# PALYNOLOGICAL AND EMBRYOLOGICAL STUDY IN Solanum nigrum L

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***Abstract :*** Pollen grains and embryology of Solanum nigrum were studied. The detailed external morphology of pollen grains have discussed. Ovules examined under this investigation showed abnormal embryo sac Developmental stages of megasporogenesis and megagametogenesis in the embryosac was typical showing normal.

***IndexTerms*** **- Solanum nigrum, palynology, Megasporogenesis, megagametogenesis.**

## INTRODUCTION

Palynology is the study of pollen grains and spores which constitute the reproductive units of plants .Researches spread over a period of four centuries have served to draw the wide spectrum of interest and application of the science in various areas of academic pursuits and human endeavors (Nair ,1960 b) .The application of palynology is made feasible , chiefly because of the unique morphological features of exine, which is not only of value in taxonomic and phylogenetic studies (Erdtman 1952,Nair 1979) but also in analytical investigations, involving grains which are in dispersed state .The outermost layer of the pollen grains i.eexine formed of an extra ordinarily resistant material known as sporopollenin was derived from carotenoids by oxidative polymerization.This layer possessed a number of characteristic details that helped in identification of plants, both extinct and extant much in the same manner as criminals could be identified from their finger prints.

Early work on the solanaceae described the occurrence of a zone of degenerating cells which developed a head of the globular or early heart shaped embryo (Beamish1955,Dnyansagar and cooper 1960,Erdelska ,1985). However a recent investigation in to early seed development in Solanum nigrum L. Revealed that the zone developing ahead of the embryo was not caused by the degeneration of the endosperm cells but was a product of living endosperm cells (Briggs 1990).

Solanum nigrum L.was selected for embryological study in the present investigation.The taxa is totally autogamous.Ovules examined under this investigation showed abnormalembryo sac. Developmental stages of megasporogenesis and megagametogenesisis the embryo sac was typical showing normal several mode of reproduction.

## MATERIALS AND METHODS

Mature opened or closed flowers of Solanum nigrum L. were collected in a test tube containing 70% ethyl alcohol. The flowers were transferred to a centrifuge tube and crushed in with aglass rod. This dispersion was passed through a sieve and was collected in two separated tubes (A and B)in the ratio of 1:2 .A few drops of safranin were added to the tube A and recentrifuged. This process was repeated. The supernatant was discarded and more water was added. Washing with water was continued until the supernatant turned colorless. After washing 2ml of dilute glycerin (50% glycerin) was added.This provided and unacetolysed preparation which could be used for various purposes,such as –

- Determination of morphological sterility.
- Comparative understanding of the chemical effect of acetolysis on pollen,and
- General understanding of the protoplasm and interval wall materials within the outer exine cover.

The detailed external morphology of pollen grains was studied from the acetolysed pollen grains. The acetolysis of pollen grains followed in this study was described by Nair (1960)as follows :-

Portion (B) which was kept in alcohol was centrifuged. The alcohol was decanted. Five ml of glacial acetic acid was added to the tube and it was again centrifuged and decanted. A mixture of acetic anhydride and sulphuric acid (H2SO4) in ratio of 9:1 was added toit. The tube was heated on a water bath to the boiling points and was later allowed to cool down. It was centrifuged, washed with water and recentrifuged. This process was repeated 6-7 times. The residue was finally mounted in glycerin jelly.This preparation was designated as “acetolysed” in which pollen morphology of the exine was better revealed in comparison to that of the unacetolysed pollen, which was minally due to removal of the protoplasm by the chemical treatment.A comparative study of acetolysed and unacetolysed pollen was done.

The fertility was calculated on the basis of scoring stainable/unstainableunacetolysed pollen grains.The NPC value I,e.

Number ,pore and character was determined after Endtman( 1952).

Both acetolysed and unacetolysedmeterials for light microscopy were mounted in glycerin jelly and measured with micrometer shape and the average size of the pollen grains were found out under light microscope on the basis of the examination of 25 pollen grains . The measurement included

1. Equatorial diameter.

2. Polar diameter.

3. Spine length.

Methods for Embryological study –

In the present study single taxa Solanum nigrum Linn as selected for embryological study. For the study of megasporogenesis and megagametogenesis two seprated method their employed. On one hand in order to obtain an accurate and complete picture of the development stages,sectioning and examination of paraffin embedded material was done. On the other hand for an ovule to ovule analysis of randomly selected flower, the embryo sac squash method was applied so that a complete information was available on the type of embryo sac.

For sectioning flower buds of different sizes were killed in formalin acetic alcohol (70% alcohol) and aspirated soon after being placed in the solution. Flower buds were embedded in paraffin by the normal butyl alcohol method, sectioned at 15µ and stained in iron hematoxylin.

