiasmata was higher in *B. napus*, while the terminalization coefficients were found to be higher in *B. nigra* and *B. oleracea* than the other four species. The interspecific hybrids showed reduction in numbers of chiasmata. The six F_1 crosses were found to exhibit lagging bivalents that ranged from one to 14 laggards. Two crosses were found to possess two to four micronuclei with average numbers of 3.85 (*B. campestris* x *B. juncea*) and 3.24 (*B. napus* x *B. campestris*), while the other crosses exhibited one to seven micronuclei. Differences in band activity between the parental cultivars and their interspecific F_1 hybrids were detected for both esterase and peroxidase. Banding patterns in the hybrids comprised bands from both parental species and additional bands which might be produced by re-association of some subunit. Results of isolated and purified DNA showed that amphidiploid parent had amount of nuclear DNA equal to those of respective diploid parents. DNA content of the allotetraploid cultivars *were* approximately twice the sum of the DNA in the diploid ones. The amount of DNA in *B. napus* was equal to the sum of the amounts of DNA in *B. oleracea* and *B. compestris*.

Key words: *Brassica* – cytogenetical variations – biochemical analysis-

INTRODUCTION

The genus *Brassica* inlcudes six species of great value worldwide. *B. nigra* (L.) (black mustard, genome BB, 2n =16) is growing as condiment. *B. oleracea* L. (genome CC, 2n = 18) comprises numerous morphotypes that are widely used as vegetables such as cabbage, broccoli, cauliflower and Chinese kale. *B. campestris* L. (genome AA, 2n = 20) is used both as vegetables (turnip and Chinese cabbage) and as oleiferous crop (turnip rape). *B. carinata* (Abyssinan mustard, BBCC, 2n = 34) is used in Ethiopia as a vegetable and as a source of oil. *B. juncea* (L.) (oriental mustard, AABB, 2n = 36) and *B. napus* L. (oil-seed rape, AACC, 2n = 38) are important oil seed crops in China, India, Canada and Europe. The oil seed *Brassica* includes four species namely *B. campestris, B. juncea, B. napus* and *B. corinata.* Understanding of the genomic relationships within the *Brassica genus* at chromosomal and molecular level is of relevance for assessing the potential of intergenomic hybridization which can be exploited in *Brassica* breeding programs (Skarzhinskaya *et al.* 1998 and Cheng *et al.*, 2002).

The chromosomes of *Brassica* are small, morphologically similar and quite numerous in allotetraploids. However, cytogenetical researches on *Brassicas* were initiated with the determination of chromosome number and genome analysis by Morinaga (1928). U (1935) established the evolutionary origin of the three amphidiploid species, *B. napus, B. juncea* and *B. carinata* as a result of interspecific hybridization among the three diploid species, *B. campestris, B. oleracea* and *B. nigra.* This hypothesis has been verified by multidisciplinary studies such as meiotic chromosome pairing (Mizushima, 1980 and Prakash and Hinata, 1980), isozymes (Coulthart and Denford, 1982 and Lazaro. and Aguinagalde, 1998), nuclear DNA restriction fragment length polymorphism (RFLP) markers (Song *et al.*, 1990 and 1991), AFLP (amplified fragment length polymorphism) (Pertl *et al.* 2002) and fluorescent in-situ-hybridization (FISH) (Howella *et al.*, 2002). Moreover, analyses of chloroplast and mitochondrial DNA restriction patterns have revealed that *B. campestris* and *B. nigra* are the cytoplasm contributors of *B. juncea* and *B. carinata* (Erickson *et al.*, 1983 and Palmer, 1988).

The phylogenetic relationships among the A, B and C genomes have been extensively studied at chromosomal and molecular level. Studies on meiotic chromosome pairing in the amphihaploids (AB, BC and AC) and digenomic hybrids (AAB, AAC and BBC) have revealed that there is a higher degree of homology between the A and C genomes than that between either of them or B genome (Prakash and Hinata, 1980; Attia *et al.*, 1987 and Kulak *et al.*, 2002). Diploid species of *Brassica* are secondarily balanced polyploids derived from a common ancestor with a lower basic chromosome number. *B. campestris* and *B. oleracea* have closer evolutionary relationship, while *B. nigra* is more distantly related (Udall *et al.*, 2005).

The present study employed both cytogenetical and biochemical approaches to investigate the variations among the parents and their F_1 hybrids, to understand the interspecific relationships among *Brassica* genomes.

MATERIALS AND METHODS

The materials used in this study consisted of three elementary diploid cultivars and three allotetraploid cultivars. The origin of such six cultivars and their characteristics are presented in Table (1). It is assumed that all these cultivars are highly pure.

Six out of 15 interspecific crosses were produced through bud pollination, while the rest ones failed to produce any hybrid seeds.

Cytogenetical techniques:

Flowering buds of appropriate size were collected and immediately fixed in Farmer's solution consisted of absolute ethanol and glacial acetic acid. Ferric chloride (about 0.5 g/500 ml fixative) was dissolved to give the fixed materials a deep straw color.

The materials were fixed for 48 hours and were stored in 70% ethanol saturated with ferric chloride traces in a refrigerator. The contents of a single anther with a drop of 1% acetocarmine solution were squeezed out with unplated iron needle (Darlington and La Cour, 1962). Permanent preparations were prepared according to (Eid, 1963).

The best preparations of diakinesis and metaphase I stages were used to determine the average number of univalents, bivalents, trivalents and quadrivalents and to calculate the chiasma frequency

and terminalization coefficient. To test the chiasma frequency, ANOVA test was conducted with four replications, each had 25 cells for each genotype.

Table (1): Origin and salient features of the parental cultivars used in this study.

Cultivars	Source	Common name	Genome constitution	Salient features
Diploid cultivars				
1- *Brassica nigra* cv. junius	Sweden *	Black mustard	BB	Condiment, plant height medium and early maturing
2- *B. oleracea* cv. botrytis	Sweden	Cauliflower	CC	Vegetable, plant height short and early maturing
3- *B. campestris* cv. 199	Egypt**	Turnip rape	AA	Oil seed, plant height short and medium maturing
Allotetraploid cultivars				
1-*B. carinata* cv. ethiopian	Sweden	Ethiopian mustard	BBCC	Vegetable/oil seed, plant medium and late maturing
2- *B. juncea* cv. 217.	Egypt	Indian mustard	AABB	Oil seed, plant height high and late maturing
3- *B. napus* cv. bakatol	Egypt	Oil seed rape	CCAA	Oil seed, plant height medium and late maturing

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Isoenzyme electrophoresis techniques:

Esterase and peroxidase isozymes were investigated in leaf-blades extracts obtained from 40 days old seedlings as described by Chu and Oka (1967).

