Chapter 4

MATERIALS AND METHODS

4.1. Materials

Plants belonging to the taxa listed below provided the material for the investigations described in the present thesis:

1. *Solanum nigrum* L. (2x, 4x, 6x) Indian
2. *S. nigrum* L. (6x) French
5. *S. nodiflorum* Jacq. subsp. *nodiflorum*
6. *S. nodiflorum* Jacq. subsp. *mutans*
7. *S. luteum* Mill.
8. *S. villasum* Mill.

The seeds of *S. americanum*, *S. luteum*, *S. villasum* and the diploid, tetraploid and hexaploid races of *S. nigrum* were obtained from the collections maintained by the Department of Botany, Aligarh Muslim University, Aligarh. The seeds of *S. nodiflorum* subsp. *nodiflorum* and *S. nodiflorum* subsp. *mutans* were obtained from R.J. Henderson, Department of Primary Industries, Brisbane, Australia. The plants grown from the
hexaploid seeds of \textit{S. nigra} obtained from France are explicitly designated as "French hexaploid \textit{S. nigra}" and the hexaploid plants grown from seeds of Indian origin are designated as "Indian hexaploid \textit{S. nigra}"

The seeds were sown in 30 cm pots. When the seedlings were 3 to 12 cm tall and had developed 3 or 4 leaves, they were transplanted to 30 cm pots, only one seedling being planted in one pot.

The plants were grown in net house for experimental use. The plants were susceptible to aphids. Therefore, they were sprayed periodically with dimecron solution (1 cc of dimecron in 10 litres of water).

4.2. Methods

4.2.1. Hybridization

Interspecific crosses were attempted in all possible combinations in order to determine the degree of genetic relationship among the species used in the present investigation.

Emasculation was done in the afternoon on buds which were expected to open the next day. In each inflorescence, buds
of the right stage, that is, buds with greenish yellow anthers were selected for emasculation and their anthers were removed. The flower buds were opened with fine forceps and anthers were taken out one by one without causing any injury to the gynoecium. After emasculation, the buds were bagged with a butter paper bag to prevent contamination by wind or insect borne pollen. Emasculated buds were tagged.

The flowers of plants used as male parent were also covered with butter paper bags before the dehiscence of the anthers with the object of preventing foreign pollen falling on them.

The flower buds were always emasculated in the afternoon and they were pollinated the following morning between 9:00 a.m. and 12:00 noon. Pollinations were carried out by taking out mature pollen artificially by splitting the anthers longitudinally with the help of a needle. The pointed end of the needle carrying the pollen was then brushed gently on the stigma. While pollinating the flowers, care was taken not to injure the stigmas. Pollen application was repeated twice on the same stigma to ensure pollination. All pollinations were done on bright, sunny days. After pollination, the flower buds
were again enclosed in a butter paper bag and labelled. The bags were removed as soon as the fruit development was initiated.

For selfing, the whole inflorescence was covered with butter paper bag. The bag was removed as soon as the fruit set began.

4.3.2. study of meiosis

For the study of meiosis, flower buds of proper size were fixed in Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) between 9.00 a.m. and 12.00 noon, for an hour and then transferred to propionic alcohol (1 part propionic acid and 3 parts absolute alcohol), the propionic acid having been saturated with ferric acetate. The flower buds were kept in propionic alcohol for 24 hours.

The material was washed thoroughly with 70 per cent alcohol and stored in it at 10°C. Meiosis was studied from the propionic-carmine squashes of pollen mother cells (Saminathan, Magoon and Mehra, 1954). Temporary preparations were sealed with wax. Preliminary observations were made from temporary slides. The temporary slides were made permanent by butyl alcohol schedule (Bhaduri and Ghosh, 1954). The wax was removed and the slide
was placed upside down in a mixture of glacial acetic acid and normal butyl alcohol (1:1). When the cover glass got separated, both the slide and cover glass were passed through normal butyl alcohol. The slide and the cover glass were reassembled using canada balsam as the mounting medium. The slides were kept in incubator at 30°C for 2 to 3 days. Data on meiosis were secured from well squashed preparations.

4.2.3. Study of pollen size and fertility

The pollen size and fertility were estimated from pollen samples. The stainability of pollen with acetocarmine was taken as an index of pollen fertility. One or two mature anthers were placed in a drop of acetocarmine and the pollen was squeezed out of the anther with gentle pressure. The pollen was stained with 1.0 per cent acetocarmine and those which took up stain and had regular outline were taken as fertile, and the empty unstained ones were taken to be sterile. The same preparations were used to measure the size of pollen. The size of pollen grain was estimated by measuring its diameter. For determining the pollen size and fertility, pollen from five plants of each type was studied.
4.2.4. **Colchicine treatment**

The growing shoot apices of 8 to 12 cm tall seedlings were treated with colchicine solution of 0.2 per cent concentration for 20 hours. The growing shoot apices were wrapped with small wads of absorbent cotton and were kept moist constantly with colchicine solution. After 10 hours treatment (8.00 a.m. to 5.00 p.m.) on the first day, there was a break for the night, and the treatment was continued on the subsequent day to make up a total duration of 20 hours.

4.2.5. **Measuring the thickness of leaf**

Thin transverse sections of leaves were cut with the help of a razor. The sections were stained with 1.0 per cent acetocarmine. The thickness of the leaf was measured by the ocular micrometer scale and the ocular divisions were converted into microns.

4.2.6. **Drawings and microphotographs**

All the cytological drawings were made at table level with a camera lucida using 10x eyepiece and 100 x objective. Microphotographs of pollen grains were taken at different magnifications.
4.2.7. Abbreviations

The following abbreviations have been used in the thesis:

\[\begin{align*}
\text{PMC} & = \text{Pollen mother cell} \\
\text{DIK} & = \text{Diakinesis} \\
M_I & = \text{Metaphase one} \\
M_{II} & = \text{Metaphase two} \\
A_I & = \text{Anaphase one} \\
A_{II} & = \text{Anaphase two} \\
T_I & = \text{Telophase one} \\
T_{II} & = \text{Telophase two} \\
\text{Ch} & = \text{Chiasmata}
\end{align*}\]

The Roman numerals I, II, III and IV are used to denote the univalent, bivalent, trivalent and quadrivalent chromosomal association respectively.