Chapter 2

MATERIALS AND METHODS

2.1. Materials

The caryomorphological studies were conducted on the following non-tuberiferous species of the genus Solanum:

1. Solanum nigrum Linn.
2. S. nodiflorum Jacq.
3. S. luteum Mill.
4. S. villosum Mill.

These species were raised from the seeds obtained from the collection maintained by the Department of Botany, Aligarh Muslim University, Aligarh. The seeds were sown in 30 cm pots. When the seedlings were 8 to 12 cm tall and had developed three or four leaves, they were transplanted to 30 cm pots. Only one seedling was planted in one pot. The plants were grown in greenhouse. The plants were susceptible to aphids. These were, therefore, sprayed frequently with Dimecron solution (1 cc of Dimecron in 10 litres of water). Herbarium specimens of the species and their hybrids have been prepared. These shall be deposited with the herbarium of the Department of Botany, Aligarh Muslim University, Aligarh.
2.2. Hybridization

In order to determine the crossability among the species used in this study, interspecific hybridization was attempted in all possible combinations. Reciprocal cross pollinations were also made among the cytotypes of *S. nigrum*. Data regarding the number of crosses of each combination and the extent of fruit and seed set were collected.

2.3. Emasculation, pollination and selfing

Since the flowers are bisexual, emasculation before dehiscence of anthers is necessary in order to cross pollinate the flowers. In each inflorescence, buds of the right stage, that is, buds with greenish yellow anthers, were left intact while others were removed. The flower buds were emasculated one day before the dehiscence of anthers. The flower buds were opened with fine forceps and the stamens were pulled out carefully. In all the cases, while emasculating the flowers, care was taken not to puncture the anthers. The inflorescences with emasculated flowers were protected from contamination with unwanted pollen by covering them properly with butter paper bags. Each bag was tied with a label.

The flowers of male parents were also protected similarly with the object of preventing foreign pollen from falling on them.
The flower buds were always emasculated in the afternoon and pollinated the following morning between 9.00 am and 12.00 noon. The pollinations were carried out by brushing the stigmas of the emasculated flowers with the fresh pollen of male parent. As very little pollen comes out in the natural course of dehiscence, pollen had to be taken out artificially by splitting the anthers longitudinally with the help of a needle. The pointed end of the needle carrying the pollen was gently brushed on the stigmas. While pollinating the flowers care was taken not to injure the stigmas. After pollination the inflorescences were again enclosed in butter paper bags. These bags were tied and labelled; they were removed only at the time of seed collection.

For selfing, the entire inflorescence was enclosed in a butter paper bag for a few days before blossoming. The bags were removed only at the time of seed collection.

2.4. Colchicine treatment

Growing tips of young branches were treated with aqueous solution of colchicine of 0.10 per cent, 0.20 per cent and 0.25 per cent concentrations for 12, 18 and 24 hours. Growing tips were covered with small wads of absorbent cotton and were kept moist constantly with colchicine solution. Constant care was taken to see that
the cotton wads did not dry up during the course of treatment. On the first day, after 9 hours treatment (8.00 am to 5.00 pm) there was a break in the evening and the treatment was continued on subsequent days to make a total treatment of 12, 18, and 24 hours.

2.5. Measuring the thickness of leaves

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 leaves was measured by the ocular micrometer scale and the ocular divisions were converted into microns.

2.6. Statistical methods

A statistical study was undertaken in an attempt to arrive at a better understanding of the morphological relationship of the species and their hybrids.

The homogeneity of means of morphological characters of random samples from each population was tested by the F test using the technique known as Analysis of Variance, developed by Fisher. However, in cases where the sample size was two, "Student's" t test was used.

The mean values of morphological characters of two populations were compared by "Student's" t test in order to determine how far their mean values differed significantly from one another.
2.7. Study of meiosis, pollen size and fertility

For a study of meiosis flower buds of proper size were fixed in Carney's fluid (6 absolute alcohol : 3 chloroform : 1 glacial acetic acid) between 9.00 am and 12.00 noon for an hour and then transferred to propionic alcohol (1 propionic acid : 3 absolute alcohol) for 24 hours. The propionic acid saturated with ferric acetate was used. The material was washed with 70 per cent alcohol and stored in it at 10°C. The material was studied as and when it was convenient.

Anthers were dissected from buds and stained by the standard propionocarmine squash technique (Swaminathan, Magoon and Mehra, 1954). Temporary preparations were sealed with paraffin wax and later made permanent by using n-butyl alcohol-acetic acid series and mounted in Canada balsam (Bhaduri and Ghosh, 1954). Meiotic data were secured from well squashed preparations.

The pollen size and fertility were estimated from fresh pollen samples. The stainability of pollen with aceto-carmine was taken as an index of pollen fertility. The pollen was stained with 1.0 per cent aceto-carmine and those which took up stain and had regular outline were taken as fertile and the empty ones without staining were counted as sterile. The same preparations were used to obtain pollen size measurements. The size of pollen grain was estimated by measuring its diameter.
2.8. **Photomicrographs, drawings and abbreviations**

Microphotographs were taken from permanent slides with an "Ultraphot" microscope (10 x eyepiece and 100 x objective) using 0.81 Gevalith Ortho safety film. All the cytological drawings were made at table level with a camera lucida using 10 x eyepiece and 100 x objective. Photomicrographs of pollen grains were taken at different magnifications.

The following abbreviations are used:

- **PMC** = Pollen mother cell
- **Diak** = Diakinesis
- **M_I** = Metaphase one
- **M_II** = Metaphase two
- **A_I** = Anaphase one
- **A_II** = Anaphase two
- **T_I** = Telophase one
- **T_II** = Telophase two
- **Xta** = Chiasmata

The univalents, bivalents, trivalents and quadrivalents have been denoted by Roman numbers as I, II, III and IV respectively.