**ABSTRACT:** Meiotic behaviour of plant chromosomes is influenced by both genetic and environmental factors. The present paper shows the affect of abnormal meiosis on pollen fertility. Meiotic restitution is considered to be a common mechanism of polyploidization in plants and hence is one of the most important processes in plant speciation. Pollen mother cells (PMCs) of diploid potato from anthers at different stages of meiotic process were analyzed for their chromosomal behaviour and irregularities. Pollen morphology was also studied through SEM as it is significantly helpful at the generic and specific level. Various meiotic irregularities were observed: univalent and multivalent formation, chromosome bridges, fragments, lagging chromosomes etc. B-chromosome was first time reported in wild diploid potato, though the frequency of the same was less. Specie was identified as Solanum chacoense.

**KEYWORDS:** Diploid potato, Male Meiosis, Meiotic Abnormalities, Palynology, Solanaceae

Meiosis is a cytological basis for gametogenesis and inheritance in eukaryotic organisms and maintains genomic stability as well as integrity over sexual life cycles. Meiosis involves specialized cell division which is a dynamic cellular process controlled by the action and synchrony of many genes (Baker et al., 1976; Schwarzacher, 2003), and action and function of these genes is also influenced by environmental factors such as high and low temperatures, chemicals, radiations and nutrition. Different genes have been identified in plants which condition the different events in meiosis. Basic steps of meiosis involve molecular and cellular events, such as DNA and chromosome replication, chromosome pairing, synapsis and recombination, chromosome segregation and cytokinesis (Cai and Xu, 2007). Balanced chromosome segregation followed by normal cell division and formation of a bipolar microtubule based spindle is required for normal meiosis. Conversely any discrepancy at molecular or cellular level during meiosis may perhaps causes male sterility. Meiotic abnormalities which generally affect the pollen viability have troubled sexual reproduction. Unreduced gametes (2n) are recognized as a common mechanism of origin of most polyploids in plants (Sang et al. 2004; Otto 2007). Generally, 2n gametes originate from deviating meiosis in plants (Ramanna and Jacobsen 2003). Meiosis is a highly dynamic cellular process controlled by a complex genetic network (Golubovskaya 1979). In plants, it has long been recognized that mutant genes can affect meiosis in various ways and that some of these may lead to the formation of 2n gametes (Golubovskaya 1979; Kaul and Murthy 1985). During meiosis, different types of cytological irregularities can lead to 2n gametes with variable genetic composition (Matsuoka and Nasuda 2004). The potato (Solanum tuberosum L.) has turned from a garden of curiosity into one of the staple food crops of mankind because of its capacity to give high out turns of food per acre. Cytological information’s on the non-d cellular level during meiosis may perhaps.

**MATERIALS AND METHODS**

Appropriate sized flower buds of wild potato were collected from different parts of Himachal Pradesh (India) and fixed in Carnoy’s fixative (6:3:1= absolute alcohol: chloroform: glacial acetic acid v/v/v) for 24 hrs and preserved in 70% alcohol at 4°C. For meiotic studies the appropriate sized anthers were squashed in 2% aceticarmine. PMCs from anthers in different stages of meiotic process were analysed. To confirm the chromosome number in case of normal meiosis, around 100 pollen mother cells (PMCs) were observed at different stages of meiosis. In case of abnormal meiosis, however, more than 200 PMCs were considered to ascertain the type and frequency of various abnormalities per plant. Apparent pollen fertility was estimated by mounting mature pollen grains in glycerol aceticarmine (1:1). Normal well filled and deeply stained pollens were taken as fertile while shrivelled up and unstained pollens as sterile. Photomicrographs of pollen mother cells at various stages and pollen grains were made from fresh and prepared slides using Leica Qwin (X290, X900 and X2180) and Nikon Eclipse 80i microscope (X330, X1340 and X3400) Digital Imaging Systems.

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\text{Pollen fertility} \% = \frac{\text{Number of fertile pollen}}{\text{Total pollen observed}} \times 100
\]
Processing of pollen grains for SEM studies: Pollen grains suspended in a drop of water were directly transferred into a fine pipette to a metallic stub using double sided cellulose tape and coated with gold in a sputtering chamber (Ion sputter JFC-1100). Coating was restricted to 150A. The S.E.M examination was carried out on a Jeol microscope JSM-T200. The measurements are based on 15-20 readings from each specimen. Polar length, equatorial diameter, colpi length and exine thickness were measured. The terminology used is in accordance with Erdtman (1952); Faegri & Iversen (1964); Kremp (1965) and Walker & Doyle (1976).

RESULTS AND DISCUSSION
With respect to meiotic chromosome number, meiotic stages as well as abnormalities observed in each stage and data are presented in Table 1. A PMC at metaphase-I showing 12 bivalents (Fig. 2) and metaphase-II showing 12:12 distribution of chromosomes (Fig. 3). A number of meiotic abnormalities in one or other form have been noticed such as, B-chromosome (Fig. 4), chromosome stickiness at metaphase-I (Fig. 5), interbivalent connection (Fig. 6), late disjunction of bivalents (Fig. 7 and laggards (Fig. 7). As a result of these meiotic abnormalities, variable sized apparently fertile and sterile pollen grains (Fig. 9) were observed. 67% Pollen fertility was recorded. The presence of such association was reported in S. tuberosum (Sangowawa, B. G. and Choudhuri, H. C. 1986) and interpreted it as resulting from the presence of heterozygosity for structural change. Amongst the presently studied taxa, interbivalent connections were seen in the PMCs at M-I and A-I (2n=2x=24). Majority of the earlier workers opined that with the advancement of meiosis, there is a reduction in the frequency of interbivalent connections and the connections disappeared before the first metaphase stage. The phenomenon was first time reported in the polyploid plants by Kwada (1910) (cf. Darlington 1965).

