Gametic embryogenesis.

Unit III

① Androgenesis. & Gynogenesis.

Introduction :-

* The ability to produce haploid plants is a tremendous benefits in genetic, plant breed plant physiology and embryology studies.

* Haploid plants can be produced by Two met

1) Androgenesis
2) Gynogenesis.

Androgenesis :- Haploid production of plants through anther or microspore culture has been referred to as 'Androgenesis'.

Gynogenesis :- Haploid production of plants fro ovary or ovule culture has been referred to as 'Gynogenesis'.

Androgenesis

it is the formation of sporophyte from t the male gametophyte on artificial medium it is most commonly found in family solanaceae and poaceae.

the androgenesis immature pollen grains are induced to follow the sporophytic mode o development by various physical and chemical stimuli.

there are two methods for in vitro production of androgenic haploids

they are:

1) anther culture.
2) pollen culture.

Anther culture.

⇒ Introduction:-

* Anther culture is the process of using anthers to culture haploid plantlets.

* The technique was discovered by Guha & Maheshwari in 1964.

* This technique can be used in over 200 sps.

→ Source of anther :- Usually anther from the flower buds will give better response during androgenesis.

→ Pretreatment of anther :- The main pretreatments applied to anther culture are

1) **Cold treatment** :- In general, cold treatment between 3-6 degree C for 3-15 days gives good response. As a result weak or non viable anthers are killed.

2) **Hot treatment** :- Plant in some species when subjected to 30°C for 24 hrs or 40°C for 1 hr stimulates embryogenesis.

* Sterilization of flower buds was carried out in the Laminar Air Flow Cabinet.

⇒ Media and Growth regulators :-

*Media :-

* Vary with species.
MS * MS media for anther culture.
* The basal medium and combinations of
Growth R. growth regulators are also important factors.
Agar * Solidified with Agar.
* Agar contains compounds inhibitory to some species.

* Growth regulators :-

Carbon source * Complete nutrient medium.
→ Sucrose * 2-3% Sucrose is added.
Ac. Charcoal * Activated charcoal to agar medium is advocated.
Aux Cyt * Cytokinin is sometimes used with Auxin.

⇒ Stages of development :-

* After inoculation, haploid plants develop from anther culture either directly or indirectly.

1) Direct androgenesis :-

* It is also called pollen derived embryogenesis.
* Pollen grains directly act as zygote.
* Similar to zygotic embryogenesis.
* At globular stage, the embryo is released

