MEIOTIC EVALUATION OF *BRASSICA CAMPESTRIS* DESI AND *BRASSICA NAPUS* HS-98 FOR CHROMOSOMAL STABILITY AND POLLEN FERTILITY

M. Islam, Salman, H. Ahmad, M. Sajid* and Inamullah

Department of Genetics, Hazara University, Mansehra, Khyber Pakhunkhawa,
*Department of Agriculture, Hazara University, Mansehra, Khyber Pakhunkhawa,
Corresponding author's email: mislamhu@hu.edu.pk

ABSTRACT: The objectives of the current study were to record and document the chromosomal behavior, stability and pollen fertility in some selected genotypes of *Brassica*. The floral buds were collected before sunrise, fixed in acetic acid ethanol for 24 hrs and preserved as a stock for cytogenetic and pollen fertility analyses. The subsequent meiotic analysis was conducted in Meiotic-I (MI) of pollen mother cells (PMC) of *B. napus* and *B. campestris* (Taylor, 1924). The results showed regular 10 bivalents, one by one synapses and one to one disjunction in *B. campestris*. A total of 450, bivalents were observed, among them, 300 were rings and 150 rods bivalents. The total numbers of chiasmata observed were 752 giving an average of 17 chiasmata per cell. The fertile pollen percentage was 94% in 74 pollen observed. Similarly, the meiotic behavior of the *B. napus* HS-98 showed regular 19 bivalents and a total of 855 bivalents were observed. Among these, 506 were ring and 349 rod bivalents. The total numbers of chiasmata observed were 1362 and an average chiasmata per cell were 30.3. The pollen fertility was 93% on the basis of 79 pollens observed. The cytogenetic analysis confirmed that both the genotypes were stable, diploids and high pollen fertility. Moreover, the genotypes can confidently be used as stable materials for further breeding program.

Key words: *Brassica napus* L., *B. campestris* L., Meiotic analysis, Chiasma and Pollen fertility.

(Received 06-05-2017 Accepted 27-06-2018)

INTRODUCTION

Brassica napus and *B. rapa* belong to the genus *Brassica* and family Brassicaceae. Worldwide, family Brassicaceae consists of 375 genera and 3200 species (Jessop and Toelken, 1986) while in Pakistan only 92 genera and 250 species are reported (Jaffery, 1973). Among *Brassica*, *B. campestris* and *B. napus* are grouped as rapeseed (Khan & Munir, 1986), while *B. juncea*, *B. carinata* and *B. nigra* are placed into mustard group. Globally, rape seed oil contributing 13% to the world's supply (Raymer, 2002), while in Pakistan, rape seed and mustard are contributing 17% to the domestic production of edible oil (Annonymous, 2017). For oilseeds and vegetable oil, China and USA are the major importer and exporter, respectively (Sharma, *et al*., 2012), while globally, the overall rapeseed production increased by 2 percent as compared to the previous data (USDA, 2018).

The cytological study of (U., 1935) confirmed and revealed that *B. campestris* composed of AA genome, (2n=20), *B. nigra* have BB genome (2n=16), *B. oleracea* have CC (2n=18) and the genome of three amphidiploids such as *B. juncea* consist of (AABB, 2n=36), *B. napus* (AACC, 2n=38) and *B. carinata* (BBCC, 2n=34). Similarly, the application of *Brassica* triangle of three diploid species and their corresponding amphiploids is an ideal model system for evaluation of homeologous recombination and polyploidization (Snowdon, 2007). For conformation of primary basic chromosome number, cross was made between two different genotypes of *B. juncea* and confirmed that the two genotypes comprises of genome A and B and evolved from six hexaploid (Paritosh *et al.,* 2014). Similarly, in another study it was concluded that the genome of *B. juncea* is relatively stable as compared with its diploid progenitor species and provide information related to evolution of *B. juncea* (Xu *et al.,* 2016). The syntenic relationship of those loci which delimit the *miR165* between *A. thaliana* and *B. rapa* and recognize paralogs among these loci and the overexpression of *miR165a* in *B. juncea* and *A. thaliana* showed altered flower development and reduced vegetative growth (Sirohi *et al.,* 2018). In recent past, a genome wise integration of *Brassica* maps has been constructed which will generate a novel data for rapeseed and Canola research (Wang *et al*., 2011).

Similarly, through cytogenetic tools, technique and better karyotype analysis accurate identification of chromosomes possessing agriculturally important traits can be carried out (Navabi *et al.,* 2010). Further, through fluorescence *in situ* hybridization (FISH), the distribution patterns and chromosomal localizations of the repeat sequences can easily be evaluated and chromosome can easily identified (Xu *et al.,* 2016). Use of microsatellite (SSR) provides fruitful information about the distribution and evolution of morphotypes of *Brassica species* and

