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# Evaluation of the Advance Rapeseed Line HS-98 for Cytogenetic and Physiological Stability

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**Abstract:** Meiotic analysis was carried out in M-I of pollen mother cells of genotype HS-98. The chromosome number was 2n = 38 (19 bivalents per cell). No univalents, multivalents, secondary association or B-chromosomes were recorded. Total number of bivalents was 950 in 50 observations. The number of rod-IIs, ring-IIs, number of chiasma and chiasma per cell were 569, 381, 1513 and 30.26, respectively. The value of rod-IIs per cell, ring-IIs per cell and chiasma/bivalent were 7.82, 11.38 and 1.59, respectively. The fertile pollen percentage remained 93% of the 604 pollen observed. The studies concluded the genotype exhibits both genetic and physiological stability and is therefore recommended for evaluation as commercial variety.

**Key words:** *Brassica napus* · meiotic analyses · pollen fertility · genotype evaluation

## INTRODUCTION

Rapeseed and mustard comprise five known species of the genus Brassica. Among which *Brassica rapa* and *Brassica napus* are grouped into rapeseed or Canola. Unfortunately its production is 6-8 times less in the subcontinent as compared to the developed countries of the Northern Hemisphere. Unavailability of the improved genotypes is the main constraint for improved yield and quality of rapeseed in the developing countries like Pakistan.

The developed countries mostly improved their genotypes through species introgression [1-3], which is not established in Pakistan. Hence a strategy to collate and introgress genes of the desired traits of different species was implemented [1, 4-7]. A number of interspecific hybrids were generated, some of which were tested for their yield and quality potential and other are in progress [8-10].

This paper is a continuity of the Brassica breeding program. An advance line of rapeseed select from the interspecific progeny of *B. napus* X *B. juncea* for early maturity, aphid tolerance and high yield was evaluated for cytogenetic stability. Results of the study are communicated here.

## MATERIAL AND METHODS

An improved rapeseed genotype HS-98 (Habib Selection-98) was evaluated for the important cytogenetic and physiological traits. Pollen Mother Cells and pollen grains were the sources of information. For cytogenetic studies floral buds were collected in the early morning and fixed in 1:3 acetic acid ethanol for overnight. The fixed buds were preserved in 70% ethanol. Preparations were made in 2.0% acetocarmine. For preparation single anthers were excised and squashed in 2% acetocarmine. The debris was removed and extra stain under the cover slip was sucked with the help of filter paper. The slides were gently heated and examined in microscope. The cytoplasm if needed was decolorized by passing 45% acetic acid through the edges of the cover slip. Slide having best spread were selected for data collection. Mounting was done in Canada Balsam and the mounted slides were placed on slide warmer at 50°C for 72 h. The studies were carried out in Genetics Research Lab of Government Postgraduate Jahanzeb College Saidu Sharif, Swat.

Fertility of the genotype was determined in 10 randomly selected plants. Mature un anthecised flowers of each plant were collected separately and preserved in

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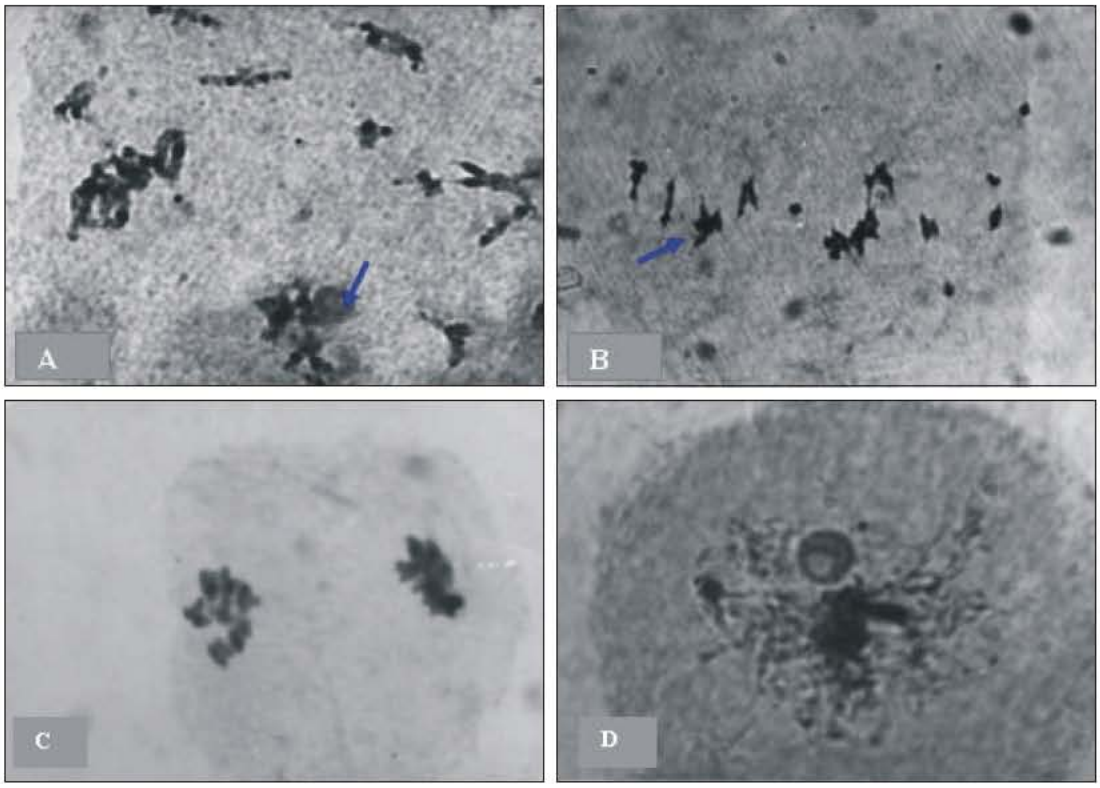