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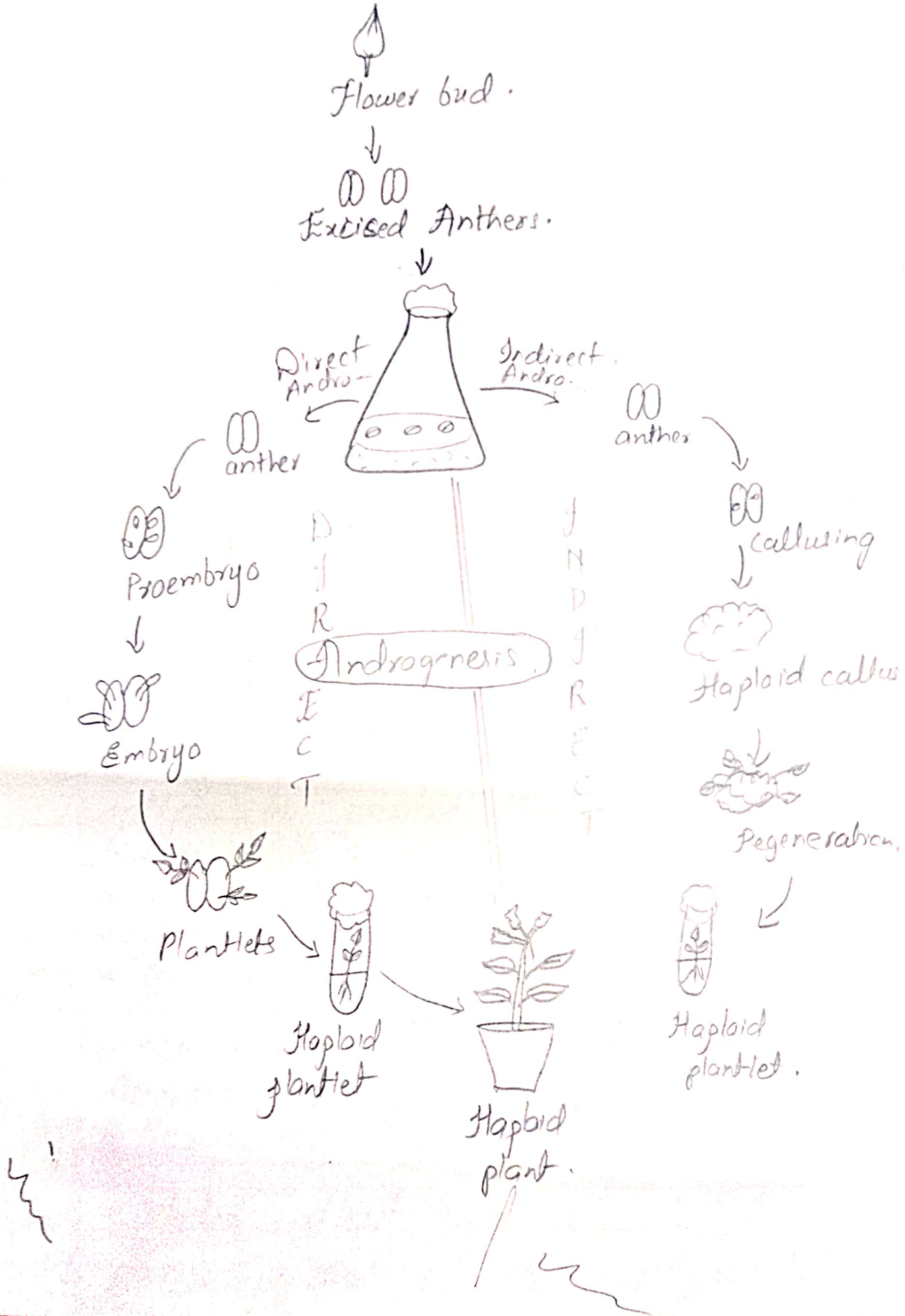
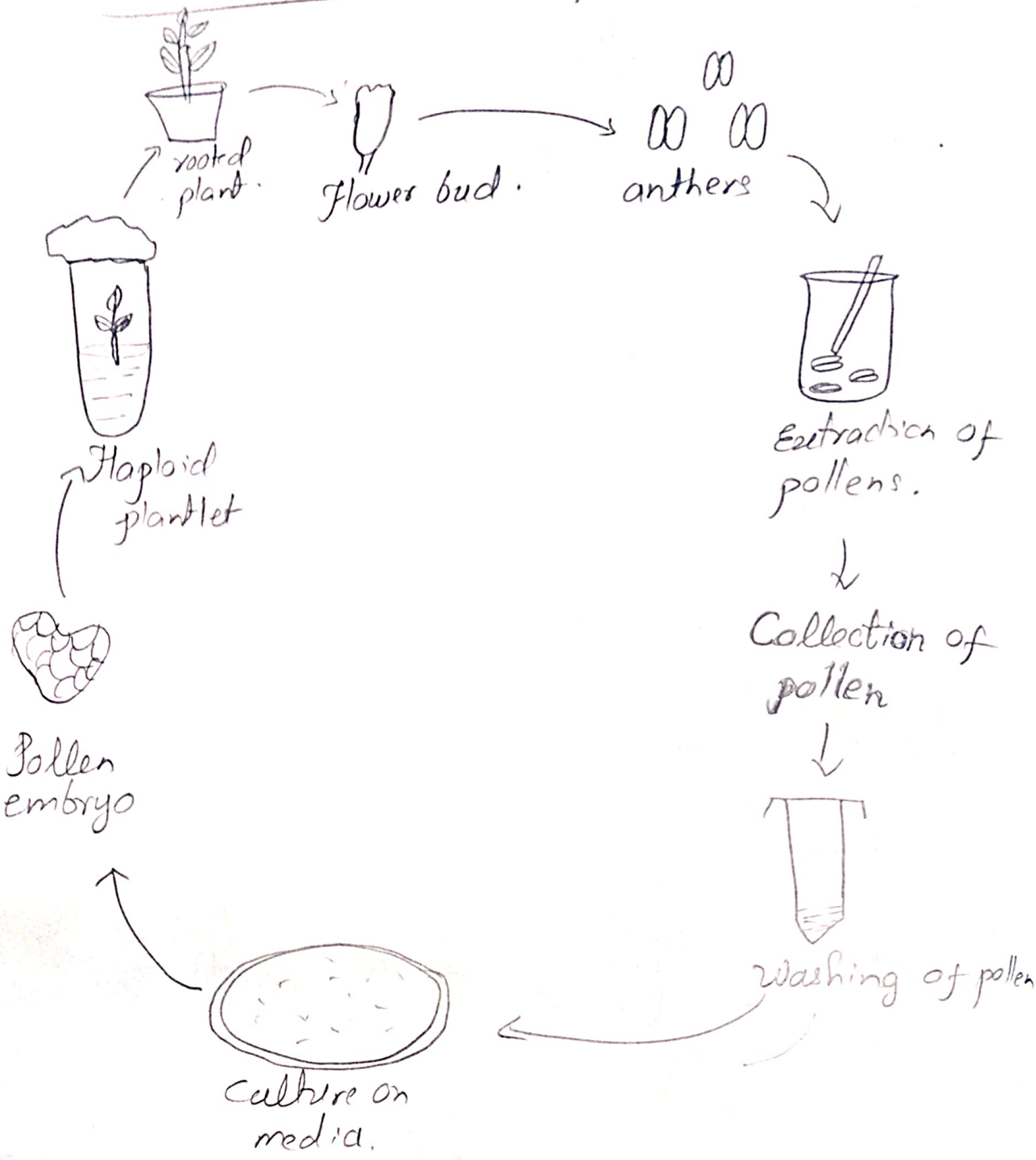
Androgenesis. [Anther Culture]



