

PRACTICAL MANUAL
on
**BREEDING AND SEED PRODUCTION OF FLOWER AND
ORNAMENTAL CROPS**

Course No. HFL-322; Credit Hrs. 3(2+1)

For

B.Sc. (Horticulture) Ill-year (2nd Semester)



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2019

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Syllabus: Breeding and Seed Production of Flower and Ornamental Crops

Practical: Study of floral biology and pollination in important species and cultivars. Techniques of inducing polyploidy and mutation. Production of pure and hybrid seeds. Harvesting, conditioning and testing of seeds. Practice in seed production methods.

Name of Student

Roll No.

Batch

Session

Semester

Course Name :

Course No. :

Credit

Published: 2019

No. of copies:

Price: Rs.

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Experiment No. 1

Objective: To study floral biology, emasculation and pollination of Rose.

Materials Required:

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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

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Calyx:

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Corolla:

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Androecium:

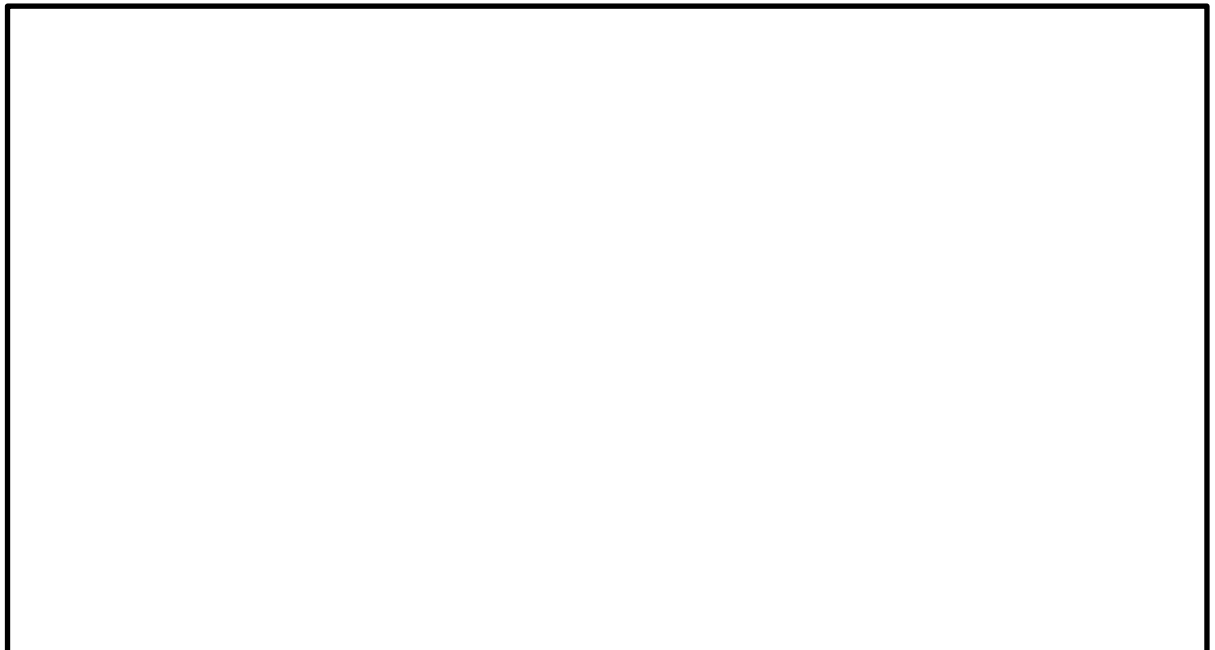
.....

Gynoecium:

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Floral formula:

Draw flower structure and floral diagram:



Experiment No. 2

Objective: To study floral biology, emasculation and pollination of Gladiolus.

Materials Required:

Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

Calyx:

Corolla:

Androecium:

Gynoecium:

Floral formula:

Draw flower structure and floral diagram:



Experiment No. 3

Objective: To study floral biology, emasculation and pollination of Tuberose (Rajanigandha).

Materials Required:

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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

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Calyx:

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Corolla:

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Androecium:

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Gynoecium:

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Floral formula:

Experiment No. 4

Objective: To study floral biology, emasculation and pollination of Hibiscus.

