FLS 322 BREEDING AND SEED PRODUCTIOZN OF FLOWER AND ORNAMENTAL CROPS (2 + 1)

Practical Manual

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The views expressed in the manual are the personal opinion of the contributors.

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FOREWORD

It is encouraging that College of Horticulture, Bermiok, Sikkim is releasing "A Manual on Breeding and seed production of flower and ornamental crops" for benefits of students, scientist and breeders. The prime objective of any plant breeding programme is to develop superior plants over the existing one in relation to their economic use. In ornamentals, there is always a quest for the development of new cultivars. Since, in flowers a specimen can't be seen for a longer duration and people have desire for the development of newer forms, colours and types of flowers. Quality is the most important attribute of the floricultural crops as a single blemish on the petal is not tolerable to the consumer. During evolution, the biodiversity has arisen on account of mutation, recombination and natural selection, which resulted in several variations in flowers. Most of these changes have been brought about by the efforts of professionals and enthusiastic amateurs most often as a result of close observations followed by selection. Thus imparting practical hand on breeding of flowers nad ornamental crops is most important for students and learners.

This manual brings the basic concepts emasculation and pollination in flowers crops to students, researchers and common person and makes available easily implementable methodologies. I congratulate the author in bring out this publication.

(Anupam Mishra)

PREFACE

The origin of plant breeding is as old as human civilization when the man started selecting superior plants and regenerating them for his use. Initially breeding started as an art, as the superior plants were selected based upon the human skill and preference. The scientific selection and development of superior plants was taken up only after the discovery of sex in plants. This process was further refined when Mendel proposed the laws of inheritance. The prime objective of any plant breeding programme is to develop superior plants over the existing ones in relation to their economic use. Like other crops, the breeding objectives of flower crops also differ from crop to crop and depend upon the nature of the plant and the part used for commercial exploitation. Flowers have many beneficial components for the consumer that can be created, enhanced, or improved by flower breeding programmes using classical or molecular techniques. This practical manual aims at to provide comprehensive practical skill to execute the theoretical classroom knowledge. Sound practical skill is utmost required in Agri-allied fields as the Agri./Hort. degree programme have to deal with farming community once they are off-campus and they should not face any problems. Therefore, it is very much vital that the students should be well acquainted with practical know-how in addition to the theoretical learning in the classroom and library.

Keeping the aforesaid points in the view, this practical manual on the course "Breeding and seed production of flower and ornamental crops" -FLS 322 (2+1) provides information on different aspects of ornamental breeding *viz* basic concept of emasculation and pollination, floral biology of cut and loose flowers, hybridization technique in flower crops, F1 hybrid seed production, seed viability test, pollen viability, educational visit to commercial seed production unit. This practical manual book will help to learn better through practical/ exercise and enrich their knowledge about the course. The faculty in the Department of Floriculture and Landscape Architecture, COH, of Central Agriculture University (Imphal) has made an earnest effort in compiling the manual as per the syllabus of the ICAR's 5th Deans' Committee. The manual content some illustrative exercises and questions for better understanding/ practiced on the subject.

It is hoped that this manual will serve as a useful document for the student.

(**Dipika Sarmah**)

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Practical No: 1

Title: Acquaintance with breeding tools for floricultural crops.

Objectives: To acquaint with important breeding tools and accessories used in floricultural crops.

Sl.No.	Tools	Uses
1	Scissors	Emasculation and removal of flower petals
2	Forceps	Emasculation, pollination
3	Needles	To study Flower structures
4	Camel's hair brush	Flower structure, pollination covering
5	Butter Paper Bags	Covering of emasculated/pollinated flowers
6	Jewel Tags	Recording information related to pollination
7	Pencils	For writing on jewel tags
8	Petri dishes	For collecting pollens
9	Magnifying lens	To study flower structure,stigma receptivity, pollination
10	Tags or labels	To properly labeled the plant

Exercise:

1. Draw the equipments with proper label.

Practical No: 2

Title: Pollen collection, its preparation and storage.

Objective: Collection of pollen for controlled hybridization.

Materials required: Scissors, forceps, petri dishes, camel hair brush, and permanent marker.

Procedure:

1. Determine the exact time of anther dehiscence by visual observation.
2. Collect the pollen from freshly opened flower.
3. To avoid contamination it is advised to collect anthers in a petriplate before dehiscence (i.e. balloon or popcorn stage)
4. Anthers at balloon stage are kept in a warm wind free area/sunlight by keeping the upper lid of petri dish slightly tilted to allow exchange of air to avoid condensation of vapours.
5. Gently tap the freshly dehisced anthers on a non sticking clean paper to dust the pollen.

Storage:

Pollen can be used fresh immediately after dehiscence or can be stored for a delayed use by transferring into small loosely stoppered glass vials. These are stored in desiccators over anhydrous calcium sulphate until use. For long term storage, keep the desiccators containingpollen vials in a deep freezer below 20^{o}C or store the pollen in small gelatin capsules or Teflonvials placed in cry flasks containing liquid Nitrogen at -196^{o}C.

Precautions:

1. Same brush should not be used for collection of pollens from more than one plant.
2. Use mask to avoid pollen allergy.
3. Keep anthers in wind free area.

Exercise:

1. Visit the field and collect pollen

Practical No: 3

Title: Determining the pollen viability through staining tests.

Objectives: To assess the potential of a given pollen parent to serve as a pollinizer.

Materials required:

Microscope, microscopic and cavity slides water glasses, cover slips, incubator, burner, stains, reagent bottles, distilled water, measuring cylinder, beakers, scissors, forceps, dessicator, glass vials, muslin cloth, refrigerator, pollen source.