Pollen culture -



* The released embryo develop cotyledons, which ultimately gives rise to plantlets.

* Eg :- Datura, Brassica campestris.

2) Indirect androgenesis :-

* In indirect androgenesis, the pollen grains divide erratically to develop callus.

* Callus tissue which is finally redifferentiate and forms haploid plantlets.

* Eg. rice, wheat, tomato.

Microspore culture

* Anthers are collected from sterilized flower buds.
* The microspores are then squeezed out of the anthers by pressing them against the side of beaker with a glass rod.
* Anther tissue debris is removed by filtering the suspension through a nylon sieve.
* This pollen suspension is then centrifuged.
* This supernatant containing fine debris is discarded.
* Resuspended in fresh media.
* Washed atleast twice.
* Then pipetted out into small petri dishes.

* Incubated at 28°C.
* 14 days of culture.
* After 14 days, the culture are transferred to suitable media.

⇒ Factors affecting androgenesis.

* Genotype of donor :- Important for determining the success or failure of androgenesis.

* Anther wall factor :- Growth inhibiting substances leaking out of the anther wall in contact with nutrient media.

* Culture medium :- The culture medium also plays a vital role in the correct amount and proportion of inorganic nutrients.

* Growth regulators :- Kinetin or cytokinins are essential for induction of pollen embryos. Sucrose plays an important role in induction of pollen haploid plants.

* Activated Charcoal :- Removes inhibitors.

* Physical factors :- Light, temperature, pH.

* Other factors :-
  → Organic supplements
  → products of proteins
  → Coconut milk.
  → Amino acids.

Date
Page

⇒ Applications of androgenesis :-

* Shortening of breeding cycle.
* Gametoclonal variations : besides yielding haploids, in vitro androgenesis provides a unique opportunity to screen the gametophytic variations.
* Induction of mutation.
* Genetic transformation : production of disease resistant plants.

⇒ Limitations :-

* Low yield only 5-8% of total pollen grains.
* Conversion of pollen embryos into plants.
* Albinism in cereals.
* Instability of genetic material during androgenesis.

Gynogenesis.

⇒ Introduction :-

* It is the formation of sporophyte from the female gametophyte on artificial medium.

* Haploid production of plants from ovary or ovule culture has been referred to as gynogenesis.

⇒ Definition :- "It is the process by which culture of unfertilized ovaries to obtain haploid plants"

⇒ History :-

* First reported on in Barley by San Noem in 1976.

* Later works done in Wheat, Rice, Maize, Tobacco etc.

⇒ Methods of gynogenesis :-

* There are two methods for invitro production of gynogenic haploids.

They are :- ① Ovary culture.
② Ovule culture.

* In vitro gynogeneses is used as an alternate technique in species where anther / pollen culture is unsuccessful.

* The gynogenic plants may arise through direct embryogenesis or the gamete cells may form a callus followed by plant regeneration.

* Both ovary slice culture and ovule culture can be carried out simultaneously for achieving in vitro gynogenesis.

① Ovary culture

* The in vitro culturing of ovaries isolated from pollinated or unpollinated flowers is called ovary culture.