varieties (Zheng *et al*., 2016). Similarly, SNP markers are used for mapping of genetically diverse *B. juncea* genome and provide information about evolution of *B. juncea* (Kumar *et al*., 2014). The cytogenetic variability in terms of variation, distribution and availability of microsatellites in some specific and motif-dependent hybridization patterns (Carmona *et al*., 2013). Through rDNA, the distribution pattern and constitutive heterochromatin regions between morphotypes can be described and compared (Zheng, 2015). In *B.rapa*, the whole genome was triplicated and the tandem repeats were fractionated when compared with tandem repeats of *B. thalayana* (Fang *et al.,* 2012). Moreover, through these modern technique and procedures, comparative genetic and cytogenetic studies can conducts karyotyping and genome evolution in members of Brassicaceae (Lysak and Koch, 2011). Further, through fine karyotyping analysis, each individual somatic metaphase chromosome can be easily identified (He *et al.,* 2015). Therefore, the current study was carried out to observe meiotic behavior and stability within the *B. campestris* Desi and *B. napus* HS-98.

MATERIALS AND METHODS

Two genotypes, one each of *B. campestris* L. and *B. napus* L. viz. Desi and HS-98 were used for this study and grown at Hazara University, Mansehra. Approved cultural practices were applied for their cultivation. For cytogenetic evaluation, young floral buds were collected before sunrise and kept in 1:3 acetic acid ethanol for 24 hrs. After fixation, the materials were preserved in 70% ethanol. A total of 45 pollen mother cells were randomly selected and cytological investigation was carried out through smear method (Taylor, 1924). For slide preparation, single anther/pollen mother cell was placed on the slide and squashed in 2% acetocarmine. The debris were removed, coverslip was placed on the squishing spot and extra stain under the cover slip was sucked with the help of filter paper. The slide was gently heated and examined under microscope by using 10x and 100x lenses. Slide having cells were randomly spread, selected for data collection. The number and morphology of chromosomes were observed and data were recorded in written as well as in pictorial form. The cover slip was carefully removed and small amount of Canada balsam was placed, heated gently and again cover slip was placed carefully. For permanent slid preparation, the mounted slides were placed on slide warmer at 50^{o} C for 72 hrs .

Pollen fertility of the genotypes was observed in ten randomly selected plants. Mature anther was taken from each genotype, cut at the middle and pressed both sides of the anther with needles in 1% acetocarmine. Debris were removed and the extra stain was sucked with filter paper. The pollens were viewed under the microscope by using 10x and 100x lens. Darkly stained pollens were considered as fertile and unstained as sterile.

RESULTS AND DISCUSSION

In the present investigation, the meiotic behaviour of the *Brassica campestris* and *B. napus* chromosomes were evaluated at metaphase-I. The number of chromosomes observed in *B. campestris* were 10 bivalents, 2n= 20. Out of total 450 bivalent 300 were ring, 150 rods and an average of 6.66 and 3.33 per cell, respectively. The total number of chiasmata in 45 sampled cells were 752 and an average 16.71 chiasmata per cell (Table-1). Similarly the meiotic behavior of the *B. napus* revealed that, it had 38 chromosomes associated with 1-to-1 combination. This association appeared in the form of 19 bivalents in pollen mother cells. The pollen mother cells had no supernumerary or B-chromosomes. No secondary association was observed at M-I. All these factors confirmed that the genotype HS-98 having its origin from an interspecific cross completely behaved as normal diploid.

Cytogenetic evidence of *B. napus* revealed that out of 855 bivalents, in 45 sampled PMC's, the number of rod bivalents, ring bivalents, number of chiasma and chiasma per cell were 349, 506, 1362 and 30.26, respectively. The value of rod bivalents per cell, ring bivalents per cell and chiasma per bivalent were 7.75, 11.24 and 1.59, respectively (Table-1).

Table-1: Chromosomal configuration and chiasma distribution in pollen mother cells of *B. campestris* Desi and *B. napus* HS-98.

***B. campestris* Desi**		**Rod Bivalents**		**Ring Bivalents**		**Chiasma**		
PMC's Scored	**No of Bivalents**	**Total**	**Cell^{-1}**	**Total**	**Cell^{-1}**	**Total**	**Cell^{-1}**	**Bivalent^{-1}**
45	450	150	3.33	300	6.66	752	16.71	1.67
***B. napus* HS-98**								
PMC's Scored	**No of Bivalents**	**Total**	**Cell^{-1}**	**Total**	**Cell^{-1}**	**Total**	**Cell^{-1}**	**Bivalent^{-1}**
45	855	349	7.75	506	11.24	1362	30.26	1.59

The fertile pollen percentage in *B. campestris* remained 94% of the 73.8 pollens observed. Similarly, the fertile pollen percentage in *B. napus* remained 93.3% and 79.2 pollens were observed (Table-2).

Table-2: Pollen fertility of *B. campestris* var. Desi and *B.napus* HS-98.