For embryo sac squash, flower buds at different stages of development were fixed in a modified freshly prepared carnoy's fluid (6:3:1 per volume of 95% ethanol ).Glacial acetic acid and chloroform. After about an hour of fixation 25 drops of a saturated aqueous solution of ferric chloride were added for energy50ml of the fixative. The ovules were ready for squashing about 48hours after the addition of ferric chloride. Flower buds could be stored in this mixture in a refrigetor for several months without any evident deterioration.The fixed flower buds were placed in a petridish containing the fixative, and beginning with the youngest flower each bud was studied serially with the help of a pair of five niddles, the pistil was taken out and placed on the slide in a drop of acetocarmine. The styles were cut off at their base and the ovary was tapped gently with the needle until the ovule popped out. With the little practice the ovule could be taken out without any difficultly. Whileviewing the slide on the microscope under low power, the top of the cover glass was pressed with a needle carefully. Gentle heating of the slide helped further to sprat the cells a part. With desired pressing and tapping of the cover glass, the intact embryo sac was separated and studied, 50-100 ovules were examined.

**Embryological Study:**

**Megasporogenesis:**

Each ovary contained a single anatropousovule. The archesporial cell was differentiated from a some of the integumentary cells and envolved by the inner integument. Along with the differentiation of archesporial cell. Several cell were observed to have become conspicuous in the nuclear epidermis due to a marked increased in size. The archesporial cells functioned as the megaspore mother cells which underwent meiosis. Meiosis was found to be normal resulting in the formation of linear tetrad. Soon after the completion of meiotic divisions all megaspores with the exception of the chalaza degenerated.

**Megagametogenesis:**

Development of 8 nucleate normal polygonum type (Maheshwari 1950) embryo sac was observed in Solanum nigrum.

The functional megaspore divided mitotically and is the two nuclate embryo sac, the two nuclei ever found to be separated to the opposite poles with a large vacuole between them. As a result of a second mitotic division a four nuclate sac was found and a subsequently a third mitotic division produced on eight nucleate embryo sac with four nuclai at each end. The 8 nucleate sac ultimately matured into a female gametophyte with two synergids and egg, two polar nuclei and three antipodal.

**RESULTS**

Pollen grains are tetracolporate (fig-1). Average size of pollens are 20.96±0.40µ(p)x18.61±0.36µ(E):-19.97±0.1(PAI). Ovary contains a single anatropes ovule. Endosperm development is ab initio cellular and becomes many celled before the first division of zygote 4-5 d after anthesis, embryo development follows the solanad type fig-2 (sexena and singh, 1969).

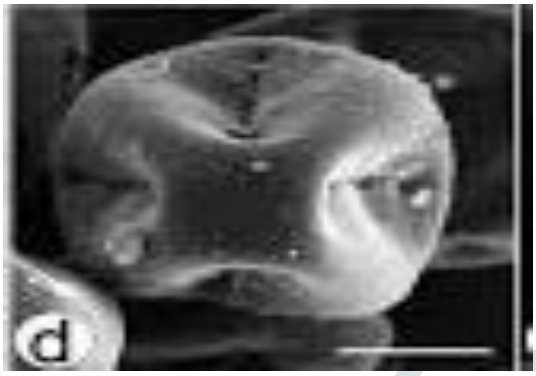

Fig -1

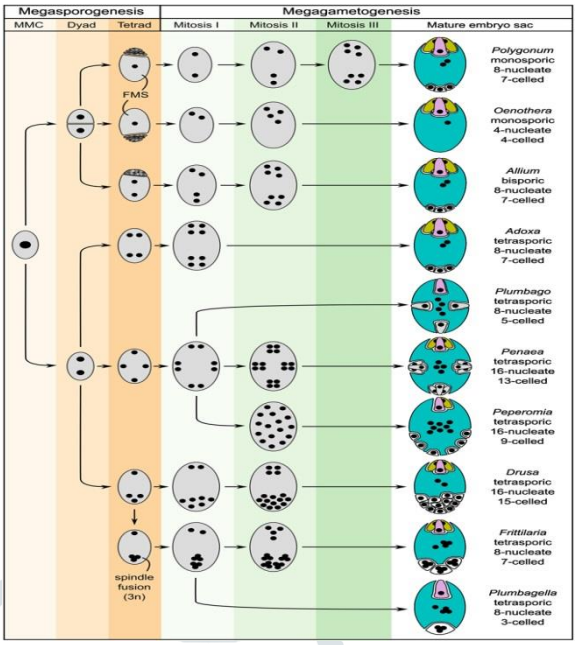


Fig -2

## CONCLUSION

The literature on palynology was replete with example of successful application of this type of study in solving various taxonomic problems (Wodehouse, 1935; Erdtman, 1957; 1966, 1969; Bassett and Crompton 1968, 1978). The angiosperms were known to exhibit two basic types of pollen grains, namely monocolpate and tricolpate. the monocolpate pollen grains with a single furrow was characteristics of the monocotyledons (chanda and Ghosh, 1979) and the woody members of the Ranales. The tricolpate pollen grains with three meridionallyplaud furrows were characteristically found in dicotyledons constituting the bassis of type from which other types were derived.

Embryological stydy in Solanum nigrum revealed that it had a monosporic8- Nucleate, polygonum type of embryo sac development. As the stydy was made in a single species, it could not be utilize in further taxonomic treatment of the family.

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