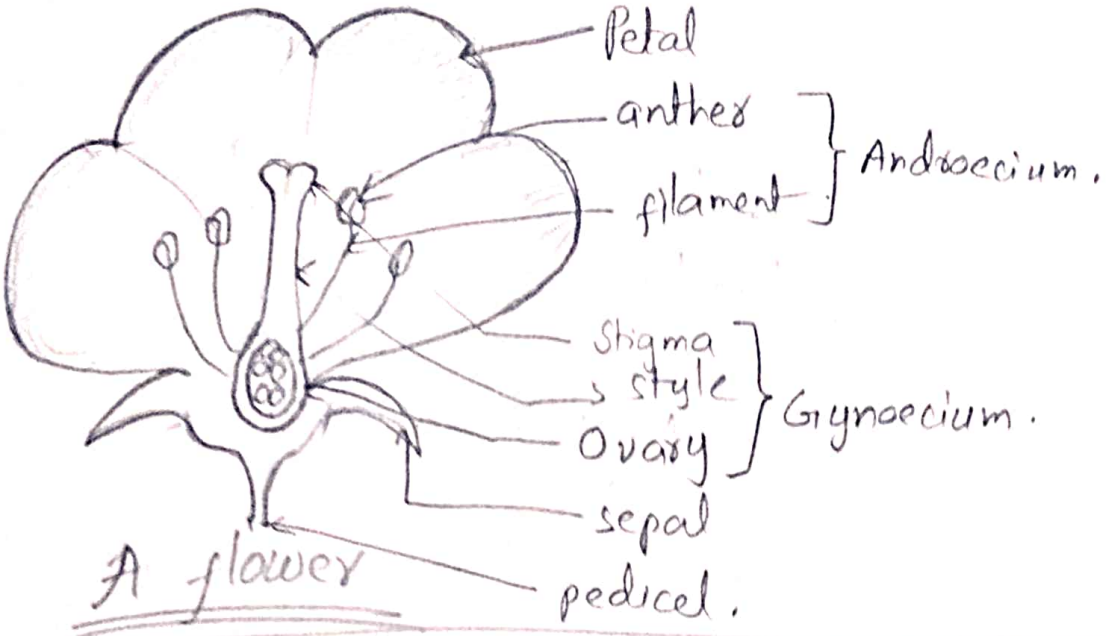
* The technique of ovary culture was developed by Nitsch in 1951. He successfully grew the ovaries of Cucumis, Lycospersicon, Nicotiana etc. on synthetic medium.

* For haploid production, flower buds are excised 24-48 hours prior to anthesis for unpollinated ovaries.
* The calyx, corolla and stamens are removed and ovaries are then surface sterilized.

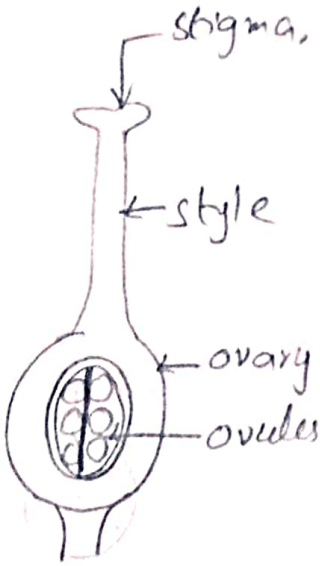
* Before culturing the tip of the distal part of the pedicel is cut off and the ovary is implanted with the cut end inserted in the nutrient medium.

* The normal Nitsch's or White's or MS or N6 inorganic salt media supplemented with growth substances are used.

* Sucrose as a carbon source is essential although maltose & lactose have been shown to be equally favorable.