Staining Tests:

1. ACETO/PROPIONO CARMINE (1-2%) TEST

- Weigh 1 or 2 g of carmine powder, dissolve it in 95ml of glacial acetic/propionic acid.
- Add distilled water to make a total of 100ml solution. Boil it, cool and filter.
- Add few drops of 10% ferric chloride solution (10 g ferric chloride dissolved in distilled water to make a final volume of 100ml), mix it and store in a refrigerator.

Testing:

- Shed the freshly collected pollen on a clean slide and put a drop or two of freshly prepared stain over it.
- Agitate the pollen grains with a needle for about 30 seconds.
- Place the cover slip, held in forceps, gently to avoid air bubbles.
- After a few minutes, examine the slide under a microscope.
- Coloured pollen grains are considered viable.
- Take 2-3 readings, avoids taking observations towards the edges of cover slip. Calculate average pollen viability percentage.

2. IODINE(0.5%)TEST

Preparation

- Dissolve 500mg each of potassium iodide and iodine in distilled water to make a final volume of 100ml.

Procedure

- Pour a little amount of pollen on a clean side.
- Put 1 or 2 drops of the dye over pollen and mix thoroughly.

- Place a cover slip and count the number of darkly stained (viable) pollen grains under the microscope.
- Take reading for several times and work out the average to give percent pollen viability.

3. Erythrosin B (0.04%) TEST

Preparation

- Dissolve 40mg of erythrocin B in distilled water to make a final solution of 100ml.

Procedure

- Follow the staining procedure as done in carmine/iodine test.
- Count the number of unstained pollen grains, which are considered viable in this method, under a microscope.
- Calculate percent pollen viability.

4. TETRAZOLIUM (MTT) TEST

Preparation

- Dissolve 10g of 3(4,5-dimethyl thiazolyl 1-2) 2,5- diphenyl tetrazolium bromide (MTT) in distilled water to make a final solution of 100ml.
- Prior to use, dilute it at the rate of 1 part stain to 10 parts of 60% sucrose.

Staining procedure

- Dust pollen on a neat and clean slide.
- Add a drop of stain and then place a cover slip.
- Keep the slide for 30-60 minutes at 30-57^{o}C.
- Count light red or red pollen as viable and colourless or deep pollen as non viable. Calculate the viability percentage.

Precautions:

- Good sanitation should be maintained in and around the area
- Use gloves while handling the chemicals.
- Freshly prepared stains should be used.
- Sterilized instruments should be used.

Excerise:

1. Collect pollen and do the viability test
2. Calculate the viability percentage

Practical No: 4

Title : Methods of emasculation and pollination

Objective: To learn how to make controlled crosses between any two parents

Materials required: Notched scissors, forceps, camel's hair brush, ladder, labels, magnifying lens, crochet thread, glass rod, butter paper bags, eye lash brush, muslin cloth bags.

Emasculation:

Select appropriate shoots/ flowers on all the sides of a plant where emasculation is to be done. Remove already opened flowers or immature flower buds and leaves. In self-pollinating or in cleistogamous crops, do emasculation 3-4 days prior to day of anthesis. All the emasculation work should be done in morning hours. Hold the shoot gently avoiding breakage, and in one attempt remove petals, distal ends of sepals in addition to stamen by using either fingernails, forceps or modified scissors with notches cut in the blades and a screw for adjusting the degree of closure. Check if any anther has fallen into calyx cup during emasculation, if so, remove it. Cover the emasculated flowers with insect-proof bags until pollination to avoid contamination.

Pollination: Apply fresh or stored pollen from a desired male parent on to receptive stigmas of emasculated flowers using camel's hair brush/sterilized glass rod/cotton plug. Judge the stigma receptivity by confirming the exudation of watery fluids from stigmatic surface as seen by naked eye or through a magnifying lens. Do not overload the stigma with pollen. To avoid this, use an eye lash brush (single eye lash picks up 5-6 pollen grains). After pollination, replace the bags to protect from winds/rain.

Bagging: Cover the recently pollinated flowers with proper insect-proof butter paper bags. Remove thebags after the ovary starts swelling.

Labelling: Tie a suitable label on each shoot below the last pollinated flower. Put the followinginformation on the respective labels:

- Parentage (female x male)
- Number of flowers emasculated and pollinated
- Date of emasculation and pollination
- Name of the breeder and location

Precautions:

1. Do all the emasculation work in morning hours.

2. Do not overload the stigma with pollen.
3. Properly label the cross mentioning the parents as female × male and date of emasculation and pollination.

Exercise

1. Visit the field and do the emasculation and pollination
2. Write the procedure of emasculation and pollination method

Practical No: 5

Title: Determination of stigma receptivity.

Objective:

1. To find out actual time and duration for which pollination is effective
2. To work out cross compatibility/incompatibility reaction between two parents.

Materials required:

Notched scissors, forceps, camel's hair brush, eye lash brush, labels, (jewel tags), magnifying lens, crochet thread, glass rod, butter paper bags, muslin cloth bags.

Simple test:

Visually observe through naked eye or with the help of a magnifying lens, several stigmas at random. Presence of exudates (water fluid) on stigmatic surface would indicate that stigma is receptive.

Seed set test:

However, more reliable rest for determination of stigma receptivity is by making controlled pollination at different stages of flower development i.e.

- Two days before anthesis
- One day before anthesis
- Day of anthesis
- One day after anthesis
- Two days after anthesis

Exercise:

1. At each of the described stages, emasculate 40-50 randomly selected flowers and pollinate immediately.
2. Count the number of flowers with successful seed set after 4-6 weeks in each.
3. On the basis of percentage of seed set, work out the initiation and duration for which stigma remains receptive.
4. Maintain record

Practical No: 6

Title: Study the floral biology of Rose

Material Required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors, Jewel Tag, Pencil etc.