Esterase and peroxidase Isoenzymes were determined in the polyacrylamid gel slabs 6% following the method outlined by Davis (1964). One-tenth gram of leaf tissues was homogenized in 0.5 ml of 0.04 M-tris-buffer (pH 7.8); supplemented with 20% sucrose, 10 ml M dithiothritol (DTI) and 0.25 ml M EDTA in ice cold pestle and mortar kept on ice. The obtained homogenate was centrifuged at

10.000 xg for 5 minutes at 4°C and the clear supernatant was used for electrophoresis. Staining procedures for esterase and peroxidase were conducted according to Scandalios (1969).

DNA isolation:

Isolation of DNA from *Brassica* plants was adapted according to Van de Ven *et al.* (1990). DNA was repurified using phenol/chloroform and chloroform/isoamyl alcohol and the DNA pellet was dissolved in 500 µl of TE buffer. The quantity and purity of the obtained DNA were determined according to the UV absorbance at 260 and 280 nm wave length values using ultraspec 1000 UV/vis spectrophotometer, Pharmacia Biotechn and calculated according to Sambrook *et al.* (1989).

RESULTS AND DISCUSSION

Cytogenetical observations:

The cytogenetical study was aimed to investigate the behavior of the parental species as well as their F_1 hybrids at different phases of the meiotic division.

Chromosome pairing at diakinesis and metaphase I stages:

Normal number of bivalents was detected, at both phases, in the diploid species, *B. nigra* cv. Junius (eight), *B. oleracea* cv. botrytis (nine) and *B. campestris* cv. 199 (ten), and also in the allotetraploid species, *B. carinata* cv. Ethiopian (17), *B. juncea* cv. 217 (18) and *B. napus* cv. Bakatol (19) (Figure 1).

Complete pairing at diakinasis and metaphase I stages was observed for the parental species. However, irregular chromosomal association was found in their interspecific F_1 hybrids. Table (2) shows the average number and percentage of chromosome pairing for the F_1 hybrids.

***B. napus* (CCAA) *x B. juncea* (AABB) (*AABC*):**

At diakinesis stage, univalents, bivalents and trivalent were detected with an average number of 15.71, 10.57 and 0.05, respectively. The results showed that about 5% of the cross exhibited 12 I, 11 II and one III, whereas 10% of the cells displayed 11 I + 13 II. About 11% of the cells contained 13 I + 12 II, and 74% of the cells exhibited 17 I + 10 II as shown in Figures (2 a, b and c).

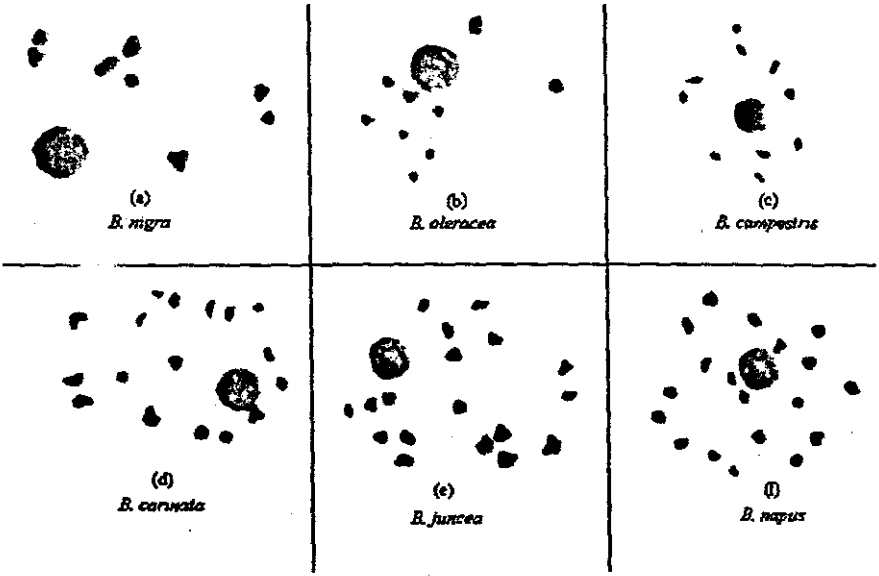


Figure (1): Photomicrographs of diakinasis phase of *Brassica* species showing regular behavior (x = 1500).

At metaphase, the average number of univalents, bivalents and trivalents were found to be 15.39, 10.76 and 0.03, respectively. However, cell percentages having different chromosomal units were as follows; 10 I + 12 II + 1 III (3%), 11 I + 13 II or 13 I + 12 II (14%), and 17 I + 10 II (69%).

At diakinesis and metaphase stages, the two homologous A-genomes paired preferentially as ten bivalents in a high percentage of the examined cells (74 and 69%, respectively, (Table 2). This configuration may represent the ten chromosomes of *B. campestris* paired with their own homologues, while the nine chromosomes of *B. oleracea* and the eight chromosomes of *B. nigra* did not pair and were considered as univalents. Allosyndetic pairing among chromosomes of *B. campestris* (A) with chromosomes of *B. nigra* (B) and *B. oleracea* (C) was observed in some meiocytes by the formation of trivalent and additional bivalent, therefore, pairing is not only restricted to the A homologous but, extends to include the added B and C genomes.

Table (2): Chromosome pairing of the interspecific F_1 hybrids between *Brassica* genotypes at diakinesis and metaphase I phases.

Genotypes	Diakinesis						Metaphase I					
	No. of examined cells	Chromosome associations				%	No. of examined cells	Chromosome associations				%
		I	II	III	IV			I	II	III	IV	
B. napus	5	12	11	1	-	5.0	3	10	12	1	-	3.00
x	10	11	13	-	-	10.0	14	11	13	-	-	14.00
B. juncea	11	13	12	-	-	11.0	14	13	12	-	-	14.00
	74	17	10	-		74.0	69	17	10	-	-	69.0
Total	100	1571	1057	5		100.0	100	1539	1076	3		100.0
Average	1	15.71	10.57	0.05		1	1	15.39	10.76	0.03		1
B. campestris	13	10	9	-	-	13.0	3	7	9	1	-	3.0
x	87	8	10	-	-	87.0	18	6	11	-	-	18.0
B. juncea								8	10	-	-	79.0
Total	100	826	987			100.0	79	761	1015	3		100.0
Average	1.00	78.26	9.87				1.00	7.61	10.15	0.03		
B. napus	8	11	9			8.0	8	12	7	1		8.00
x	14	7	11			14.0	18	11	9			18.00
B. campestris	78	9	10			78.0	74	9	10			74.00
Total	100	888	1006		100	100.0	100	952	958			100.0
Average	1.00	8.88	10.06				1.00	9.52	9.58	0.08		
B. nigra	4	8	7		1	4.0	3	10	6		1	3.0
x	96	10	8			96.0	4	9	7	1		4.0
B. juncea							93	10	8			93.0
Total	100	992	796		4	100.0	100	996	790	4	3	100.0
Average	1.00	9.92	7.96		0.04		1.00	9.96	7.90	0.04	0.03	
B. carinata	3	8	7	1	-	3.0	4	8	7	1	-	4.0
x	24	11	7	-	-	24.0	24	11	7	-	-	24.0
B. nigra	73	9	8	-	-	73.0	72	9	8	-	-	72.0
Total	100	945	773	3		10.0	100	944	772	4		100.0
Average	1.00	9.45	7.73	0.03			1.00	9.44	7.72	0.04		
B. carinata	11	10	8			11.0	4	9	7	1		4.0
x	89	8	9			89.0	10	6	10			10.0
B. oleracea							86	8	9			86.0
Total	100	822	889			100.0	100	7.84	902	4		100.0
Average	1.00	8.22	8.89				1.00	7.84	9.02	0.04		