Materials Required:

Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

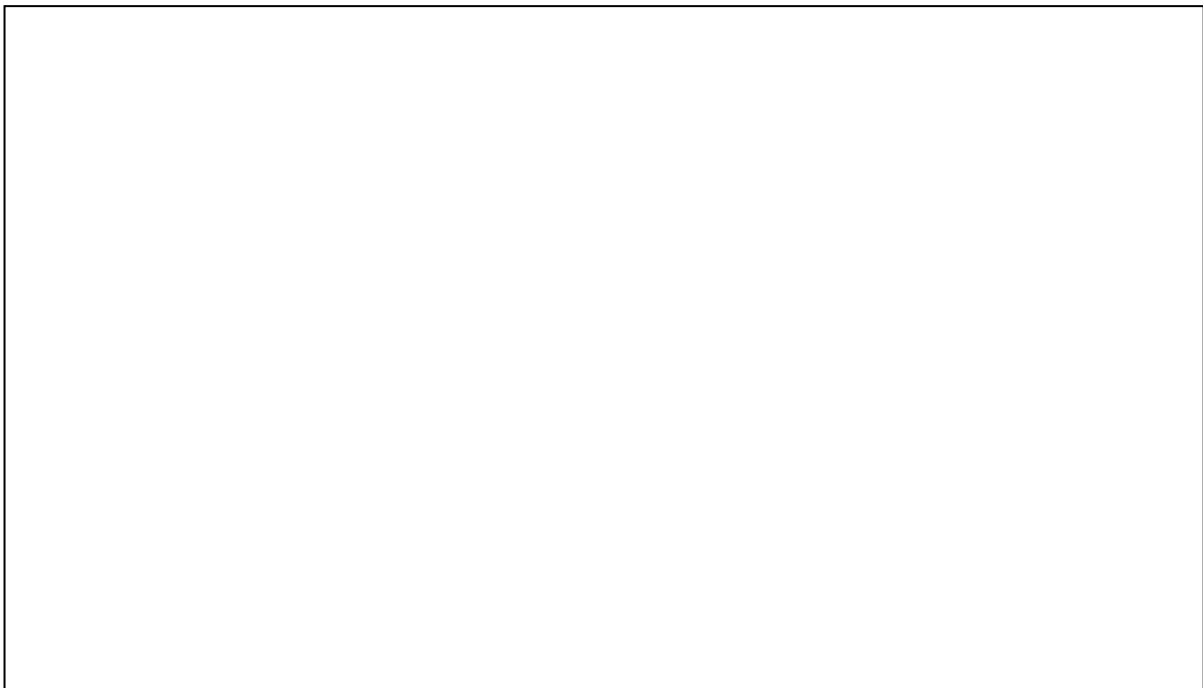
Calyx:

Corolla:

Androecium:

Gynoecium:

Floral formula:



Experiment No. 5

Objective: To study floral biology, emasculation and pollination of Marigold.

Material required:
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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

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:

Calyx:

Corolla:

Androecium:

Gynoecium:

Floral formula:

DISC FLORETS:

Calyx:

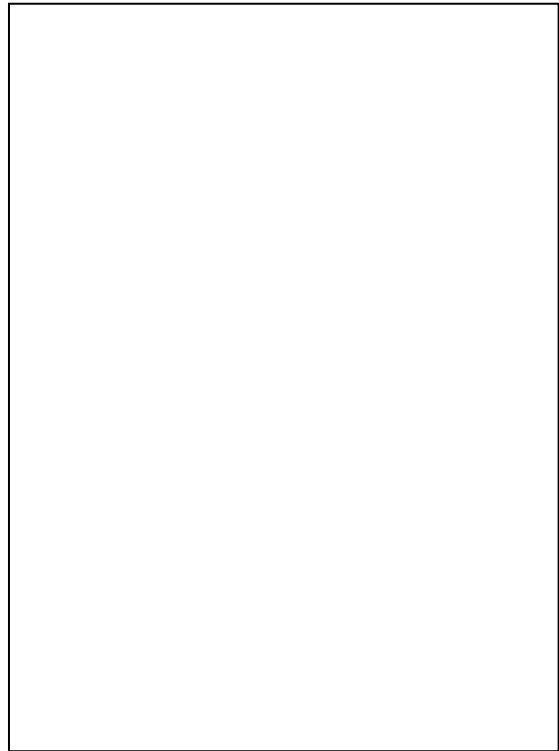
Corolla:

Androecium:

Gynoecium:

Floral formula:

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Emasculation and crossing techniques:

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Objective: To study floral biology, emasculation and pollination of *Petunia*.