Botanical name: *Rosa* sp.

Family : Rosaceae

Floral Biology:

Inflorescence: Roses have determinate inflorescence that may assume corymb, paniculate, or solitary form. When flowers are borne singly, as in many hybrid tea cultivars, there are still underdeveloped flower buds in the axils of the flowers immediately below the terminal flower which can develop into short flowering shoots under favourable environmental conditions. Flowers bracteate or ebracteate, pedicellate, complete, regular, actinomorphic, bisexual, perigynous with deep cup like thallamus, and sweetly scented.

Calyx: 5 in number gamosepalous, tubular, sometimes campanulate, calyx lobes sometimes show transition to leaves.

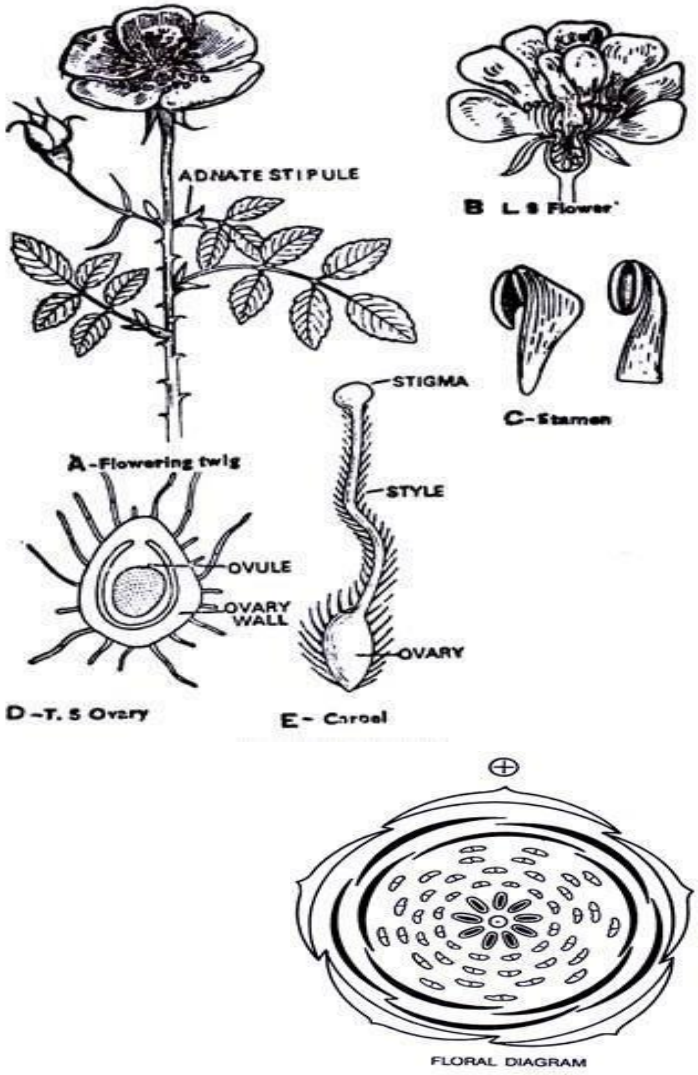
Corolla: 5 to many, free, imbricate, large and showy, fragrant, rosaceous, inserted on the rim of the thalamus cup. There is transition between petals and stamens and vice-versa.

Androecium: Many, polyandrous (free), unequal, inserted on the disc that lines the calyx tube, filaments of equal length, anthers basifixed, dehiscence longitudinal.

Gynoecium: Polycarpellary, superior at the bottom of the thalamus cup, each carpel having one basal ovule. Each carpel having a single almost terminal style, stigma thickened.

Fruit: Each carpel matures into an archive, the archives are enclosed within the thalamus cup which become orange or red colored and fleshy. The cup enclosing the archives is called the hip.

Floral Formula: $\oplus \male\female K_{(5)}\, C_5$ or $\infty\, A\infty\, \underline{G}\infty$



Exercise:

1. Draw the flower structure and floral diagram

Practical No: 7

Title: Study hybridization technique in Rose (*Rosa Hybrida*)

Materials required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors and Pencil etc.

Procedure:

Emasculation and pollination:

- For emasculation flower buds which have opened ¾ of its petals are selected. Calyx is removed with the help of a sharp scissor.
- Then the petals are plucked out exposing the stamens. The emasculation is done by cutting away the anther filaments with scissors. Care should be taken not to cut the anthers. In case the anthers are cut, scissors should be sterilized in rectified spirit before emasculating the next flower.
- After emasculation the stigma is exposed and protected by covering with butter paper bag securely tied to the flower stalk.
- Next day the butter paper bag is removed and stigmatic surface is examined for receptivity. If it is sticky, then it is ready for pollination.
- For pollination only those male parents are selected which produce fertile pollen. The flowers are removed in the morning before the anthers dehisce. Their petals are removed and the tip of the dehisced anther are kept in clean pert dish and kept in sun so that the anther dehisced easily.
- Anthers are rubbed on stigmas of female flower to affect successful pollination.
- In general one male flower could be used for pollinating three or four female flowers.
- After the pollination, female flower is covered with butter paper bag and tied securely to the stalk of the flower. Then, the names of male and female parents including date and other information are noted down and tied on stalk of flowers.
- The hip is allowed to develop for 3 to 4 months for attaining maturity. When the hip turns pink, it is ready for harvesting.