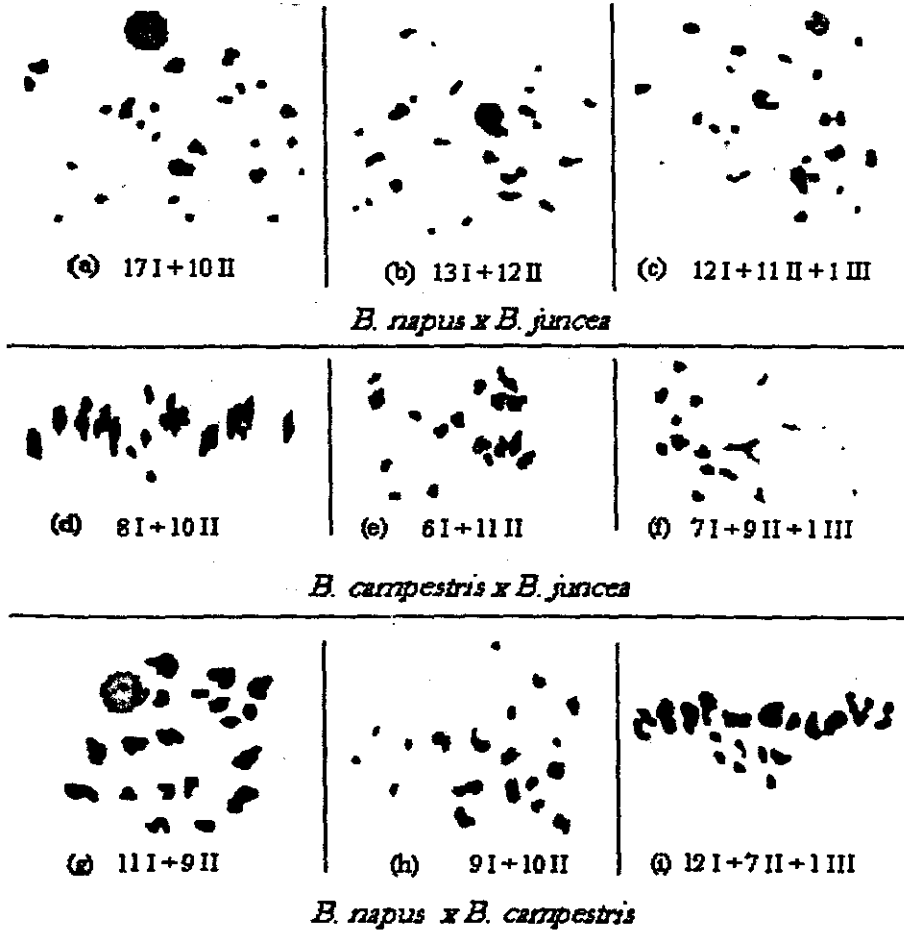


Figure (2): Photomicrographs of diakinasis and metaphase I phases of the F_1 hybrids (*B. napus x B. juncea*), (*B. campestris x B. juncea*) and (*B. napus x B. campestris*) (x = 1500).

***B. campestris* (AA) x *B. juncea* (AABB):**

At diakinesis stage, univalents and bivalents were scored with an average of 8.26 and 9.87, respectively,. At metaphase I stage, however, three different chromosomal configurations as univalents, bivalents and trivalents were observed with an average number of 7.61, 10.15 and 0.03, respectively as illustrated in Figures (2 d, e and f).

The most common configuration in the last cross was 10 II+8 I, this observation assumes that genome A from *B. juncea* and *B. campestris* have maintained relatively close homology. Similar results were obtained by Mohapatra and Bajaj (1987).

Nevertheless, two A chromosomes failed to pair with their own homologue as 13% of PMCs presented less than ten bivalents (Table 2, diakinesis). On the other hand, based on RFLP marker, Slocum *et al.* (1990) and Udall *et al.* (2005) reported that genome rearrangements have occurred during the evolution of *B. campestris* and *B. napus*, respectively This could explain the difference between genome A in each of *B. juncea* and *B. campestris*. Also, allosyndetic pairing between bivalents of *B. campestris* (A) with chromosomes of *B. nigra* (B) was observed by the formation of trivalents. However, the allosyndetic pairing was very rare. Song *et al.* (1993) observed multivalent configurations in the hybrid (A x B). At the same time, the presence of more than ten bivalents confirmed autosyndetic pairing, whereas *B. nigra* chromosomes tended to pair autosyndetically with each other in about of 18% of cells.

***B. napus* (CCAA) *x B. campestris* (AA):**

At diakinesis, univalents and bivalents were observed with an average number of 8.88 and 10.06, respectively. However, at metaphase I stage, univalent and bivalents were recorded with an average of 9.52 and 9.58, respectively, while trivalent was obtained once (Figures 2 g, h and i).

The percentage of PMCs forming 9 I+10 II was strikingly high and reached 78% in diakinesis and 74% in metaphase I. This type of association had been expected to be the rule if the ten chromosomes of *B. campestris* that actually prefer to pair only with their own homologues, leaving the nine chromosomes of *B. oleracea* as univalents. Chang and Tai (1986), Attia *et al.* (1987) and Heneen *et al.* (2001) reported the same chromosome configurations between *B. napus* and *B. campestris* with percentages of 95 75.1 and.89, respectively.

***B. nigra* (BB) x *B. juncea* (AABB):**

At diakinesis stage, the average number of observed univalents and bivalents was found to be 9.92 and 7.96, respectively, whereas quadrivalent was observed once. Percentage of cells showing 10 I + 8 II was 96, while the percentage of cells having 8 I

+ 7 II + 1 IV was four as shown in Figure (3 a). However, at metaphase I stage, there were four different chromosome configurations such as univalent, bivalent, trivalent and quadrivalent with an average number of 9.96, 7.90, 0.04 and 0.03, respectively. While the percentage of cells having 10 I + 6 II + 1 IV was 3.0%, it was 4% of cells had 9 I + 7 II + 1 III and 93% of the cells had 10 I + 8 II as shown in Figure (3 b and c).