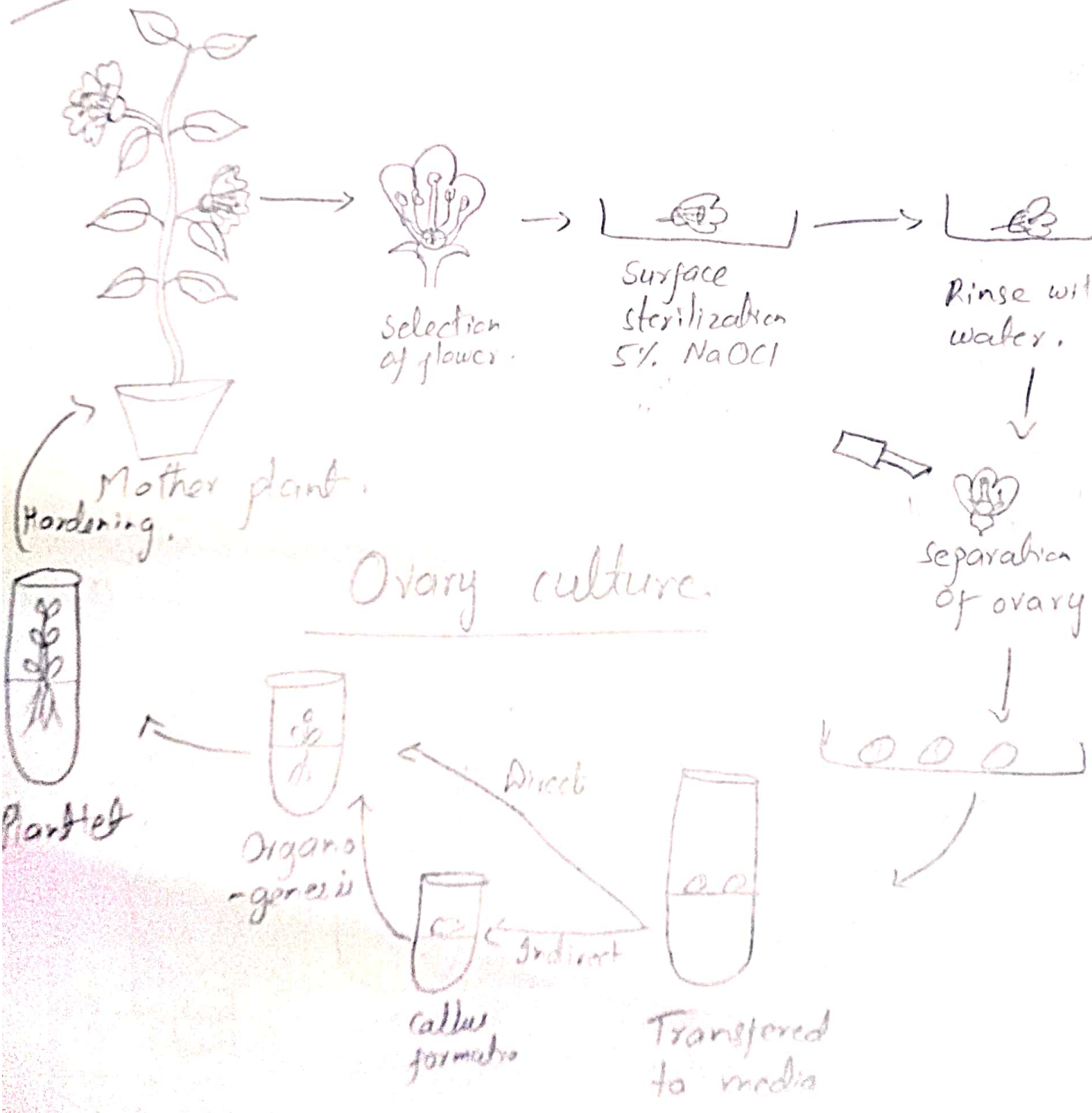


A flower

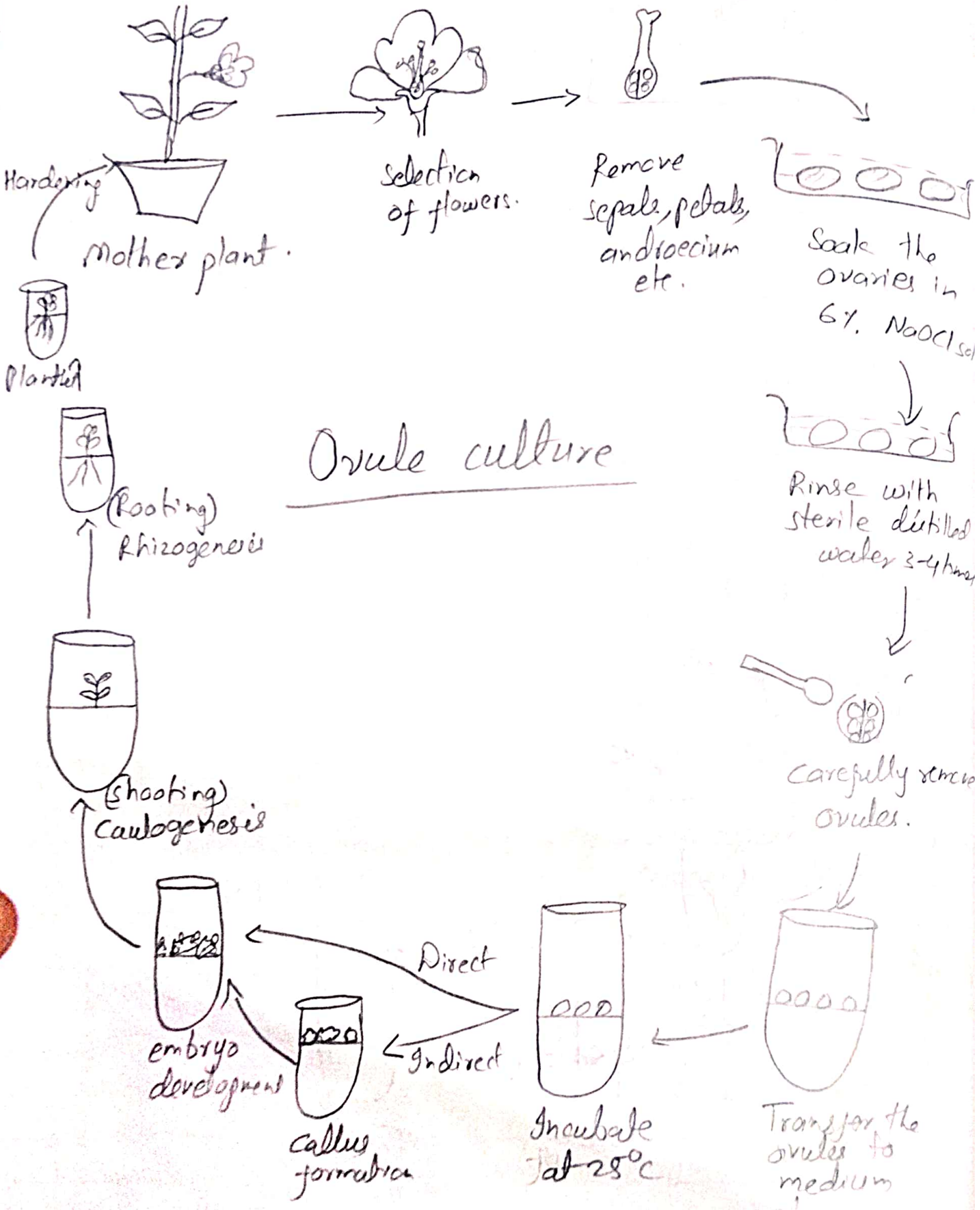


Gynoecium.

Ovary culture.



Ovule culture



Ovule culture

* Ovule culture is an elegant experimental system by which ovules are aseptically isolated from the ovary and are grown aseptically on chemically defined nutrient medium.

* Ovule culture was carried out for the first time by White (1932) in *Antirrhinum majus*.

* However, the technique of ovule culture was perfected by Maheshwari (1958).

* Ovule culture is useful to study the behaviour of zygote (or) very young embryos which are difficult to culture.

* In vitro culture of unfertilized ovules has been the most efficient and reliable technique for the production of haploid and doubled haploid plants of sugar beet.

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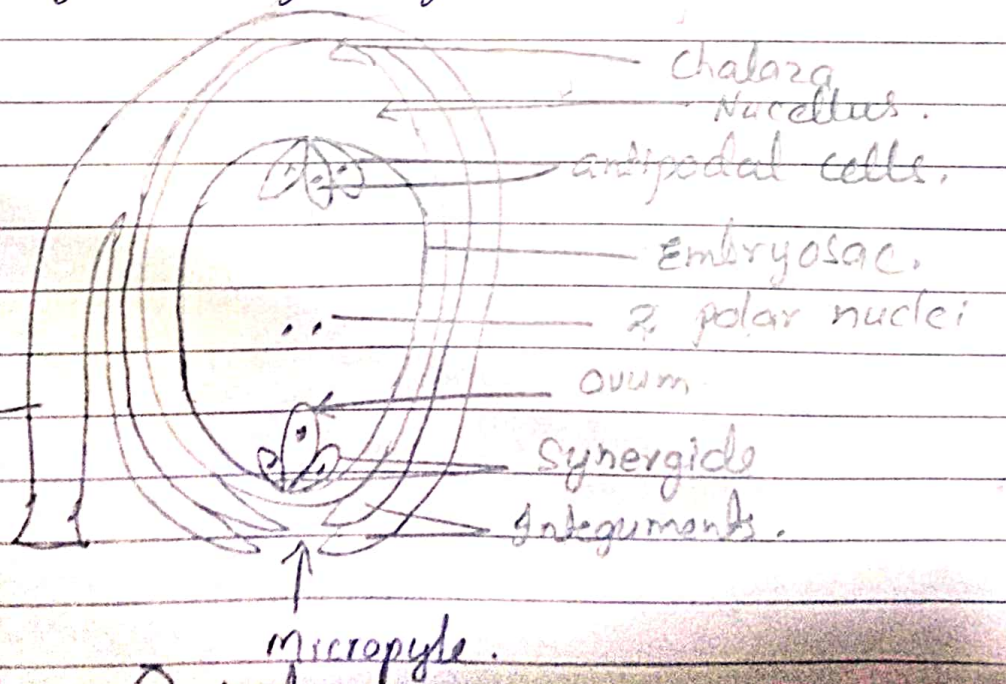


Ovule.

→ Procedure :-

① Collect the flower

② Remove sepals, petals, androecium etc.

③ Soak the ovaries in 6% NaOCl solution

④ ~~Rinse~~ Rinse the ovaries 3-4 times with sterile distilled water.

⑤ Carefully remove ovules with spatula.

⑥ Ovules are gently transfered to liquid or solid medium at 45°C of culture vial

⑦ Incubate the culture in either dark or light at 25°C.

Sem IV Paper I Unit III

Chromosome Doubling / Diploidization :→

Haploids can be Depolarized when haploid cell undergo chromosome doubling to produce homozygous plants.

Methods :

Chr Colchicine treatment :

Colchicine has been extensively used as a Spindle inhibitor to Induce chromosome duplication

→ It can be applied in the following ways:

→ The plantlets when still attached to the anther are treated for 24 - 48 h with 0.5% Colchicine Solution, Washed throughly and replanted.

