ANTHER CULTURE [Haploid prodⁿ]

INTRO → Shimakura 1st cultured anther 'in vitro' with a view to investigating the physiology of meiosis. Tulecke (1953) 1st noticed that the mature pollen grains of Ginkgo triloba could be induced to form a callus on medium containing inorganic salts, sucrose, vit B and coconut milk. This method is now applied with succes on various plant sps. Such as Atropa, Brassica, Hordeum, lycopersicon, Nicotiana, oryza, Solanum, Triticum, Zea etc.

Nutrient medium → Anthers can be cultured on a medium containing sucrose (usually 2%) ion, vitamins, hormones etc. Auxin, cytokinin etc are added for growth. Anthers cultured on a medium containing coconut milk or kinetin develop embryoids which later form haploid plantlets.

In raising haploid plants from isolated pollen culture of Nicotiana tobacum and Datura Nitsch (1974) used a synthetic medium containing glutamine, l-serine, inositol.

FACTORS AFFECTING ANTHER CULTURE

1) Activated charcoal → It has a stimulatory effect on embryogenesix and this has been observed in anther cultures of Potato, rye, tobacco etc. This may be due to removal of inhibitory substances from agar by activated charcoal. Anther cultures of Petunia and Nicotiana indicate that activated charccal removes both exogenous and endogenous growth hormones from

culture medium :

2) Temperature → Temperature has significant effect on embryoid formation.

Pretreatment of anthers at 3-10°c for 2-30 days stimulates embryogenesis (Sunderland and Thoberts 77). In N. tobaccum if the buds are pretreated at 5°c for 72 hours then 58% anthers produce embryoids (Nitsch 1974). In Brassica compestris pretreatment of anthers at 35°c for 24 hrs helps embryoid formation.

Centrifugation of the anthers at 2-5°c for approximately 30 minutes helps embryoid formation.

3) Stage of the anther → Particular stage of the anther at the time of culture is very important. Suitable stages of anthers for culture are pre-mitotic, mitotic and post mitotic.

a) Pre-mitotic stage → Anthers at this stage have microspores which have just completed the 1st meiotic division & the pollens are immature, uninucleate & starch free. Anthers of Hordeum Vulgare and Hyoscyamus at this stage are suitable for culture.

b) Mitotic stage → In some plants, anthers at first pollen division stage are most suitable for culture eg - Nicotiana tobaccum and Datura innoxia.

c) Post mitotic stage → Early binucleate stage of pollen development is most suitable for culture in Atropa belladonna and Nicotiana

[illegible] etc

4) Photoperiod and light intensity → Higher no. of embryoids are formed when anthers are taken from plant grown under short days (Dunwell and Perry, 1973) and high light intensity.

5) Flowering time → Anthers taken from flowers at the beginning of the flowering periods of the plant are most suitable for culture.

6) Endogenous level → Embryogenic pollens are found near the tapetum within the anthers. The tapetum may release some substance which initiates embryogenic development in pollens.

7) Age of the plant → Usually anthers from younger plants are most suitable for culture.

TECHNIQUE OF ANTHER CULTURE

1) Selected plants are cultivated until they reach flower bud stage.
2) In some cases flower buds are chilled few days prior to culture.
3) Flower buds of proper size & developmental stage are taken and surfaces sterilized with alcohol or hypochlorite solution for 10-20 minutes. Buds are rinsed several times in sterile double distilled water.
4) The anthers are carefully excised from flower buds using forceps and dissecting needle. Filaments must be removed from the anthers otherwise callus may be formed

at the cut ends.

5) Anthers may be cultured either on agar solidified culture medium (Sunderland and Wicks, 1971) or placed on a filter paper bridge over a liquid medium (Sunderland and Roberts 77).

4) Anthers are cultured at 25°c in presence or absence of light. Light is essential after plantlets are formed continuous illumination from cool white fluorescent lamp of 300 lux is satisfactory.