Precautions:

- During emasculation care must be taken not to injure the gynoecium.

- At the time of pollination, one must ensure that the stigma is receptive which is shown by sticky ooze from the stigma.
- Pollen must be collected from freshly dehisced anthers
- Tagging with proper labels must be attached to every pollinated flower.
- Instruments used for breeding must be clean and sterile to avoid chance pollination.
- Proper staking must be done after the whole programme to avoid toppling down of the flower after or at any stage of development.
- Good sanitation should be maintained in and around the area. All the debris and anthers petals etc. should be removed during emasculation and must be disposed off properly.

Exercise:

1. Visit the field and do the hybridization in rose
2. Collect the rose seeds and store

Practical No: 8

Title : Study the floral biology of Gladiolus.

Botanical name : *Gladiolus* sp.

Family : Iridaecae

Floral Biology :

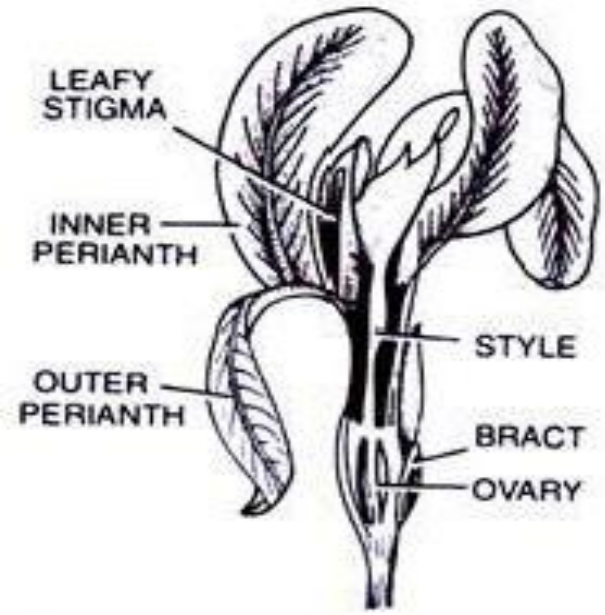
Inflorescence : Spike the individual florets are directly attached to the axis .

Flower: Sessile, complete, zgyomorphic, bisexual, epigynous, trimerous, large. **Perianth:** Six on two whorls, gamophyllous.

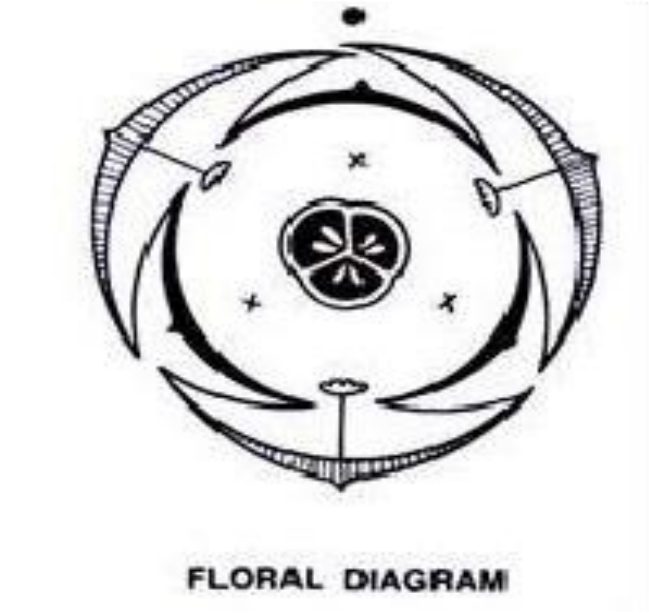
Androecium: Three, free, opposite the outer parianth lobes, filament long and of equal length, anthers bithceous, dehiscence by lateral longitudinal shits. Stamens are epiphyllous. **Gynoecium:** Tricarpellary pistil with a three forked stigma. Ovary contains about 15 to 150 ovules.

Fruit: Capsule

Foral formula : $\%\,\male\,\female\; P_{(3+3)}\, A_3\, \underline{G}_{(3)}$



V.S. FLOWER



FLORAL DIAGRAM

Exercise:

1. Draw the flower structure and floral diagram

Practical No: 9

Title : Study hybridization technique in Gladiolus (*Gladiolu*s sp.)

Materials required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors and Pencil etc.

Procedure:

- Hybridization in gladiolus is done by hand emasculation and hand pollination to protect the pollen parent from any contamination; the florets are covered with butter paper bags. Each floret which is to be used as seed parent must be emasculated prior to its opening.
- All the anthers should be removed and tips of the floret should be tied with soft thread.
- Emasculation is done preferably in the afternoon or morning (before 7.30 a.m.) stigma is receptive between 10 a.m. and 1pm when it becomes feathery.
- Pollen from the male parent is dusted on the sticky stigmatic surface.
- This process is repeated on the following day of emasculation to ensure pollination.
- Four or five lower florets should be used for hybridization and the rest part of the spike should be removed as soon as the pollination of the 4th or 5th bud is completed.
- After pollination the florets are again tied at the top with thread and tags mentioning the parentage and date of crossing should be tied with the florets.
- If the pollination is successful the style will begin to wilt within 48 hours. The fully matured capsule when turn brown in colour is harvested.

Precautions

- During emasculation care must be taken not to injure the gynoecium.
- Pollen must be collected from freshly dehisced anthers
- Tagging with proper labels must be attached to every pollinated flower.
- Instruments used for breeding must be clean and sterile to avoid chance pollination.
- Proper staking must be done after the whole programme to avoid toppling down of the flower after or at any stage of development.
- Good sanitation should be maintained in and around the area. All the debris and anthers petals etc. should be removed during emasculation and must be disposed off properly.