→ Anthers can be plated directly on a Ch Colchicine Supplemented medium for a week and when the first division has taken place these are transferred to colchicine free medium for ~~a week and when the first~~ androgenesis process to take place.

→ This method can be followed in maize where male & female flowers are Individual present [monoecious].

→ Colchicine - Lanolin Paste [0.4 %) may be applied to the axis of Leaves when the plants are mature

→ The main axis is Decapitated to stimulate the axillary bud to grow into diploid and fertile branches

→ Repeated colchicine treatment to axillary buds with cotton wool plugs over a period of time [Eg: 14 days in Potato]

→ In Cereals Vigorous plants at 3-4 tillers stage are collected, Soil is washed from the Roots and are cut back to 3cm below the crown

→ The plants are placed in glass jars or vials containing Colchicine Soln (2.5g Colchicine dissolved in 20 ml dimethyl Sulfoxide & made up to a liter with water]

→ The Crowns are covered with colchicine solution

→ The plants are kept at room temperature light for 5h, the roots are washed thoroughly with water & potted into Light soil.

- plants should be handled with extra care after colchicine treatment for few days & Should be maintained under high humidity.

Endomitosis :

- Haploid cells are in general unstable in culture & have a tendency to undergo endomitosis to form diploid cells

→ This property of cell culture has been Exploited in Some species for obtaining homozygous plants or for diploidization

→ A Small Segment of Stem is grown on an auxin and cytokinin medium to induce callus formation

→ During callus growth & Differentiation there is a doubling of Chromosomes by endomitosis to form diploid homozygous to form diploid homozygous cells & ultimately plants

fusion of pollen nuclei:

→ Homozygous Diploid callus or Embroids may arise from the Spontaneous nuclear fusion in microspore is high in culture

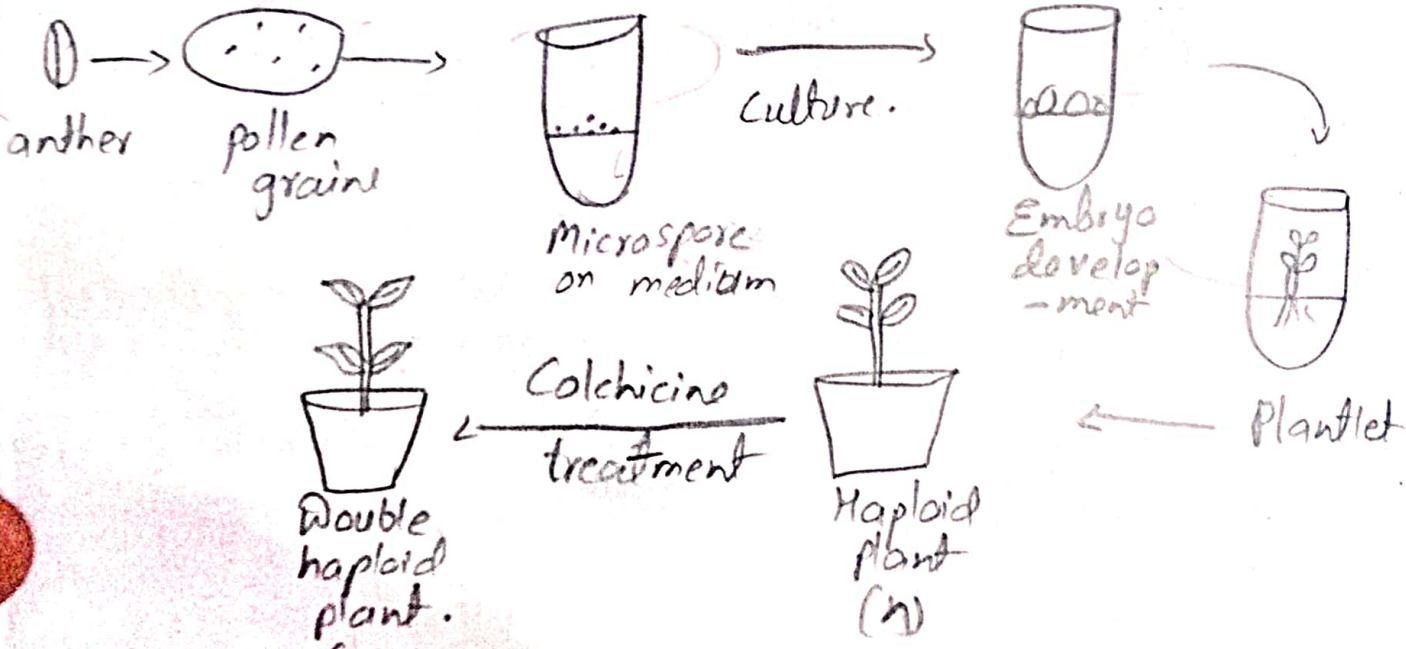
→ Uses :→

* production of homozygous Lines in the shortest possible time
* The study of homologous chromosome pairing in the haploids to gets ~~diploids~~ polyploids
* Over come Self incompactability
* New genotypes
* Breeding programme
* Genetic maps are very important to understand the structure & Organization of genomes
* Chromosome behaviour

