**Dr. Rima Kumari: Date: 30/09/2020**

Online class and e- content for BSc IInd year students

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| Date and Time | Online class medium  | E. content topic |
| 30/09/202001:30 p.m to 2.10 p.m | Via Google meetLink: Meeting URL: https://meet.google.com/zew-xekx-kgg | **GOLGI APPARATUS** |

**Anther Culture and the Establishment of Haploid Plants:**

Anther culture is used to develop instant homozygous inbred lines, avoiding the lengthy time needed using traditional self crossing methods including bud pollination. Anther culture results in haploid plants, easily identified by their smaller sterile flowers. Chromosome numbers are doubled using colchicine to give doubled haploids. Spontaneous diploids can also occur. Depending on incompatibility status, doubled haploids can be used as parents for hybrids or as ‘cultivars’ in their own right.

This is a technique by which immature **pollen** is made to divide and grow into tissue (either callus or embryonic tissue), primarily to produce haploids (plants with an N chromosome number) known to be **anther culture**. Datura stramonium was the first haploid plant that was **discovered** by Bergner in 1921. But Successful **anther culture** was first reported in the 1970s through in vitro methods by Guha and Maheshwari. The advantages include a high frequency of haploid plants, easy to induce cell division in most species, and no requirement of a high level of expertise. It involves selecting suitable parents in a [breeding program](https://www.sciencedirect.com/topics/engineering/breeding-program) depending on the goals and conducting a cross.  In this culture Anthers may be removed aseptically from sterilized flower buds and placed in culture. A proportion of pollen within these anthers — particularly those from Solanaceous species — grow and proceed through a series of developmental stages, eventually giving rise to haploid embryos.

This phenomenon has been designated as “androgenesis”. In addition, there is also possibilities of haploid embryo formation, the ultimate resultant is the formation of regenerated haploid plant.

**Materials and Methods:**

1. Flower buds (freshly collected) of Nicotiana tabacum;

2. Modified Ms culture media;

3. Sterilized glass goods like petridish, beakers, glass vial with lids;

4. Sterilized scalpel, forceps, Bunsen burner etc.;

5. Surface stimulants like 5% teepol, 70% ethanol (v/v), sterilized distilled water.

**Procedure**

The steps of anther culture are:

1. The flower buds of Nicotiana tubacum (measuring 15-20 mm), is collected from flowering plants only;

2. Then selected buds are taken for surface sterilization by immersing in 5% teepol solution for 40-60 seconds. The buds are immediately washed in sterilized distilled water;

3. Subsequently, the buds are immersed in 70% ethanol (v/v) for 10 seconds followed by 2% sodium hypochlorite (v/v) for 10 minutes. Finally, the buds were washed thoroughly in sterilized distilled water (three times) and transferred to pre-sterilised petridishes;

4. By sterile forceps and scalpel, the anthers were aseptically removed and then either anthers are directly placed on culture media aseptically or they are crushed for isolation of pollen through centrifugation in sterilized buffer and then suspension of pollen is plated on agar media for embryogenesis;

5. The cultures are kept initially in dark. After 3-4 weeks they undergo embryogenesis or androgenesis and then the culture vials are kept in light for proliferation of callus tissues;

6. At this stage, the culture was incubated at 24-28°C for 14 hrs. in light at about 2,000 lux. intensity, then plantlets are regenerated from young differentiated embryo.

The detailed stages of anther culture techniques are shown in Fig 1.2.Stages of anther sulture technique