The main pairing configuration was eight bivalents plus ten univalents and this suggests that a complete pairing had achieved between all B genome chromosomes in *B. juncea* and *B. nigra* leaving the ten chromosomes of *B. campestris* as univalents. Moreover, Mizushima (1980) and Olsson (1960) have shown that two B genomes exhibited complete homologues pairing in the presence of one A genome resulting in 8 II + 10 I as the model configuration. On the other hand, allosyndetic pairing also happened between chromosomes of the B genome with those of the A genome to form a trivalent and quadrivalent and occurred in 4% and 7% of the examined PMCs at diakinesis and metaphase I stages. This finding confirms previous result obtained by U (1935) and Mizushima (1950) for the same combination.

Also, in fact, the probability that the additional quadrivalents may also be formed from the synapsis between-chromosomes of *B. nigra* only can not be excluded whereas this conclusion agrees with the conclusion reported by Attia *et al.* (1987) or between two chromosomes from *B. nigra* and two chromosomes of *B. campestris*.

***B. carinata* (BBCC) x *B. nigra* (BB):**

At diakinesis stage, The percentage of cells having 8 I + 7 II + 1 III was three. On the other hand 24% of the cells had 11 I + 7 II and 73% of the cells had 9 I + 8 II as shown in Figure (3 d). At metaphase I stage, the percentage of cells which exhibited 8 I + 7 II + 1 III (Figure 3 e) was proven to be 4%. On the other hand 24% of the cells had 11 I + 7 II (Figure 3 f) and 72% of the cells exhibited 9 I+8 II.

The common configuration was eight bivalents plus nine univalents and this suggests complete pairing of all B chromosomes from the two parents. Such observation assumes that the B genomes from *B. carinata* and *B. nigra* have close homology. This finding confirms the previous results reported by U (1935) and Mizushima

(1950) for the same combination. Nevertheless, one to two B chromosomes failed to pair with their own homologue as 24% of PMCs presented less than eight bivalents in each of diakinesis and metaphase I stages. This finding could be a result of lesser degree of homology between the two B genomes of *B. carinata* and *B. nigra* and this suggests that genome rearrangements have occurred during the evolution of *B. nigra* (Slocum *et al.*, 1990).

It can not be excluded that in rare cases a trivalent within a 8 I+7 II+1 III configuration may also result from three autosyndetically paired C chromosomes. This conclusion is in agreement with the previous observations that most bivalents in *B. oleracea* can be in association of 2 or more (Heneen *et al.*, 2001). However, the maximum pairing in the haploids of *B. oleracea* was 1III+1II+4I (Armstrong and Keller, 1982). Also, this is in agreement with the formula of Attia and Röbbelen (1986) who reported that the C genome was tetrasomic for three linkage groups.

***B. carinata* (BBCC) *x B. oleracea* (CC):**

Univalents and bivalents were observed at diakinesis stage with an average of 8.22 and 8.89, respectively. While there was 11% of the cells exhibited 10 I+8 II, there was 89% of the cells had 8 I + 9 II (Figure 3 g). At metaphase I, univalents, bivalents and trivalent were detected with an average of 7.84, 9.02 and 0.04, respectively. The percentage of the cells having 9 I + 7 II + 1 III (Figure 3 h) was 4%; whereas the percentage of cells exhibited 6 I + 10 II was 10% (Figure 3 i), 86% of the cells exhibited 8 I + 9 II. The most common configuration was 8 I + 9 II and this suggests preferential homologous pairing of all chromosomes from the two parents. This observation assumes that the genome C from *B. carinata* and *B. oleracea* has maintained relatively close homology. This coincides with earlier results from the same digenomic combination CB-C elaborated by Mizushima (1950). The failure of homologous pairing within the single set of C genome suggests that structural changes and rearrangements happend through the course of its evolution as 11% of PMCs presented less than 9 bivalents. Chevre *et al.* (1989) reported that some PMCs of diploid *B. oleracea* have a configuration of 2 I+8 II owning to the earlier separation of one bivalent. This could explain the difference between the C genomes of *B. carinata* and *B. oleracea*. .

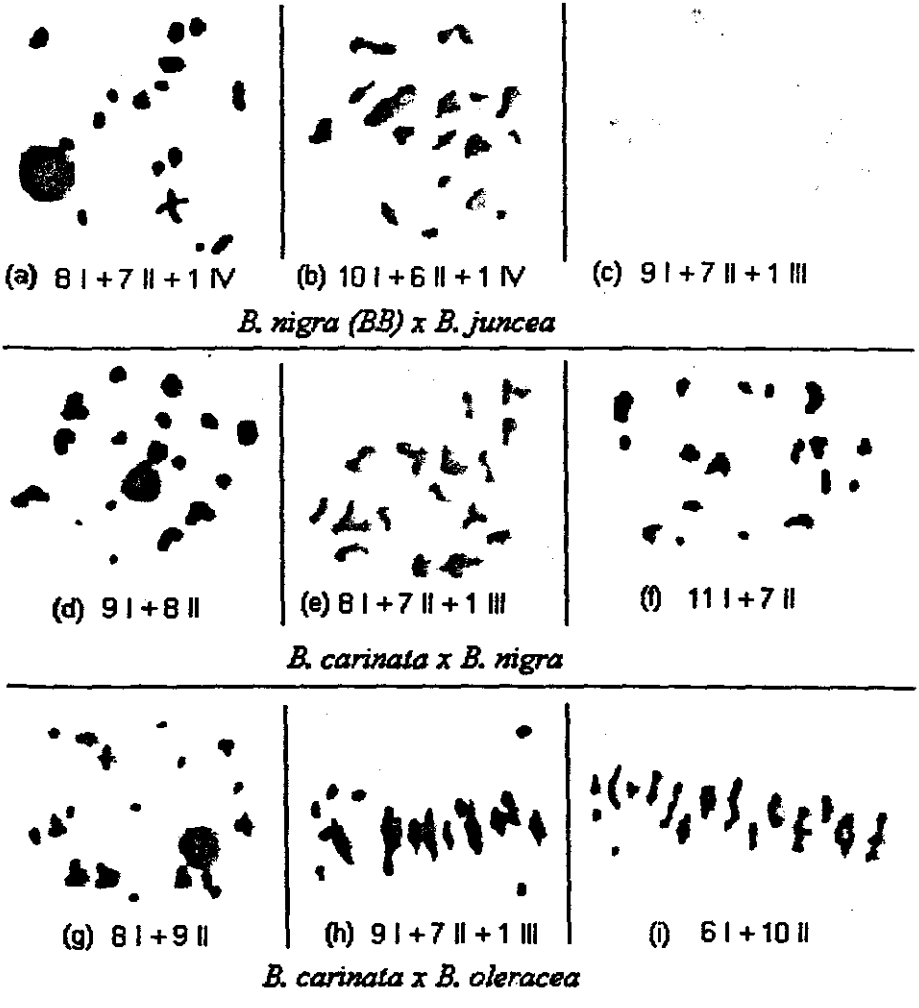


Figure (3): Photomicrographs of diakinasis and metaphase I phases of the F_1 hybrids *B. nigra* x *B. juncea*, (*B. carinata* x *B. nigra*) and (*B. carinata x B. oleracea*) (x = 1500).