***B. campestris* var. Desi**				***B. napus* HS-98**			
Non fertile	**Fertile**	**Total Number**	**Fertility Percentage**	**Non fertile**	**Fertile**	**Total Number**	**Fertility Percentage**
7	93	100	93	3	91	94	97
1	46	47	98	6	88	94	94
5	65	70	93	1	67	68	98
7	87	94	92	7	56	63	89
9	91	100	91	9	98	107	91
4	59	63	94	4	59	63	93
3	47	50	94	5	77	82	94
2	61	63	97	1	67	68	98
5	78	83	94	8	89	97	92
4	64	68	94	7	49	56	87
Avg. 4.7	69.1	74	94	5	74	79	93

The diploid nature of *B. campestris* (2n= 20), have been reported by Roobbllen (1960). The evolution of *Brassica* species U (1935), also conformed by Waminal *et al.,* (2016), Parkash (1974) was put forward a theory that *B. campestris* has evolved from a six paired parent and karyotyping of *B. campestris* genome was presented which was confirmed by (Paritosh *et al*., 2014; Xu *et al*., 2016). Moreover, the whole genome triplication play a vital role in speciation and morphotype diversification of Brassica, Cheng *et al.,* (2014).

According to Robbellen (1960) the genomic formula for *B. campestris* is "ABBCDDEFFF" which signifies that *B. campestris* behaves as diploid for chromosomes A, C and E, tetraploid for B and D and hexaploid for F chromosomes. A 10 major *Brassica* repeats appeared and occupied more than 50% of each respective unassembled genome sequence Waminal *et al.,* (2016) which support the finding of Robbellen. Similarly, chromosomal specificity of molecular markers, SSR, karyotyping can easily be investigated, Waminal *et al.,* (2018). The present investigations support the work of Venkateswavlu and Kamala (1971) and therefore multivalent or univalent were observed at metaphase-I.

The meiotic behaviour of the *B. napus* under observation revealed its true diploid nature, 2n= 38, 19 bivalents at metaphase-I of meiosis which confirm the previous result of U (1935), whole genome duplication facilitate high diversification Tank *et al*., (2015). Similarly, Xiong and Pires (2011) also conformed the chromosomal comparison, recombination homologous and genome evolution in *Brassica* through novel chromosomal painting technique.

In the current study, chromosomes segregated as a normal diploid and at anaphase-II 19 chromosomes per gametic cell were observed. No secondary associations were observed, which confirms that *B. napus* behaves as normal diploid. Whatever its origin and phylogeny may be, it has diploidized its chromosomal behavior. It is thought that it might be originated from a six-paired ancestor but its 19 pairs may or may not be due to segment polyploidization. In short, whatever the phylogeny may be *B. napus* currently behaves as a diploid species because it has no univalent or multivalent formation at Metaphase-I in meiosis. Similarly, a karyotypic diversity of three genotypes of *B. juncea* was carried out and result showed that the genome is relatively stable as compared with its diploid progenitor species Xu *et al*. (2016).

Conclusion: The current cytological investigation concluded that both *B. campestris* Var. Desi and *B. napus* -HS-98 were 10 and 19 bivalents, respectively and *B. napus* HS-98 is the most suitable genotype for further breeding program and should be released as a variety.

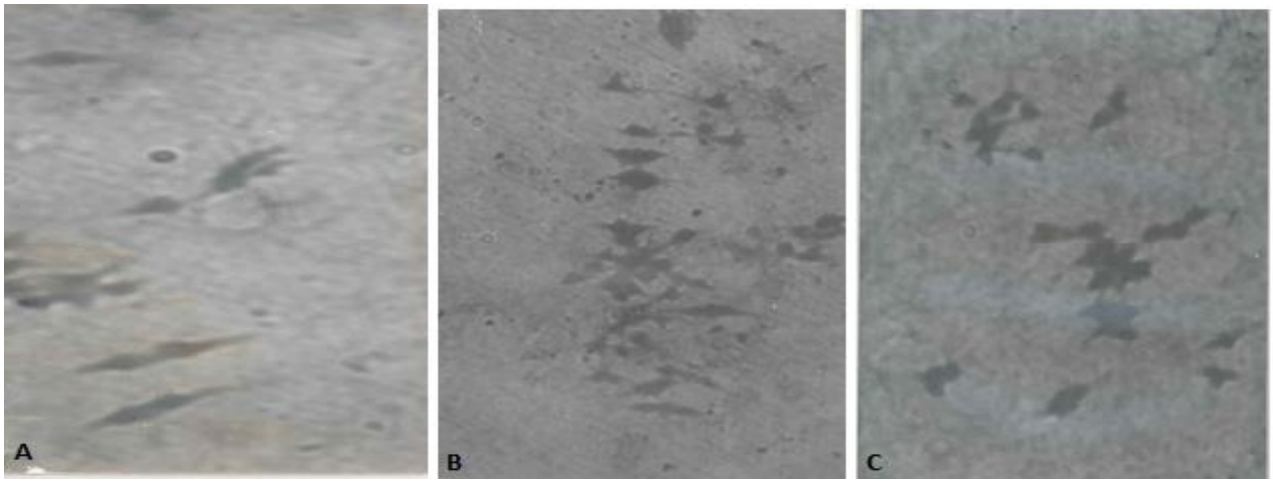

Figure-1: A. MI of *B. campestris* 2n=20 B. MI of *B. napus,* 19 bivalents
C. *B. napus*, overlapping behavior.

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