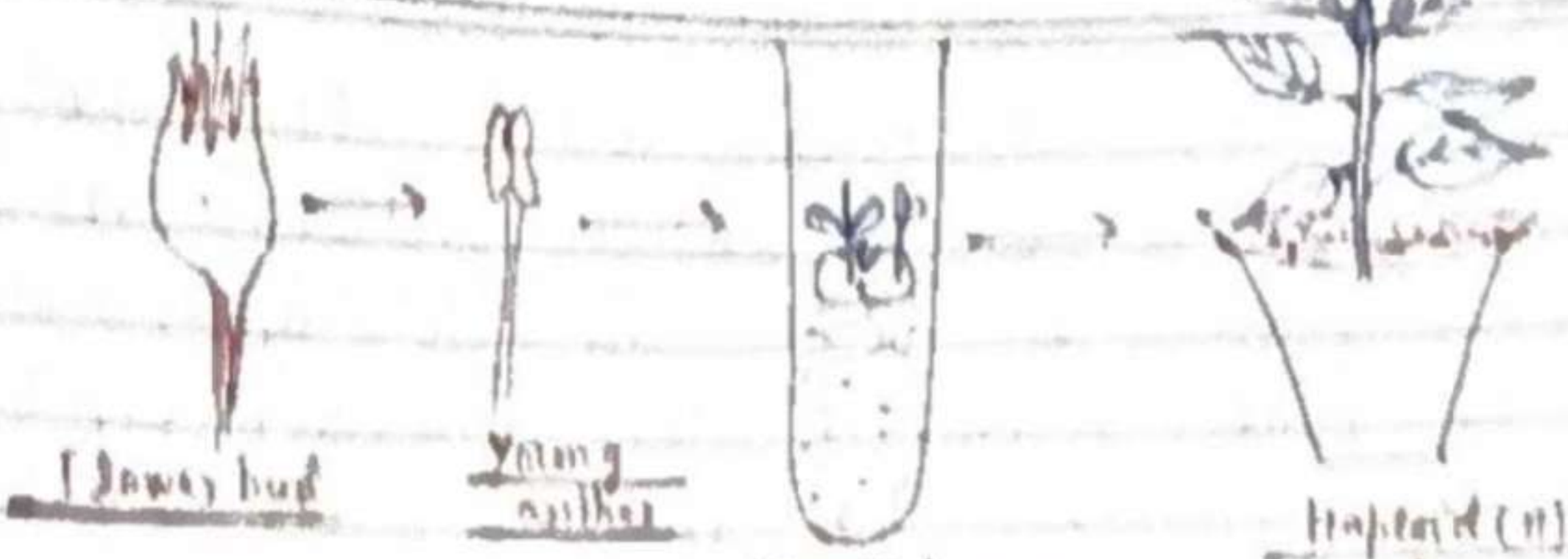
3) After a period of 4-5 weeks in culture plantlets are formed. From a single anther many plantlets are formed.