Allosyndetic pairing to form a trivalent between chromosomes of the C genome with those of the B genome was very rare and occurred in 3 and 4% of the examined PMCs at diakinesis and metaphase I stages, respectively. It can not be excluded, however, that in rare cases a trivalent within a 9 I+7 II+1 III configuration might also result from three autosyndetically paired *B.*

chromosomes. This conclusion was confirmed by Attia *et al.* (1987). These results agree with those reported by Song *et al.* (1993).

Allosyndesis, observed in interspecific F_1 hybrids between the different allotetraploid *Brassica* species, allowed stimulating deductions regarding the relationship of the different genomes. Attia *et al.* (1987) concluded that a high amount of pairing was found in the AC amphihaploid while amphihaploid AB and BC showed a low pairing. Also, Jahier *et al.* (1989) observed, in the F_1 hybrids (ABC), that 18% of chromosomes paired as quadrivalents or pentavalents. Also this observation supports the hypothesis on the polyploid nature of the genomes A, B and C. Also, Mizushima (1950) concluded that *B. oleracea* and *B. campestris* are more closely related to each other than to *B. nigra*. Previous observations reached the same results where the presence of nine bivalent in AC amphihaploid plant was reported (Prakash and Hinata, 1980; Attia and Röbbelen, 1986; Palmer, 1988 and Song *et al.*, 1990).

The probability that the trivalent and additional bivalent may also be formed from only C or B or A chromosomes can not be excluded. Whereas, the studies on chromosome pairing in haploid of *B. campestris* proved that the maximum chromosome pairing observed in haploid was two bivalents plus one trivalent (Armstrong and Keller, 1982). Also pachytene chromosome analysis by Venkateswarla and Kamala (1971) revealed that *B. campestris* genome is represented by AABC DDE FFF and *B. nigra* by AABC DDEF. Attia and Röbbelen (1986) reached the same results whereas they found tetravalents and pentavalents in the amphihaploid involving *B. campestris, B. nigra* and *B. oleracea* which suggests the existence of autosyndetic pairing.

The evolution of any differentiation of the two genomes A and C appears to be too small to secure a conspicuous preferential pairing. This conclusion was confirmed by analysis of ACC ($2n = 28$) hybrids (Gotoh, 1959), and crosses of *B. campestris* with an autotetraploid *B. oleracea* (Inomata, 1980). These results agree with those reported previously for synthetic amphihaploids (Prakash and Hinata, 1980) since they found that in synthetic F_1 *B. napus* lines (A x C and C X A) chromosomes from the A and C genomes paired very well in metaphase I with 0-3 univalents.

Chiasmata frequency/cell at diakinesis and metaphase I stages:

Data presented in Table (3) revealed that *B. napus* species possessed the highest mean value for chiasmata frequency/cell at both stages, followed by *B. juncea* then *B. carinata*, however, the lowest mean value was recorded for *B. nigra*.

At both stages, F_1 hybrids had lower mean values than their respective parents. The highest mean values at both stages was recorded for (*B. napus x B. juncea*) F_1, and the lowest mean values at both stages was found for *B. carinata x B. nigra*) F_1.

Chiasmata frequency/bivalent at diakinesis and metaphase I:

The highest mean value was recorded for *B. napus*, followed by *B. juncea*, and then *B. carinata*, however, the lowest mean value for such trait was recorded for *B. oleracea* as presented in Table (3). In the F_1 hybrids, the mean values were found to be lower than those of their parental species at both stages. Comparisons between F_1 hybrids showed that the highest mean value was recorded for (*B. nigra x B. juncea*) F_1 while the lowest mean value was recorded for (*B. carinata x B. oleracea*) F_1 at diakinesis. At metaphase I, however, the highest mean value for such trait was recorded for (*B. carinata x B. nigra*) F_1, followed by (*B. nigra, x B. juncea*) F_1 while, the lowest mean value was recorded for (*B. carinata x B. oleracea*) F_1.

Terminalization coefficient at diakinesis and metaphase I:

At diakinesis stage, Table (3) revealed that there were no significant differences between the parental genotypes themselves and/or their hybrids. However, the (*B. carinata x B. oleracea*) F_1 possessed the highest mean estimate at diakinesis.

At metaphase I, the highest mean estimate of terminalization coefficient was recorded for *B. oleracea* cultivar, whereas, *B. napus* possessed the lowest one. In F_1 hybrids, the mean values for terminalization coefficient were proven to be significantly higher than those of their respective parents. Again, the (*B. carinata* x *B. oleracea*) F_1 possessed the highest estimate.

Table (3): Mean performance of chiasmata frequency per cell; per bivalent and the terminalization coefficient at diakinesis and metaphase I phases for six interspecific F_1 hybrids and their parental cultivars.

Genotypes	Diakinesis			Metaphase I		
	Chiasmata frequency / cell	Chiasmata frequency / bivalent	Terminalization Coefficient	Chiasmata frequency / cell	Chiasmata frequency / bivalent	Terminalization Coefficient
B. nigra	14.3 f	1.78 abcd	0.57 ab	12.6 de	1.57 ab	0.86 abc
B. oleracea	14.9 def	1.65 d	0.57 ab	12.7 de	1.54 bc	0.87 ab
B. campestris	18.6 c	1.86 abc	0.54 ab	15.5 c	1.55 bc	0.85 abc
B. carinata	32.2 b	1.89 ab	0.55 ab	26.48 b	1.58 ab	0.84 bc
B. juncea	34.3 ab	1.91 ab	0.55 ab	28.8 ab	1.60 ab	0.84 bc
B. napus	36.6 a	1.93 a	0.55 ab	30.8 a	1.62 a	0.83 c
B. napus x B. juncea	17.3 cd	1.63 cd	0.58 ab	15.1 cd	1.39 e	0.88 ab
B. campestris x B. juncea	16.3 cd	1.65 cd	0.57 ab	13.8 cde	1.41 e	0.88 ab
B. napus x B. campestris	17.2 cd	1.709 bcd	0.58 ab	14.4 cde	1.48 d	0.87 ab
B. nigra x B. juncea	14.1 ef	1.72 abcd	0.58 ab	12.2 e	1.53 c	0.87 ab
B. carinata x B. nigra	13.2 f	1.69 bcd	0.58 ab	11.9 e	1.55 bc	0.88 ab
B. carinata x B. oleracea	14.2 ef	1.59 d	0.59 a	12.2 e	1.37 e	0.89 a

In the same column, means followed by the same letter are not significantly different at 1% level.

Number of lagging chromosomes:

At the first meiotic anaphase stage, regular behavior was observed in all parental species. In the F_1 crosses, however, from five to fourteen laggards were observed in the interspecific (*B. napus x B. juncea*) F_1 with an average of 12.66 (Table 4 and Figure 4 a).