Materials Required:
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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:
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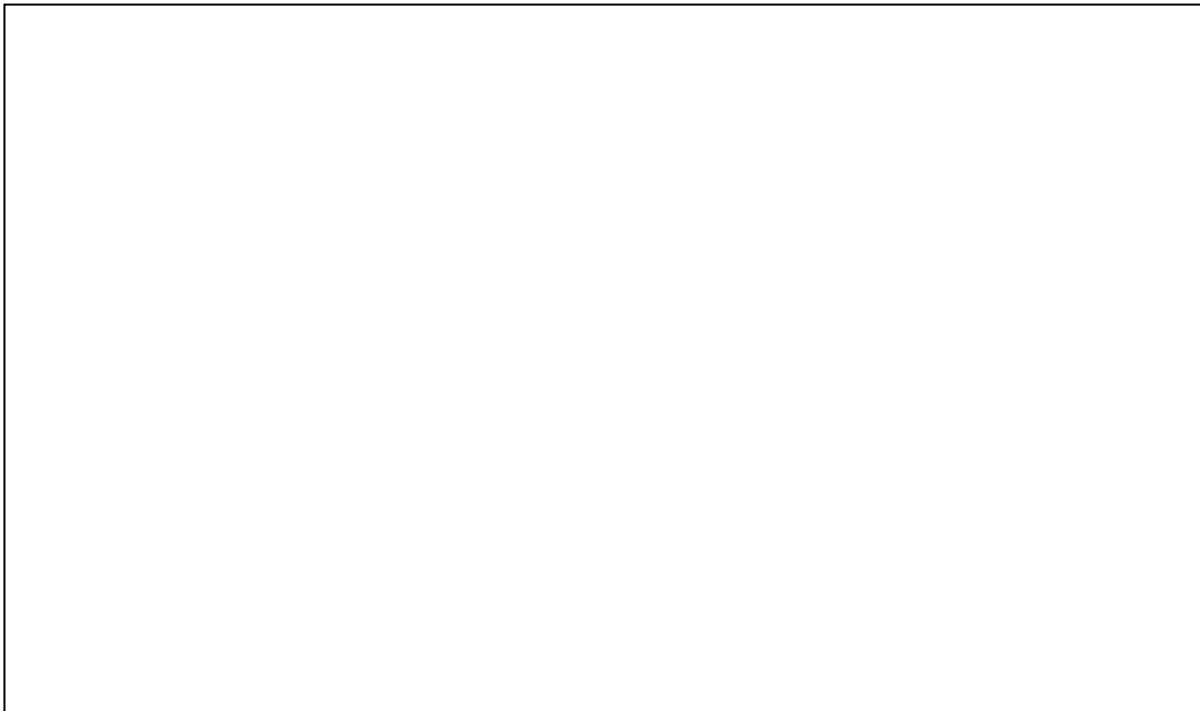
Calyx:
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Corolla:
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Androecium:
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Gynoecium:
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Floral formula:



Emasculation and crossing techniques:

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Tips

- Pollinate petunias early in the morning during cool and dry weather.
- As an alternate to removing the stamen, swipe a paint brush or the pollen from the parent flower and brush this pollen on the stigma of the receiving plant.

Experiment No. 7

Objective: To study floral biology, emasculation and pollination of China aster.

Material required:

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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

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:.....

Calyx:

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Corolla:

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Androecium:

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Gynoecium:

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Floral formula:

DISC FLORETS:.....