Exercise:

1. Visit the field and do hybridization in gladiolus.

Practical No: 10

Title: Study the floral biology of Tuberose.

Botanical name : *Polianthes tuberosa*.

Family : Amaryllidaceae

Floral Biology :

Inflorescence: Cyme

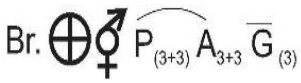
Flower: Bracteate, pediceelate, hermaphrodite, actinomorphic, epigynous

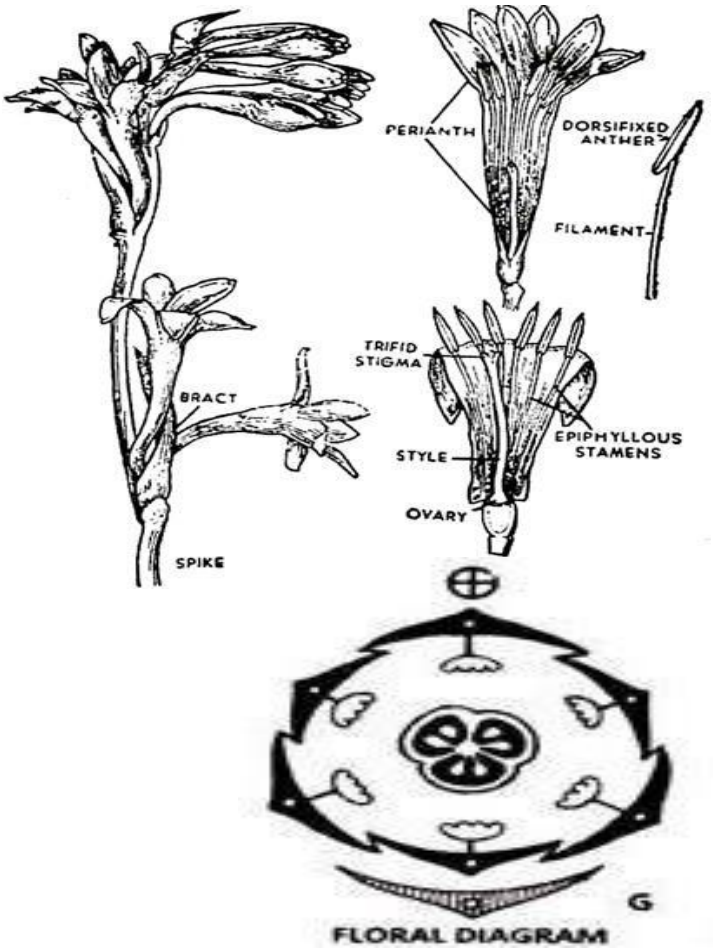
Perianth: 6 tapels in two whorls of 3 each gamophyllus, tubular, long tube, fragrant

Androceium: Stamens 6, polyandrous, epiphyllous- as the tepals arranged in two whorls; anthers bithecous, introrse, filament distinct, sometimes connate basally by stamina tube.

Gynoceium: Tricarpellary, syncarpous, ovary inferior, trilocular, axile placentation with numerous ovul;es in each loculus; style slender, trifid.

Fruit: Loculicidal capsule

Foral Formula: 



Exercise:

1. Draw flower structure and floral diagram of tuberose .

Practical No: 11

Title : Study hybridization technique in Tuberose.

Materials required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors and Pencil etc.

Procedure:

- In tuberose both male and female parents are born on the same florets. The stamen from the female parent are gently removed when the floret start opening. The process is called emasculation. The emasculated florets are covered with paper bag.
- Stigma become thick and stigma surface become sticky 1 or 2 days after florets opening.
- The hairs of stigma help to catch the pollen that fall on it. This is the stage when stigma becomes receptive.
- The mature dehiscing anthers from the desired pollen are gently rubbed against lobes of stigma of the female parent with the help of forceps.
- The transfer of pollen to the stigma is known as pollination. The pollinated florets are labeled properly.

Exercise:

1. Visit the field and do hybridization.

Practical No: 12

Title: **Study the floral biology of Hibiscus**

Botanical name : *Hibiscus rosa chinensis*

Family: Malvaceae

Inflorescence: Solitary axillary.

Flower: Ebracteate, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous, hypogynous, large, showy, red, mucilaginous.

Epicalyx: Five to seven, green, linear.

Calyx: Sepals 5, gamosepalous, ovate, campanulate, valvate aestivatin.

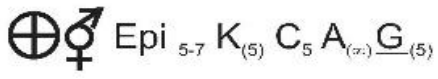
Corolla: Petals 5, polypetalous, mucilaginous, twisted, red, united at the base and adnate to the staminal tube.