In the F_1 of (*B. campestris x B. juncea*) (Figure 4 b), (*B. napus x B. campestris*), (*B. nigra x B. juncea*) (Figure 4c), (*B. carinata x B. nigra*) and (*B. carinata x B. oleracea*), laggards were detected with averages of 6.14, 6.38, 7.28, 5.36 and 6.12, respectively (Table 4).

Table (4): Meiotic chromosome behaviour at anaphase I, telophase I, anaphase II, telophase II and quartet stages, number and average of lagging chromosomes and micronuclei of six interspecific F_1 hybrids and their parental cultivars.

	Anaphase I		Telophase I		Anophase II		Telophase II		Quartet	
	No. of cells	No. of laggards	No. of cells	No. of laggards	No. of cells	No. of laggards	No. of cells	No. of laggards	No. of cells	No. of micronuclei
Brassica nigra, B. oleracea, B. campestris, B. carinata, B. juncea and *B.*										
	100	0	100	0	100	0	100	0	100	0
Total	100	0	100	0	100	0	100	0	100	0
Average		0.0		0.0		0.0		0.0		0.0
I (*B. napus x B. juncea*)										
	53	14	72	5	52	10	46	7	36	7
	30	13	18	4	26	9	32	5	30	6
	11	9	10	3	12	8	20	3	23	5
	6	5			10	5	2	2	11	4
Total	100	1266	100	462	100	900	100	546	100	591
Average		12.66		4.62		9.0		5.46		5.91
II (*B. campestris x B. juncea*)										
	53	7	88	4	67	4	58	4	69	4
	23	6	36	3	30	3	27	3	20	3
	19	5	6	2	3	2	15	1	11	2
	5	2								
Total	100	614	100	3652	100	364	100	328	100	358
Average		6.14	1	3.52		3.64		3.28		3.58
III (*B. napus x B. campestris*)										
	61	7	60	4	63	4	68	4	33	4
	22	6	24	3	25	3	21	3	58	3
	11	5	16	2	12	2	11	2	9	2
	6	4								
Total	100	638	100	344	100	351	100	357	100	324
Average		6.38		3.44		3.51		3.57		3.24
IV (*B. nigra x B. juncea*)										
	57	8	59	5	55	4	61	4	59	2
	31	7	36	4	31	3	31	3	38	1
	7	5	5	3	14	2	8	2	3	0
	5	4								
Total	100	728	100	454	100	341	100	353	100	156
Average		7.28		4.54		3.41		3.53		1.56
V (*B. carinata x B. nigra*)										
	58	6	65	3	62	3	49	4	95	1
	31	5	35	2	32	2	30	3		
	11	3			6	0	18	2	5	0
							3	0		
Total	100	5.36	100	265	100	250	100	322	100	95
Average		5.36		2.65		2.50		3.22		0.95

Table (4):Continued

	Anaphase I		Telophase I		Anophase II		Telophase II		Quartet	
	No. of cells	No. of laggards	No. of cells	No. of laggards	No. of cells	No. of laggards	No. of cel's	No. of laggards	No. of cells	No. of micronuclei
VI (*B. carinata x B. oleracea*)										
	44	7	66	5	62	6	60	4	51	2
	33	6	28	4	34	4	34	3	36	1
	14	5	6	2	4	2	6	0	13	0
	9	4								
Total	100	612	100	454	100	516	100	342	100	138
Average		6.12		4.54		5.16		3.42		1.38

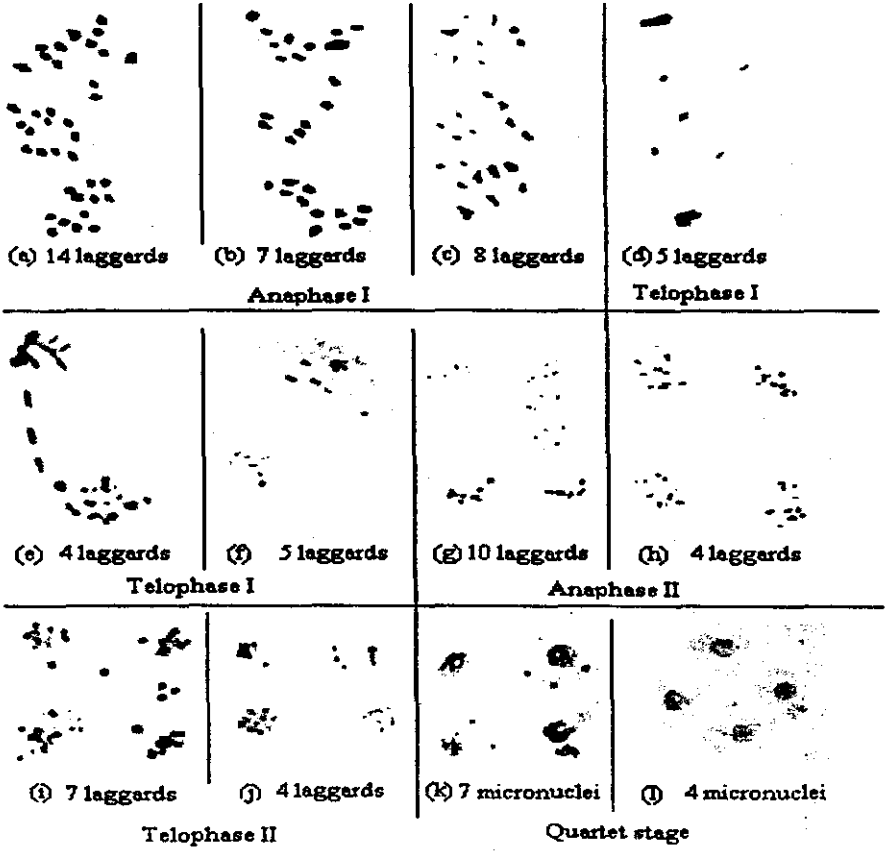


Figure (4): Photomicrographs of different meiotic stages: **a-**, **d-**, **g-**, **i-** and **k-** (*B. napus* x *B. juncea*), **b-**, **h-**, and **l-** (*B. campestris x B. juncea*), **c-** (*B. nigra x B. juncea*)), **e-** and **j-** (*B. napus* x *B. campestris*) and **f-** (*B. carinata* x *B. oleracea*) (x = 1500).

At telophase I, lagging chromosomes were observed in the F_1 crosses of (*B. napus* x *B. juncea* (Figure 4d), (*B. nigra* x *B. juncea*), (*B. campestris* x *B. juncea*) and (*B. napus* x *B. campestris*, (Figure 4e)) with an average of 4.62, 4.54, 3.52 and 3.44, respectively. The remaining two F_1's of (*B. carinata x B. nigra*) and (*B. carinata* x *B. oleracea* (Figure 4f)) possessed laggards with an average of 2.65 and 4.54, respectively.