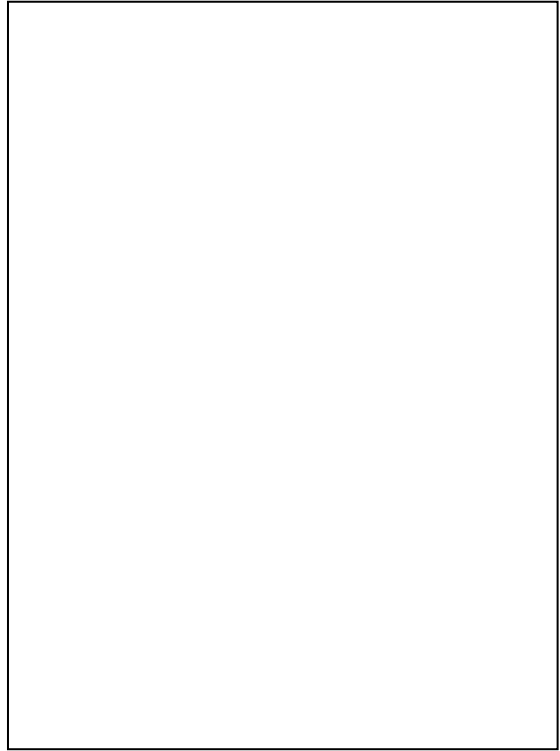
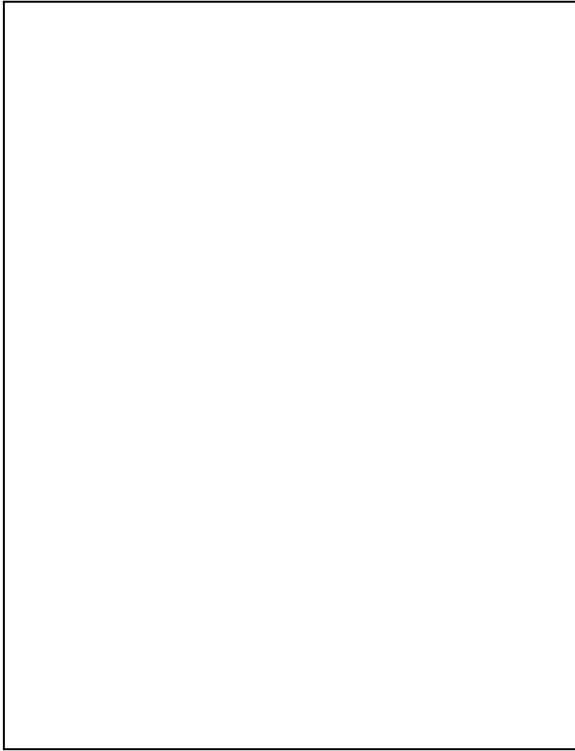
Calyx:

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Corolla:

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Androecium:

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Gynoecium:

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Floral formula:



Emasculation and crossing techniques:

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Objective: To study floral biology, emasculation and pollination of Pansy.

Materials Required:

Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

Calyx:

Corolla:

Androecium:

Gynoecium:

Floral formula:

Experiment No. 9

Objective: To study floral biology, emasculation and pollination of Sweet pea.

Materials Required:

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Family:

Botanical name:

Chromosome no.:

Floral biology and floral structure:

Inflorescence:

Flower:

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Calyx:

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Corolla:

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Androecium:

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Gynoecium:

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Floral formula:

Experiment No. 10

Objective: To study techniques of inducing polyploidy.

Introduction:.....
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Materials Required:
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Procedure:
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Colchicines treatment may be imposed on seeds, seedlings or growing shoot apex.

Seed treatment:
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Seedlings treatment:
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Shoot apex:
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Experiment No. 11

Objective: To study the effect of mutagens to induce mutation.

Introduction:.....
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Materials Required:

Procedure:

Observation:

Percentage of germination
Untreated: **Treated:**

Experiment No. 13

Objective: To study harvesting stages of seed of annuals.

Introduction:

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Stage of seed harvest of different annual flower crops

	Plant	Stages of collection
1	<i>Alyssum maritimum</i>	
2	<i>Antirrhinum majus</i>	
3	<i>Arctostoechadifolia</i>	
4	<i>Calendula officinalis</i>	
5	<i>Campanula</i> spp.	
6	<i>Celosia</i> spp.	
7	<i>Chrysanthemum coronarium</i>	
8	<i>Clarkia elegans</i>	
9	<i>Cosmos bipinnatus</i>	
10	<i>Dahlia variabilis</i>	

11	<i>Delphinium</i> sp.	
12	<i>Dianthus</i> sp.	
13	<i>Dimorphotheca</i> sp.	
14	<i>Gaillardia pulchella</i>	
15	<i>Gazania splendens</i>	
16	<i>Godetia grandiflora</i>	
17	<i>Gomphrena globosa</i>	
18	<i>Gypsophilia elegans</i>	
19	<i>Helianthus annuus</i>	
20	<i>Iberis amara</i>	
21	<i>Helichrysum bracteatum</i>	
22	<i>Impatiens balsamina</i>	
23	<i>Lathyrus odoratus</i>	
24	<i>Limonium sinuatum</i>	

25	<i>Linaria bipartita</i>	
26	<i>Linum grandiflora</i>	
27	<i>Lupinus hartwegii</i>	
28	<i>Mathiola incana</i>	
29	<i>Mesembryanthemum crinifolium</i>	
30	<i>Molucella laevis</i>	
31	<i>Papaver roeas</i>	
32	<i>Petunia hybrida</i>	
33	<i>Phlox drumondii</i>	
34	<i>Pimpinella monoica</i>	
35	<i>Portulaca grandiflora</i>	
36	<i>Rudbeckia bicolor</i>	
37	<i>Salvia splendens</i>	
38	<i>Tagetes sp.</i>	

39	<i>Tithonia speciosa</i>	
40	<i>Tropaeolum majus</i>	
41	<i>Viola wittrockiana</i>	
42	<i>Venidium fastuosum</i>	
43	<i>Zinnia elegans</i>	

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.