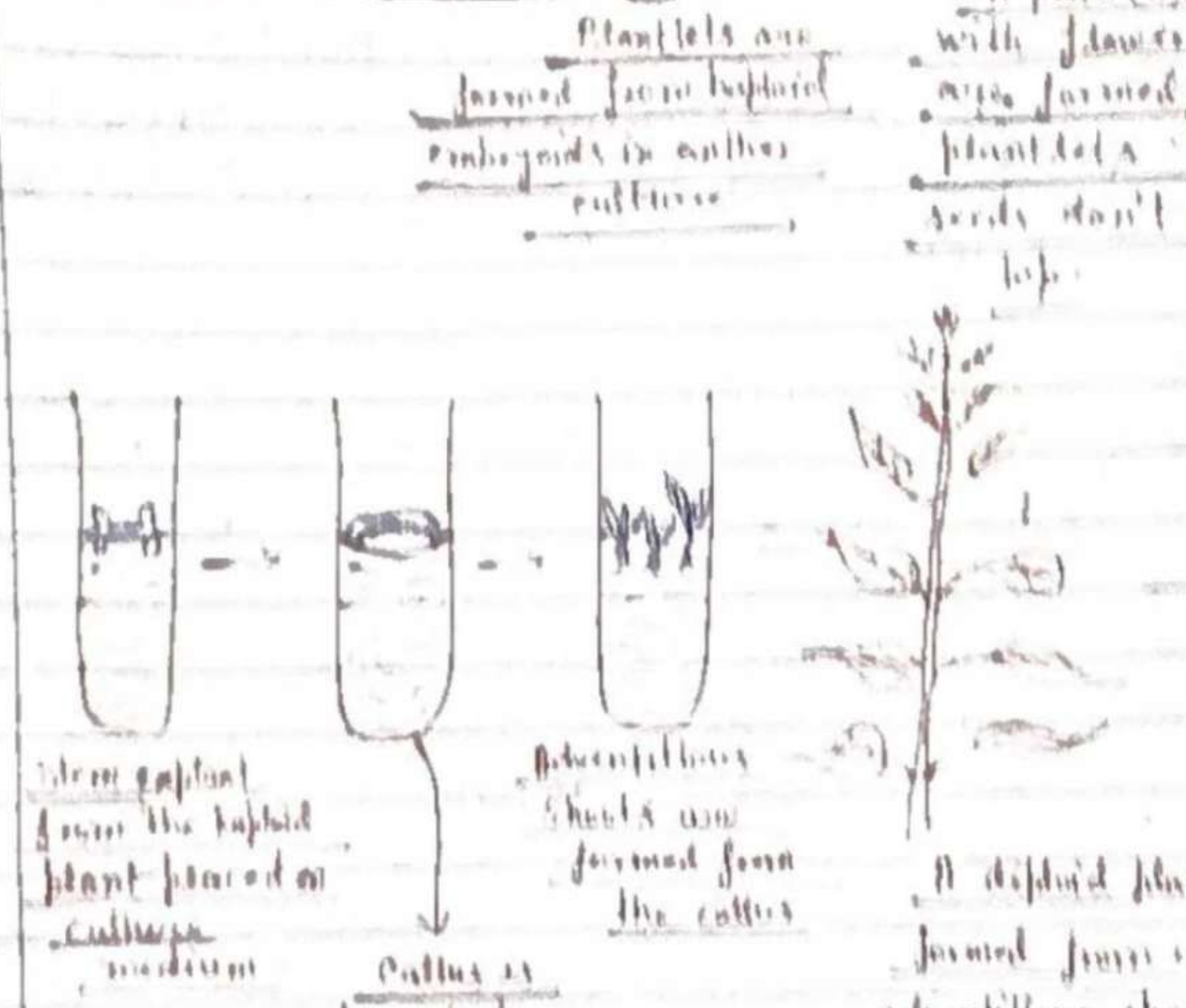
2) These plantlets are carefully separated quite early and cultured on a fresh root inducing medium containing 0.5% agar and all other components in half-strength to that of the anther culture medium.

1) After formation of proper root system they are transplanted to pots. These pots are preferably kept in humid condition for few days.

Nitsch (1972) cultured anthers of tobacco and obtained in 75%. The cultured anthers 1-100 plantlets per anther plants arising from an anther are heterogenous.

Fig:





Production of primary gene diploids : By doubling the chromosome of the haploid plants produced by anther culture completely homozygous diploid fertile plants are produced. Chosen number of haploids can be double by various methods

1) In chemically induced doubling of the haploid plantlets, the plantlets are treated with colchicine. In Nicotiana tobacum plantlets are immersed in aq. solution of colchicine upto 96 hours. Plantlets are transferred to

culture medium after treatment. In mature plants 0.4% colchicine in lanoline paste is used on the upper axillary buds. Terminal bud is removed. This encourages the growth of the lateral buds (Nakata and Taneka 1970).

2) In regeneration by tissue culture diploids may be produced due to endomitosis in callus.

Significance →

1) By Anther culture many haploid plants can be produced very rapidely.
2) Homozygous diploid plants obtained by doubling the chromosome of haploids have great importance in plant breeding and crop improvement.