At anaphase II, lagging chromosomes were observed in the F_1 of (*B. napus x B. juncea*, (Figure 4g)), (*B. campestris x B. juncea*, (Figure 4h)), (*B. napus x B. campestris*), (*B nigra x B. juncea*), (*B. carinata x B. nigra*) and (*B. carinata x B. oleracea*) with an average of 9.00, 3.64, 3.51, 3.41, 2.50 and 5.16, respectively.

At telophase II, lagging chromosomes were again observed in the F_1 crosses of (*B. napus x B. juncea* (Figure 4i)), (*B. campestris x B. juncea*), (*B. napus x B. campestris* (Figure 4j)), (*B. nigra x B. juncea*), (*B. carinata x B. nigra*), (*B. carinata x B. oleracea*) with an average of 5.46, 3.28, 3.57, 3.53, 3.22, 3.42, respectively (Table 4).

Number of micronuclei:

At quartet stage, no micronuclei and/or microcytes were observed in the parental species; however from zero to seven micronuclei were found in the F_1 crosses. In the F_1's of (*B. napus x B. juncea*, Figure (4k)), (*B. campestris x B. juncea*) and (*B. napus x B. campestris*), micronuclei with an average of 5.91, 3.58 and 3.24, respectively were recorded. While the F_1's of (*B. nigra x B. juncea*) and (*B. carinata x B. oleracea*) possessed micronuclei with an average of 1.56 and 1.38, respectively, three and thirteen percent of the tetrads in such two F_1's showed no micronuclei (Table 4). On the other hand, micronuclei were observed in the F_1 cross of (*B. carinata x B. nigra*) with an average of 0.95, while five percent of the examined tetrads in this F_1 were found to be free from micronuclei.

Concerning the chiasmata frequency per cell and per bivalent at both diakinesis and metaphase I stages, the obtained result revealed that the six interspecific F_1 hybrids, generally exhibited reduction of chiasmata frequency per cell and per bivalent compared with their corresponding parents. The results also indicated that the terminalization of chiasmata in such six F_1 crosses was faster than those detected for their parental cultivars.

The results also revealed that the six F_1 crosses showed varied number of laggards at each of the first and second meiotic anaphase and telophase stages as well. These delayed chromosomes are a usual consequence of the existence of univalents of the single genomes which often appeared unpaired (non conjunction) or probably resulted from the early disjunction of chiasmata at metaphase I stage. Furthermore, such laggards usually travel to the poles but sometimes arrive too late to be included with the daughter nuclei.

At quartet stage, micronuclei were observed in the six interspecific F_1 hybrids. Such micronuclei are the result of laggards at the meiotic stages prior to quartet stage.

Esterase (EST) isozyme patterns:

The results of electrophoretic isozyme patterns of EST of the parental species, *B. oleracea, B. carinata, B. nigra; B. juncea, B. napus* and *B. campestris* and their F_1 crosses are presented in Table (5a) and Figure (5a).

The maximum number of six bands for EST was recorded for *B. campestris* followed by *B. carinata* and *B. napus* with five bands for each. On the other hand, it was found that the lowest number of three bands was recorded for *B. juncea*. These isozyme bands were found to be distributed in two zones in these parental species. In the first zone, three, five, four, two, five and four bands were recorded for *B. oleracea, B. carinata, B. nigra, B. juncea, B. napus* and *B. campestris*, respectively. In the second zone, two, one, and two were detected in *B. oleracea, B. juncea* and *B. campestris*, respectively.

A maximum number of 14 bands was detected for the (*B. carinata x B. oleracea*) F_1 followed by (*B. nigra x B. juncea*) F_1 which exhibited eleven bands. However, the lowest number of five bands was observed for (*B. campestris* x *B. juncea*) F_1.

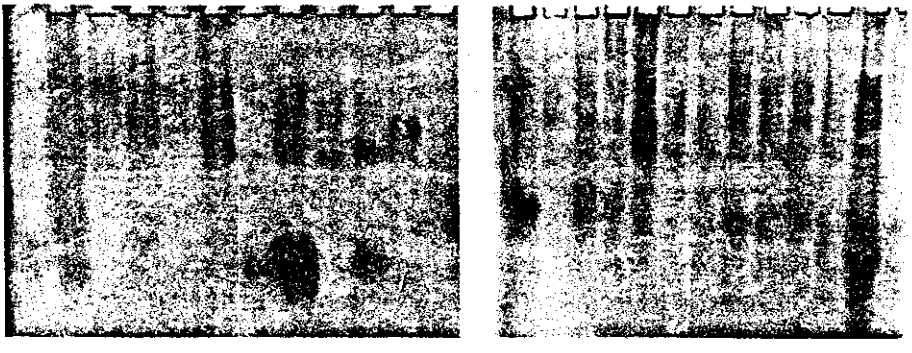
These results revealed that the Egyptian cultivar *B. campestris*, which was the highest polymorphic cultivar, exhibited a unique band (no. 6) and shared band no. 7 with the Swedish cultivar *B. carinata*. The other Egyptian cultivar; *B. juncea* had also a unique band (no. 13). The F_1 hybrids; (*B. carinata* x *B. oleracea*) was found to be the highest polymorphic hybrid (100%) followed by (*B. carinata* x *B. nigra*) and (*B. nigra* x *B. juncea*) F_1's (64.2%).

Peroxidase (PER) isozyme patterns:

Data of the PER patterns of the interspecific F_1 crosses and their corresponding parents are presented in Table (5b) and Figure (5b). The results showed that *B. nigra* was found to be highly polymorphic (84.6%) with eleven bands, followed by *B. napus* (76.9%) with ten bands and the lowest polymorphic cultivar was *B. campestris* (53.8%) with seven bands.

The highest polymorphic hybrid was (*B. campestris x B. juncea*) (100%) with 13 bands, followed by (*B. carinata x B. nigra*) F_1 with ten bands (76.9%). The lowest polymorphic hybrid was (*B. carinata x B. oleracea*) (38.5%) with five bands.

While the hybrid (*B. carinata x B. oleracea*) possessed 14 esterase bands with high activity and was the highly polymorphic one, it had poor peroxidase activity and was the lowest polymorphic hybrid in peroxidase analysis. On the opposite side, while the hybrid (*B. campestris x B. juncea*) acted very well with peroxidase and was the highest polymorphic hybrid, it acted very poorly with the esterase isozyme.



(a) (b)

Figure (5): Photographs of esterase (a) and peroxidase (b) isozyme patterns of the six parental *Brassica* species and their six interspecific F_1 hybrids. Samples numbers (lanes) are presented as follows: (a & b) 1, 3, 5, 7, 9 and 11, respectively, showing the parental species; *B. oleracea, B. carinata, B. nigra, B. juncea, B. napus* and *B. campestris*, 2, 4, 6, 8, 10 and 12 showing the F_1's of (*B. carinata x B. oleracea*), (*B. carinata x B. nigra*), (*B. carinata x B. oleracea*), (*B. carinata x B. juncea*), (*B. napus x B. campestris*) and (*B. campestris x B. juncea*), respectively.