Experiment No. 14

Objective: To study seed germination test.

Materials Required:

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Procedure:

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The percent germination is calculated is given below:

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seed taken}} \times 100$$

Substratum:

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Germination counts:

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Experiment No. 15

Objective: To study Tetra-azolium or TZ test for seed viability.

Introduction:

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Usefulness:

1. This is generally conducted on the samples showing low germination to verify germination test results.
2. It determines viability of dormant seeds those would otherwise require months of chilling or other long duration pre-treatment to overcome dormancy.
3. It enables to evaluate the causes for low germination such as empty seeds, dormancy, dead or dying tissues mechanical injury, pre-sprouting and inadequate germination test procedures.

Material required:

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Procedure:

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The viability per cent can be calculated by the formula as mentioned below:

$$\text{Seed viability \%} = \frac{\text{No. of viable seeds (stained seeds)}}{\text{Total no. of seeds taken}} \times 100$$

Experiment No. 16

Objective: To study pollen viability test.

Introduction:

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Procedure:

1. Aceto-carmin test
2. Counting of germinated pollens in 25% sugar solution with 0.5% agar at 24-27°C.
3. Counting of pollen tubes after pollination of receptive stigma with fixed number of pollen grains.

Acetocarmine test:

Material required:

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Method:

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The pollen viability % at any given time after dehiscence of anthers workout as:

$$\text{Pollen viability \%} = \frac{\text{No. of stained pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

Observation:

Crop	Stained pollen (no.)	Unstained pollen (no.)	Pollen viability (%)

Experiment No. 17

Objective: 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:

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Spacing:

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Isolation Requirements:

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Planting Ratio:

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Seed Rate:

Rouging:

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Supplementary Pollination:

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Harvesting:

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

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3. Production of Hybrid marigold Seed:

Isolation Requirements:

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Planting Ratio:

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Seed Rate:

Harvesting:

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Objective: To study methods of seed storage.

Principle:
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Methods:

1. Storage at ambient temperature and humidity:
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2. Dry storage with control of moisture content but not temperature:
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3. Dry storage with control of both moisture content and temperature:
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4. Dry storage for long-term gene conservation:
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5. Moist storage without control of moisture content of temperature:
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FORAL BIOLOGY, EMASCULATION AND POLLINATION OF ROSE (*ROSA INDICA*).

Inflorescence: Solitary flowers.

Flower: Pedicellate, hermaphrodite, complete, actinomorphic, pentamerous, perigynous, white or red.

Calyx: Sepals 5, gamosepalous, green, persistent, narrowly lanceolate, odd sepal posterior, quincuncial aestivation.

Corolla: Petals 5, polypetalous, large, rosaceous, quincuncial aestivation.

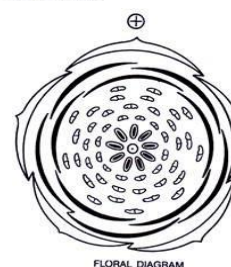
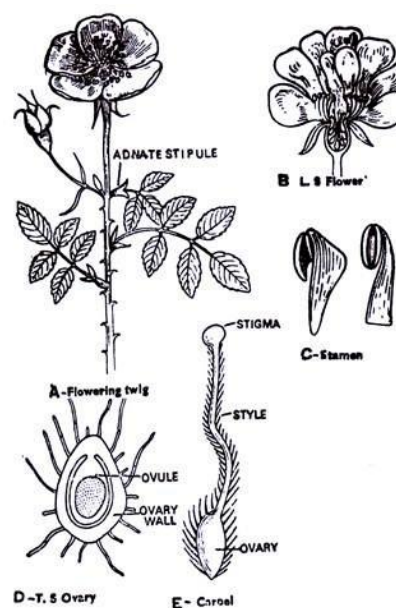
Androecium: Stamens indefinite, polyandrous, anthers yellow, versatile, filament slender, dithecous, introrse.

Gynoecium: Polycarpellary, apocarpous, ovaries enclosed in calyx tube, basal placentation, styles free, stigma terminal.