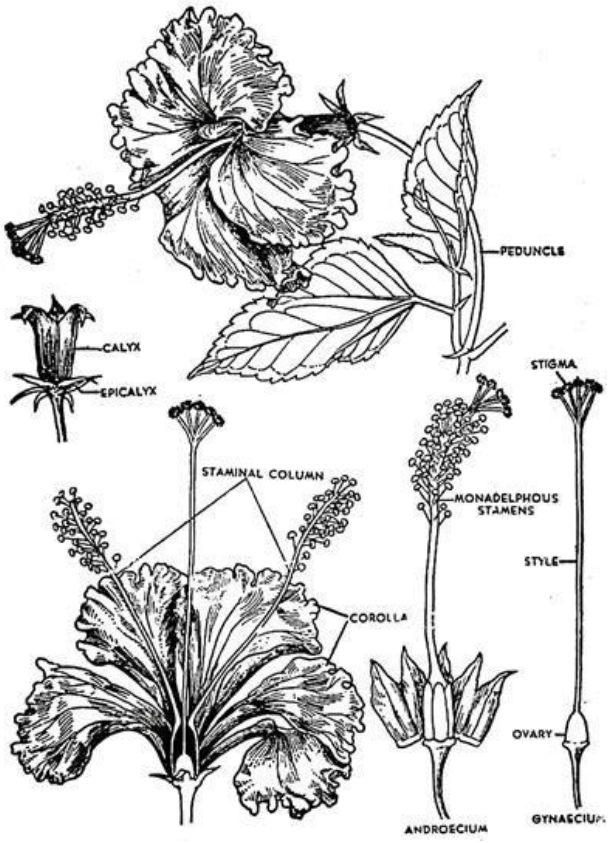
Androecium: Stamens numerous, forming a tube, monadelphous, epipetalous, anthers monothecous, yellow, reniform, extrorse, transversely attached to the filament, pollen grains multiporate.

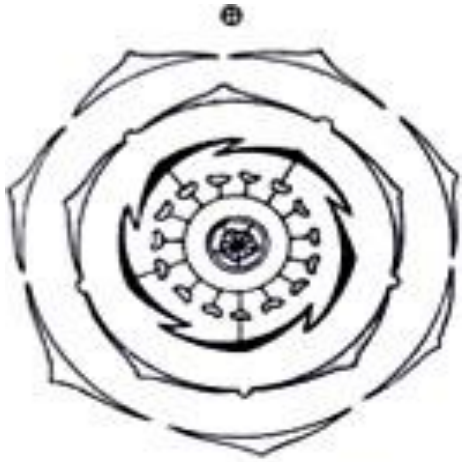
Gynoecium: Pentacarpellary, syncarpous, superior, pentalocular, axile placentation, many ovules in each loculus; style passing through the staminal tube; stigma 5 capitate.

Fruit: Capsule.

Floral formula:







FLORAL DIAGRAM

Exercise:

1. Draw flower structure and floral diagram.

Practical No: 13

Title: **Study hybridization in Hibiscus**

Materials required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors and Pencil etc.

Procedure:

- Matured flower buds of the desired female parent are emasculated one day prior to flower opening and bagged with butter paper cover.
- The dehisced pollen plant is also bagged.
- Pollen from male parent is smeared on the sticky stigmatic surface of the emasculated flower of the female parent on the next day.
- The crossed flower is bagged with butter cover to prevent natural cross pollination.
- The flower is properly labelled indicating the seed and the pollen parents and also dates of crossing.
- The butter paper bags are removed after a week of crossing and the fruits are allowed to develop to maturity.

Exercise:

1. Visit the field and do the hybridization in Hibiscus

Practical No: 14

Title: **Study the floral biology of Marigold**

Botanical name: *Tagetes sp.*

Family: Asteraceae

Inflorescence: Capitulum surrounded by involucre.

Capitula large with two kinds of flowers:

(a) The peripheral flowers or ray florets, which are large, attractive and ligulate.

(b) Disc florets, in the centre and tubular.

Ray florets: Bracteate, sessile, incomplete, zygomorphic, ligulate, pistillate or neuter, epigynous.

Calyx: Pappus-2-3, persistent.

Corolla: Petals 5, gamopetalous, a short basal tube and a large flat strap shaped limb, with 5 teeth (sometimes lesser) indicating the number of petals.

Androecium: Absent.

Gynoecium: Absent, or if present then bicarpellary, syncarpous, inferior, unilocular, basal placentation, simple style, bifid stigma.

Floral Formula:

Br % Neuter or ♀ K pappus $C_{(5)}$ A_0 $\overline{G}_{(2)}$

Disc florets: Bracteate, sessile, complete, hermaphrodite, tubular, actinomorphic, pentamerous, epigynous.

Calyx: Pappus or reduced, modified into 2-3 scales, persistent.

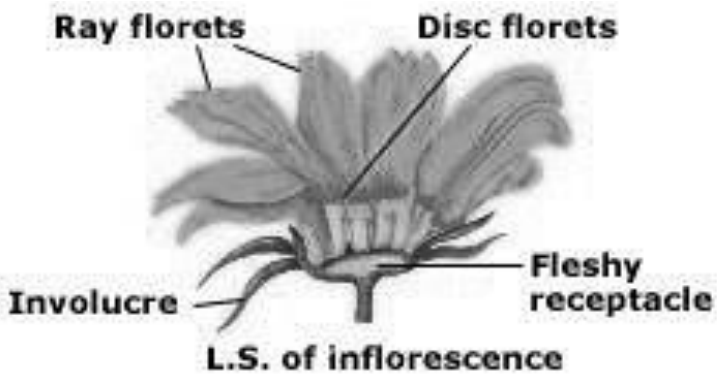
Corolla: 5, gamopetalous, tubular, 5 toothed, teeth represents the number of petals, valvate.

Androecium: Stamens five, epipetalous, filaments free, short, alternating with the petals, anthers syngenesious, basifixed, dithecous, introrse.