According to Viinikka & Nokkala (1981), the fusion of heterochromatic regions of some of the chromosomes together early in the first meiotic division probably resulted in the formation of chromatic knots, which lead to the formation of prominent interconnections that were observed at various stages of meiosis. Viinikka & Nokkala (1981) have also opined that the main function of the interconnections is to keep the bivalents in contact to each other before the spindle fibers are formed, in order to guarantee the proper movements and orientation of the chromosomes in division plane. B-chromosome (Fig. 4) was first time observed in diploid potato as relatively small sized chromosomes in addition to the normal chromosomes. These do not pair with other chromosomes, thus remaining free entities and mostly peripheral in position. B-chromosomes are supposed to change the chiasma frequency, thereby affecting the genetic changes in the gametes and affecting the next generation as reported in several species (Jauhar & Crane 1990; Sheidai et al. 2003). B-chromosomes are also reported to cause chromosome stickiness in different plant species (Jauhar & Crane 1990; Plowman & Bougourd 1994). But at present the data is too small to infer any significance like that.

Genetic and environmental factors and several agents have been reported to cause chromosome stickiness (Pagliarani 2000). Sticky chromosomes along with laggards were found in different stages encountered in the PMCs of S. chacoense that occurred from the early stages of Diakinesis to anaphase-II. However, the frequency of PMCs showing chromatin stickiness was observed to be significantly higher during metaphase-I (Fig. 5). Chromosome stickiness either involved a few bivalents or the whole complement at metaphase-I. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao, 1996). Certain laggards were observed which were unable to reach the poles at anaphase-I/anaphase-II or telophase-II/II (Fig. 8) either due to the presence of multivalent, late disjunction of bivalents or stickiness of chromosomes. Inter bivalent connections (Fig. 6) are the result of non-synchronous behavior of bivalents in a way that some of the bivalents may fail to be arranged on spindle apparatus. This results in precocious and late disjunctions (Fig. 7) and as high as 27.78% inter bivalent connections was observed at metaphase-I whereas 39.28% of bivalents showed late disjunction at anaphase-I.

Table 1. No. of cells analyzed during Meiosis and percentage of abnormal cells observed in each meiotic phase

| Meiotic Phase | Total PMCs observed | Abnormal PMCs Number (%) | Abnormalities |
|---------------|---------------------|--------------------------|---------------|
| Diakinesis    | 35                  | 10 (28.57)               | Stickiness    |
| Metaphase-I   | 119                 | 28 (23.54) 33 (27.78) | Stickiness  Interbivalent connections |
| Anaphase-II/  | 140                 | 55 (39.28) 16 (11.43)  | Early/Late Disjunction Laggards |
| Telophase-I   |                     |                         |               |
| Anaphase-II/  | 82                  | 10 (31.67) 19 (23.17)   | Laggards  Stickiness |
| Telophase-II  |                     |                         |               |

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Meiosis is highly coherent and the process is genetically programmed which comprises of pairing homologous chromosomes, crossing over, reduced in chromosome number, and lacking of S period between the two divisions. Similar to any other biological process, all the sequential steps involved in meiosis are controlled by a large array of genes (Ramana 1974; Mok and Peloquin 1975; Mok et al. 1976; Koduru and Rao 1981; Falistocco et al. 1994). Mutation in any of these genes that govern micro or megasporogenesis from pre-meiotic to post meiotic events can lead to serious anomalies in the whole process resulting in the genetically aberrant end products having adverse impact on fertility and overall reproductive efficiency of the species (Lattoo et al. 2006). Furthermore, many abnormalities affecting plant fertility or causing total male sterility have been detected during the evaluation of meiotic behavior in some species.

Pollen characters have received more attention in taxonomic and pollen morphology. However, the ultra-structure of the pollen wall, its stratification and

Figure 1-9: Male meiosis in wild potato (*Solanum sp.*)
1. A specimen, 2. A PMC at metaphase-I showing 12 bivalents, 3. A PMC at metaphase-II showing 12:12 distribution of chromosomes, 4. A PMC at anaphase-I with B-chromosome (first report), 5. A PMC at metaphase-I showing stickiness, 6. A PMC at metaphase-I showing interbivalent connections, 7. A PMC showing late disjunction of bivalents at anaphase-I, 8. A PMC at anaphase-I showing laggard, 9. Fertile and sterile pollen grains. Scale Bar=10µm.
internal structure can hardly be studied by light microscope (Zavada 1990). Therefore Scanning and Transmission Electron microscopy become necessary in examining these characters. El-Ghazaly (1990) and Harky et al. (2000) reported on the morphology of pollen grains of many plant species.

Pollen grains were usually radially symmetrical, isopolar, prolate-spheroidal, or subprolate to prolate often oblate-spheroidal with 19.073x18.163 µm dimension and 1.05 P/E ratio. Generally tri-colporate (rarely 4-colporate), colpi with costae, colpal membrane psilate to sparsely or densely granulated, ora lalongate, sexine as thick as nexine, or slightly thicker or thinner than nexine. Pollen morphology of Solanaceous species was studied by Anjum Perveen et al. (2007) and M. A. Lashin (2012). This study was just a beginning to study the relationship between cytology and palynology relationship of the species under consideration. The data derived from cytological and palynological characters of the examined specie could contribute to the taxonomy of Solanaceae.

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