Table (5): Description of esterase and peroxidase isozyme patterns of the six parental species and their interspecific F_1 hybrids.

a. Esterase isozyme patterns

No. of bands	Genotypes											
	B. oleracea	*B. carinata* x *B. oleracea*	*B. carinata*	*B. carinata* x *B. nigra*	*B. nigra*	*B. nigra* x *B. juncea*	*B. juncea*	*B. napus* x *B. juncea*	*B. napus*	*B. napus* x *B. campestris*	*B. campestris*	*B. campestris* x *B. juncea*
	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-				-						
2		-				-						
3		++		-		-						
4		+++	++	-	-	++		++	-	-		
5	-	+++	++	++	-	++		++	-	-	-	
6	-	+++	-	++	-	+++	-	++	-	-	++	-
7		+++	++	+++	-	+++	-	++	-	+++	+++	-
8		++	-	++		++		-	-		-	-
9		++		-		-						-
10		+++		-		-						-
11	++	+++		-		-		+++		-		
12	++	++++						+++		+++	-	
13		++					++	+++		++	-	
14		+++						++				
Total	5	14	5	9	4	11	3	9	5	7	6	5

b. Peroxidase isozyme patterns

1	-			-	++	-	++	-	++		++	++
2	-			-	++	-	-		-			-
3	-	-	-	-	++	-		-	-	-	-	++
4	++	-	++	-	++	-	-	++	-	-		++
5	++	-	++	++	++	-	-	++	++	++	-	++
6	-	-	++	++	+++	++	++	++	++	++	++	++
7	-		-	-	++	-	-	++	++	++	-	++
8	+++	-	+++	++	+++	-	-	++	++	++	++	++
9	-		++	+++	+++	-	-	++	-	-	-	+++
10			-	-	++		-	-	-	-		+++
11					-							+++
12												++
13												-
Total	9	5	8	10	11	9	9	9	10	8	7	13

The obtained results showed that heterozygosity was higher in the Egyptian cultivar than that of the Swedish ones in ES patterns. Analysis of EST, PER and phosphorylases (PHO) isoenzymes by Kato and Tokumasu (1979) and Schek and Wolf (1986) showed that banding patterns in the hybrids comprised bands from both parental species, plus additional bands which may have been produced by subunit re-association. Studies on *B. napus, B. campestris* and *B.*

alboglabra (a form of *B. oleracea*) by Chen *et al.* (1989) found that peroxidase (PER) was monomorphic unlike the present results and the result reported by Thorpe *et al.* (1987). Simonsen and Heneen (1995) repotred that ten out of 17 isozyme loci were polymorphic in *B. campestris* and six in *B. oleracea.* They found that the level of heterozygosity was lower in the Swedish cultivars of *B. campestris* than that of the Chinese landraces and the other cultivars of *B. campestris.* However, the level of heterozygosity in *B. oleracea* was even lower than that in the Swedish cultivars of *B. campestris.* Lazaro and Aguinagalde (1998) studied genetic variation in 36 populations of *B. oleracea* using isozyme variation at 11 loci for five enzyme systems (IDH, 6-PGD, PGM, PGI, and MDH) and found that the highest polymorphism was 54% among these populations.

DNA content:

Results obtained in Table (6) showed that *B. napus* cultivar possessed the highest amount of nuclear DNA in the parental *Brassica* species followed by *B. juncea* while, the lowest amount of nuclear DNA was for *B. nigra.* Furthermore, DNA content of *B. napus*, *B. juncea* and *B. carinata* are approximately twice the sum of the DNA in the parental cultivars, *B. campestris, B. oleracea* and *B. nigra.* These results are in a good harmony with those obtained by Sundberg and Glimelius (1986) and Verma and Rees (1974) since they reported that the amount of nuclear DNA in the amphidiploid Brassica species was equal to those of respective diploid parents.

Table (6): DNA content of the six parental cultivars.

Species	Concentration µg/ml
B. nigra	350
B. oleracea	380
B. campestris	420
B. carinata	730
B. juncea	770
B. napus	780

Intergeneric and interspecific hybridization may bring about genomic changes such as gene inactivation, chromosome elimination, and chromosome addition (**Namai, 1987**)

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الملخص العربى

التباينات السيتووراثية والبيوكيميائية بين بعض أنواع جنس البراسكا وهجنها

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تمت دراسة التباينات السيتووراثية والبوكيميائية بين ستة أصناف منزرعة لجنس البراسيكا *Brassica* ssp. وهجنها . وقد وجد أن السلوك الكروموسومي كان طبيعيا في انواع البراسيكا الثنائية (*B. nigra* و *B. oleracea* و *B. campestris*) والانواع الرباعية (*B. carinata* و *B. juncea* و *B. napus*).وقد وجدت شذوذات كروموسومية تتضمنت الوحدات الكروموسومية الثنائية ، والثلاثية والرباعية في الهجن الستة ، ووجد ان عدد الكيازما كان عاليا في النوع *B. napus* بينما كان معامل الانزلاق عاليا في النوعين *B. nigra* و *B. oleracea* . كما اظهرت الهجن بين النوعية نقصا في عدد الكيازمات ، ووجد أن هذه الهجن تحتوي علي عدد يتراوح بين 1 و 14 وحدات متلكئة ، ووجد ايضا ان هناك هجينين احتويا علي عدد من النويات الصغيرة تراوح بين اثنتين إلي أربعة بمتوسط 3.85 في الهجين (*B. campestris* x *B. juncea*) ومتوسط 3.24 في الهجين (*B. napus* x *B. campestris*).بينما احتوت باقي الهجن علي عدد تراوح بين نوية إلي سبع نويات صغيرة . وقد تم التعرف علي اختلافات في نشاط الحزم لكلا من مشابهي الانزيمات الاستيريز والبيركسيديز بين الانواع الابوية وهجنها . كما لوحظ أن نماذج الحزم فى الهجن النوعية ما هى إلا محصلة الحزم من كلا الأبوين الداخليين فى تكوين الهجين. كما لوحظ وجود حزم إضافية والتى يحتمل أن تكون ناتجة عن إتحاد تحت الوحدات مع بعضها. أظهرت نتائج عزل ونتقية وقياس المحتوى الجينى لكل الأصناف الأبوية المذكورة عالية أن كمية الـ DNA لكل من الأصناف الرباعية وهى *B. napus, B. carinata, B. juncea* ما هى إلا محصلة لمجموعة كمية الـ DNA للأباء الثنائية الداخلة فى تكوين هذه الأصناف الرباعية والتى تتضمن *B. nigra, B. oleracea, B. campestris* حيث وجد أن كمية الـ DNA فى *B. napus* هى مجموع كمية الـ DNA لكل من *B. carinata, B. oleracea, B. compestris* تقريبا. وكذلك بالنسبة لكل من *B. juncea*