* Segregant analysis
* QTL analysis
* Genetic transformation at haploid level has been studied in several ways

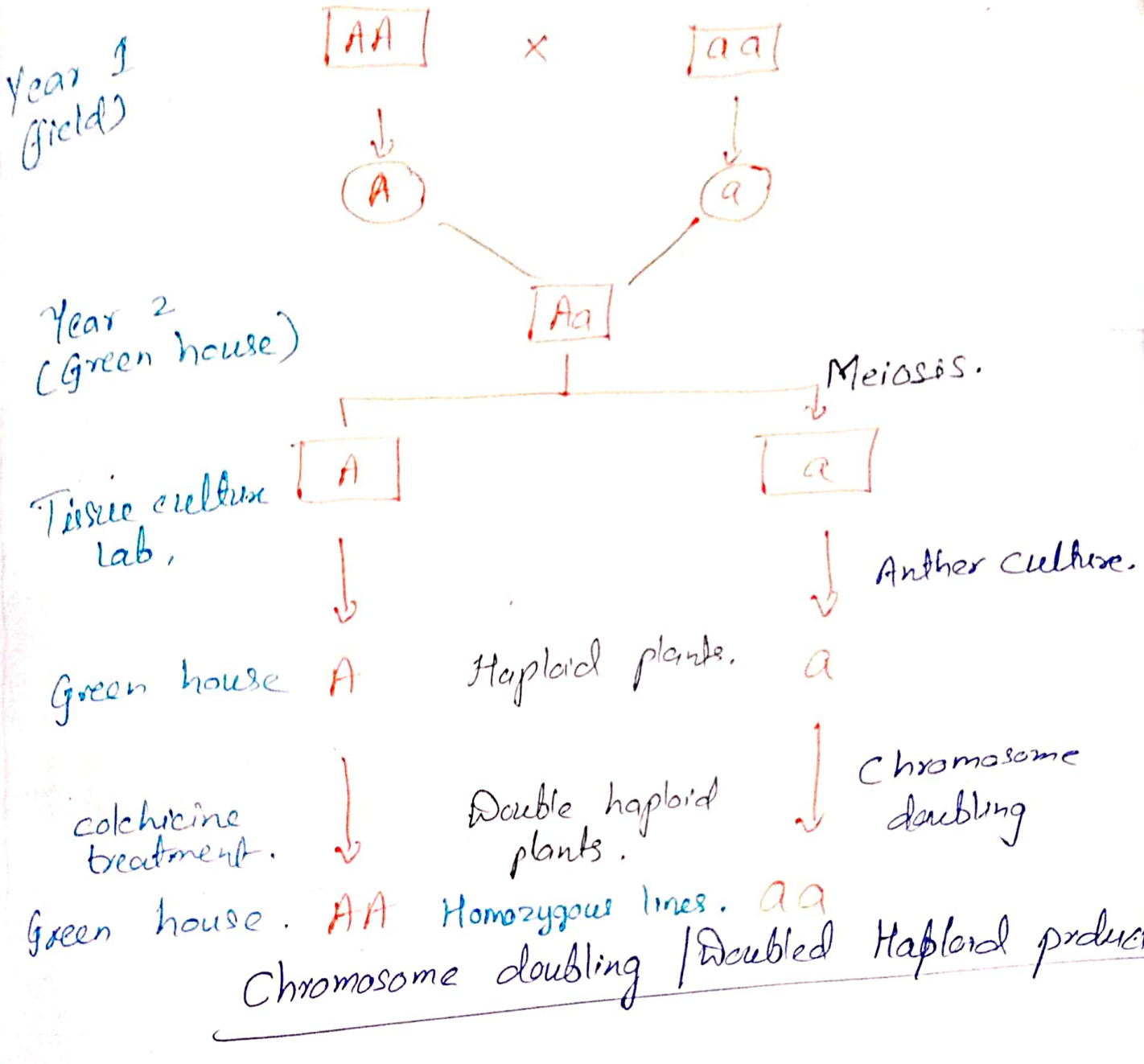
Applications:

* Development of purelines
* Development of Cultivars
* Development of hybrid as parents
* Construction of genetic maps
* Gene tagging / Locating genes
* Identification of molecular markers for trait Selection



Chromosome doubling
Doubled haploid production.

Parent 1 × Parent 2.



Chromosome doubling / Doubled Haploid production