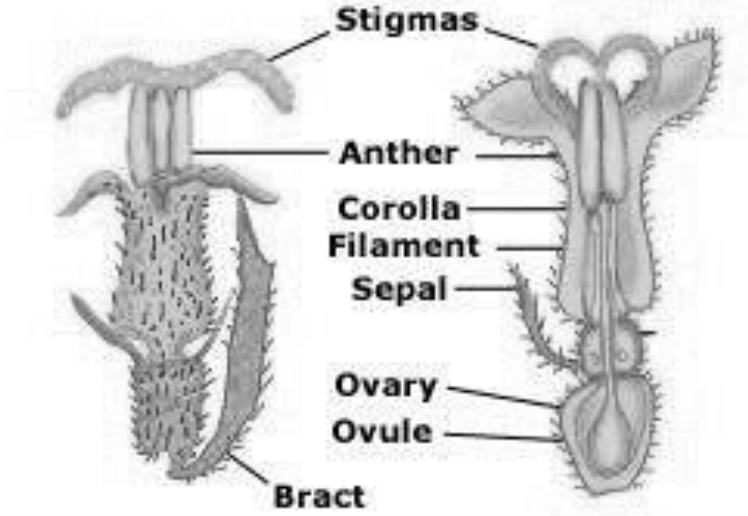
Gynoecium: Bicarpellary, syncarpous, unilocular, inferior, basal placentation, single basal ovule, style single long; stigma bifid.

Floral Formula:

Br ⊕ ⚥ K (pappus) $\widehat{C_{(5)}\ A_{(5)}}$ $\overline{G}_{(2)}$

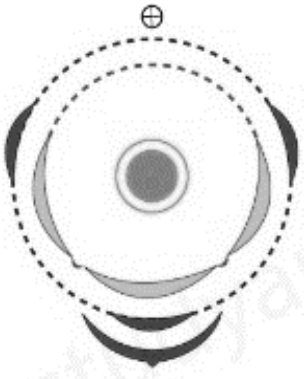


L.S. of inflorescence

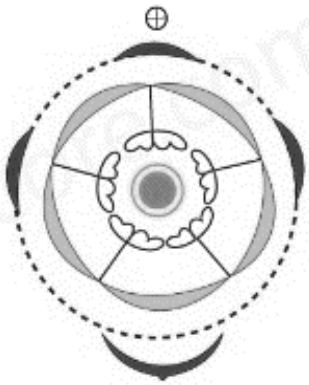


Disc floret

L.S. of disc floret



Floral Diagram of Ray floret



Floral Diagram of Disc floret

Exercise:

1. Draw flower structure and floral diagram

Practical No: 15

Title: **Study hybridization in Marigold**

Materials required: Forceps, Needle, Petridish, Brush, Butter paper bags, Scissors and Pencil etc.

Procedure:

- Emasculation is done by removing the anther tubes with forceps early in the morning that the flowers open.
- Un-emasculated flowers are removed.
- Pollination is carried out by collecting pollen from heads which are already bagged prier to flowering.
- Pollen can be collected from flowering heads into proper bags.
- Pollination is usually done in the morning after emasculation.
- Pollen can be applied by a small piece of cotton, a camel hair brush, a small section of leaf, paper or surface stigma.
- Freshly collected pollen is more effective in pollination. After each cross, care must be taken to avoid contamination by wiping the hands with alcohol and cleaning or discarding the pollen applicator.

Exercise:

1. Visit the field and do the hybridization in marigold

Practical No: 16

Title: Study harvesting stages of seed of annuals.

Harvesting of Seeds

Optimum time of harvest is a critical factor in the production of optimum quality seeds. When the crops are judged ready for harvesting the plants are either cut as a whole or seeds harvested with different procedures.

In floricultural crops, the stage of harvest varies from crop to crop. Some important annual flower crops with their optimum stage of harvest are presented in the following table.

S.No.	Plant	Stages of collection
1.	*Alyssum maritimum*	The seeds shatter easily. Remove pods when just about to dry.
2.	*Antirrhinum majus*	Cut when just about to dry, spikes mature from lower branches onwards.
3.	*Arctotis stoechadifolia*	Cut the whole plant when maximum amount of seed matures and dry then on canvas.
4.	*Calendula officinalis*	Seeds shatter when too dry. Collect heads when partially dry.
5.	*Companulla sp.*	Whole plants may be collected
6.	*Celosia sp.*	Collect the heads when dry on the plant. Protect drying heads from rain.
7.	*Chrysanthemum coronarium*	Cut when almost all the flower heads dry.
8.	*Clarkia elegans*	Remove seeds as they begin drying
9.	*Cosmos bipinnatus*	Collect seeds as pods dry.
10.	*Dahlia variabilis*	Collect the seeds as the heads dry on the plants. Collect heads of flowers as they dry. Take out tubers when plant almost dry. Store in dry and cool place.
11.	*Delphinium*	Cut the whole plant when the lower capsules begin to dry and dry in shade.
12.	*Dianthus*	Seeds may shatter if allowed to dry too much on the plant. Collect individual heads as they begin to dry.
13.	*Dimorphotheca*	Cut the whole plant when the maximum amount of seed is mature.