Fig. 1: (A) Diplotene in HS-98 showing partially associated chromosome arrowhead shows nucleolus in the PMC, (B) MI showing the spread of 19IIs arrowhead shows the position of extra chiasma, © telophase I showing no sign of laggards or bridges, (D) Leptotene showing a single nucleolus confirming diploidized nature of the genotype

Table 1: Chromosomal configurations and chiasma distribution in PMC's of HS-98

| | | Rod bivalents | | Ring bivalents | | Chiasma | | |
|---|---|---|---|---|---|---|---|---|
| PMC's scored | No. of bivalents | Total score | Cell$^{-1}$ | Total score | Cell$^{-1}$ | Total score | Cell$^{-1}$ | Bivalent$^{-1}$ |
| 50 | 950 | 569 | 7.62 | 381 | 11.38 | 1513 | 30.26 | 1.59 |

Table 2: Pollen fertility of HS-98 observed under microscope

| S. No | Sterile pollen | Fertile pollen | Total count | fertility percentage |
|---|---|---|---|---|
| 1 | 8 | 49 | 57 | 85 |
| 2 | 7 | 50 | 57 | 87 |
| 3 | 6 | 65 | 71 | 91 |
| 4 | 2 | 55 | 57 | 96 |
| 5 | 1 | 53 | 54 | 98 |
| 6 | 3 | 58 | 61 | 95 |
| 7 | 2 | 60 | 62 | 96 |
| 8 | 4 | 62 | 66 | 93 |
| 9 | 2 | 56 | 58 | 96 |
| 10 | 4 | 57 | 61 | 93 |
| Mean | 3.9 | 56.5 | 604 | 93 |

70% ethanol. Mature anthers was cut in the middle on microscopic slide and pressed with the help of needles in a drop of 1% acetocarmine. Debres were removed and the extra stain was sucked with filter paper. The pollen were viewed under the microscope. Darkly stained pollens were counted as fertile and the unstained pollens were considered as sterile.

## RESULTS AND DISCUSSION

Results obtained for the subject study are summarized in Tables 1 and 2. Meiotic behaviour of the genotype revealed that, it had 38 chromosomes associating in 1-to-1 combination, appearing 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 MI. All these factors confirms that the genotype HS-98 having its origin from an interspecific cross completely behave as normal diploid.

Cytologenetic evidence (Table 1) revealed that out of the 950 bivalents, in 50 PMC's the number of rod-IIs, ring-IIs, number of chiasma and chiasma per cell were 569, 381, 1513 and 30.26, respectively. The value of rod-IIs per cell, ring-IIs per cell and chiasma/bivalent were 7.62, 11.38 and 1.1.59, respectively. The fertile pollen percentage remained 93% of the 604 pollen observed as shown in Table 2.

It is a general observation that interspecific hybiridazition is associated with anomaly of inconsistency of chromosomal pairing. Osborn *et al*., [11] confirms even chromosomal transposition *Brassica napus*. Ahmad and Khan [12]; and Ahmad and Hasnain [5] reported that the process of diploidization bring stability in chromosome pairing. The process of diploidization is easy in the case of homoeoploid genomes [1, 6]. Jenczewski [7] is of the opinion that high level pairing in the oil seed rape haploids isolates different varieties.

All these findings proved that HS-98 has introgressed genes of earlyness from *B. juncea* and that of high yield from *B. napus*. Further more the 1-to-1 disjunction (Fig. 1) and high male fertility of the genotype (Table 2) shows that the line is completely stable with respect to its genetics and physiology. It is therefore recommended for registration as improved commercial variety.

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