14.	*Gaillardia pulchella*	Cut the entire plant when the maximum amount of seed is mature and dry on canvas.
15.	*Gazania splendens*	Cut the entire plant when the maximum amount of seed is mature and dry on canvas.
16.	*Godetia grandiflora*	When lower capsules open and begin drying, cut the entire plant and dry in shade.
17.	*Gomphrena globosa*	When the heads dry, collect individually.
18.	*Gypsophila elegans*	When the majority of the capsules have turned brown, cut whole plant and on canvas.
19	*Helianthus annus*	When the flower heads dry, collect individually.
20.	*Iberis amara*	Cut the whole plant and dry in sun set at the first signs of seeds becoming dry9self incompatible)
21.	*Helichrysum bracteatum*	When the heads become fuzzy, collect individually.
22.	*Impatiens balsamina*	Cut pods burst at the slightest touch. Collect individually when turning yellow brown. Place in a box the half matured seeds to dry.
23.	*Kochia scoparia*	Cut the entire plant when the maximum amount of seed is mature and spread on canvas to dry.
24.	*Lathyrus adoratus*	When the lower pods commence drying remove the entire plant and dry in shade.
25.	*Limonium sinuatum*	Cut the whole plant when the maximum amount of seed is mature and spread on canvas to dry.
26.	*Linaria bipartita*	When lower pods begin drying, remove the entire plant and dry in shade.
27.	*Linum grandiflora*	Cut the entire plant when the maximum amount of seed is mature and dry on canvas.
28.	*Lupinus hartwegii*	Remove individual seed pods as they dry. If allowed to dry in excess on the plants, they would burst.
29.	*Mathiola incana*	Remove the plant when seed pods begin drying. Dry in sun or shade. Single flower seeds produce 50% or more double flowering plants.
30.	*Mesembryanthemum crimifolium*	Whole plant should be harvested and seed should be extracted.
31.		
32.	*Molucella laevis*	Whole plant should be harvested and seeds should be extracted by beating the plants with stick.
33.	*Papaver roheas* *Petunia hybrid*	Remove seed pods as they begin drying. Double flowers require hand pollination.
34.	*Phlox drummondii*	Remove seeds when just about to dry to prevent shattering.
35.	*Pimpinella monoica*	Harvest umbels when completely dry and collect seeds by thrashing.

36.	*Portulaca grandiflora*	Collect when capsules begin to dry.
37.	*Rudbeckia bicolour*	When flower heads fuzzy collect them and dry in shade.
38.	*Salvia splendens*	When seed cap dries, remove plant and dry in shade.
39	*Tagetes sp.*	Collect flower-heads as they dry. Plants of dwarf species should be removed when all the flower heads are dry.
40.	*Tithonia speciosa*	Collect the flower heads as they become fuzzy and dry in shade.
41.	*Tropaeolum majus*	Collect seeds as they dry. If allowed too long on the plant they fall off.
42.	*Viola wittrockiana*	Collect seed pods when just about to dry. They shatter when over dried on the plant
43.	*Venidium fastuosum*	When flower heads become fuzzy collect them and dry in shade.
	Zinnia elegans	Cut flower heads as they dry. Dwarf varieties may be removed when the entire flower heads dry out.

Precautions:

1. Collect seeds at the right stage to avoid shattering loss.
2. Immature seeds should not be collected.
3. Seeds should be dried in shade before processing.

Exercise:

1. Collect the seeds from the field
2. Mention about the appropriate stage of harvesting

Practical No: 17

Title: Hybrid seed production using cytoplasmic genetic male sterility of marigold

Principle of Hybrid Seed Production:

- Hybrid marigold is produced by using cytoplasmic genetic male sterility and genetic fertility restoration system.
- The male sterile line (A line) contains sterile cytoplasm and recessive genes for fertility restoration. This is maintained by a male fertile counterpart (B line) which also contains recessive genes, but has fertile cytoplasm.
- For production of hybrid seed male sterile line (A line) is crossed with a fertility restoring line (R line) which has the dominant genes for fertility restoration, but may have either sterile or fertile cytoplasm.
- The restorer line (R line) should nick well with A line to produce F1 hybrid seed.

1. Production of Male-Sterile Line (A line) Seed:

Selection of site: A sunny location is ideal for marigold cultivation. Under shade, it produces more vegetative growth and do not produce any flowers. Highest yields are obtained when planting is done during August - September. Rainfall during rainy season and high temperatures during summer will affect the flower quality.

Spacing: Proper spacing between plants is required for better development of plant and higher flower yield. The following spacing is recommended for marigold.

1) African marigold: 40 X 30 cm or 60 X 30 cm.
2) French marigold: 20 X 20 cm or 20 X 15 cm

Isolation Requirements: Seed fields must be isolated from other marigold fields, same line increase fields not conforming to varietal purity requirements of certification at least by 600 meters.

Planting Ratio: The proportion of female line (A line) and male line (B line) should be 3:1. However, the first two border rows on either side may be sown with the male line (B line) seed to ensure enough pollen supply.

Seed Rate: A line: 200 g/ha and B line: 75g/ha

Rouging: The male-fertile plants in the female parent lines should be removed each day during the entire flowering period. This is best done in the morning hours before the bees have removed the pollen.

Supplementary pollination: For supplementary pollination (Hand Pollination) the palm is first gently rubbed on the male parent flowers and then on the stigmas of the female line to transfer the pollen.

Harvesting: The male parent rows should be harvested prior to harvest of female rows to avoid contamination. No male parent heads should be left intermingled with the female parent rows.

2. Production of Maintainer line (B line) and Restorer line (R line) seed:

The seed is produced in an isolated field in the manner similar to that described for open pollinated varieties. The isolation requirements however are higher and shall be same as given for production of "A" line seed above.

3. Production of Hybrid marigold Seed:

Isolation Requirements: Seed fields must be isolated at least by 400 meters from the fields of other varieties, commercial hybrid of the same variety, fields of same hybrid seed production not conforming to variety purity requirements of certification.

Planting Ratio: The proportion of female parent (A line) : Male line (R line) should be kept at 3:1 however, the first two border rows on either side may be sown with the male parent seed to supply enough pollen.

Seed Rate: A line : 200g per ha & R line 75 g per ha.

Harvesting: The male parent rows should be harvested prior to harvest of female rows to avoid contamination. No male parent heads should be left intermingled with the female parent rows.

Practical No: 18

Title: Visit to commercial seed production unit

Exercise:

1. To visit commercial seed unit
2. To observe and study the different seed production unit
3. To maintain a detailed self prepared record of all the observations made on such visit.