Floral formula: $\oplus \ominus \text{K}_{(5)} \text{C}_5 \text{ or } \infty \text{A}_{\infty} \text{G}_{\infty}$

Emasculation and crossing techniques:

- Anthers or stamens are removed before anthesis with the help of fine scissors or forceps.
- Selection of male parent. Petals are removed. Anthers at bursting stage are rubbed gently on the pistil of the flower. Pollen may be applied with the help of fingers or soft brush.
- After crossing, the flower is protected by covering with butter paper bag. The female flower is properly labeled, mentioning details about the crossing.
- Fruits (hips) are collected from successful crosses. On maturity, these become either brownish/yellow/orange/red or even remain green. Generally, three or four months are required for seed maturation.

**FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF GLADIOLUS (*GLADIOLUS* SPP.).**

Inflorescence: Cyme

Flower: Hermaphrodite, trimerous, regular, zygomorphic, epigynous, emerging from a spathe of 2 or more bracts

Perianth: Six, in two whorls, three outer ones (sepals) often petal-like all the six perianth leaves, gamophyllous.

Androecium: Stamens three, epiphyllous attached to the outer whorl of perianth lobes, inner whorl of three suppressed, anthers extrorse.

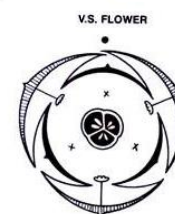
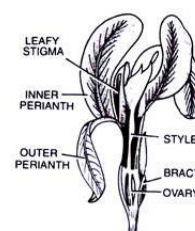
Gynoecium: Tricarpellary, syncarpous; ovary inferior, trilocular; axile placentation; style single; stigma three,

Fruit: capsule.

Floral formula: $\% \text{P}_{(3+3)} \text{A}_3 \overline{\text{G}}_{(3)}$

Emasculation and crossing techniques: In gladiolus, both male and female parts are born on the same floret. The stamens from the female parent are gently removed when the florets start opening. The process is called emasculation and is practiced to prevent inbreeding or self pollination. The emasculated florets are covered with butter paper bags.

Stigma become thick and the stigma surface becomes sticky, one or two days after floret opening. The hairs of stigma help to catch the pollens that fall on it. This is stage when stigma become receptive. The mature dehiscent anthers from the desired pollen are gently rubbed against lobes of stigma of the female parent with the help of forceps. The pollinated florets are labeled properly.



FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF TUBEROSE (*Rajanigandha, Polianthes Tuberosa*)

Family: Amaryllidaceae

Inflorescence: Cyme

Flower: Bracteate, pedicellate, hermaphrodite, actinomorphic, epigynous.

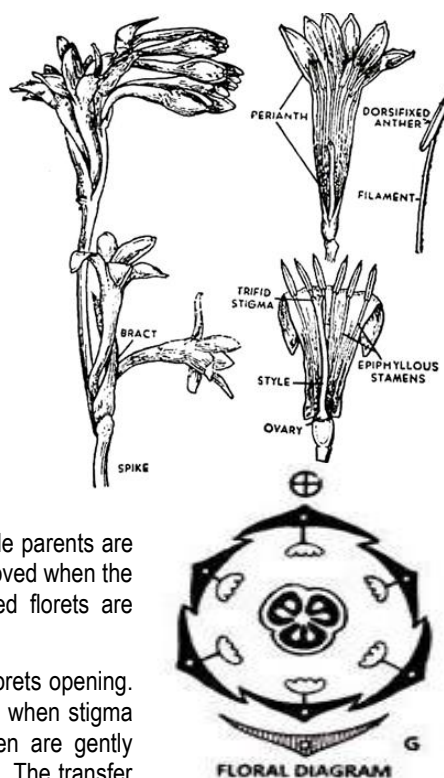
Perianth: 6 tepals in two whorls of 3 each gamophyllus, tubular, long tube, fragrant.

Androecium: Stamens 6, polyandrous, epiphyllous – as the tepals arranged in two whorls; anthers bitheous, introrse, filament distinct, sometimes connate basally by staminal tube.

Gynoecium: Tricarpellary, syncarpous, ovary inferior, trilocular, axile placentation with numerous ovules in each loculus; style slender, trifid.

Fruit: Loculicidal capsule

Floral formula: $\text{Br. } \oplus \text{ } \text{P}_{(3+3)} \text{A}_{3+3} \overline{\text{G}}_{(3)}$



Emasculation and crossing techniques: 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 dehiscent 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.

FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF HIBISCUS (*HIBISCUS ROSA-SINENSIS*)

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.

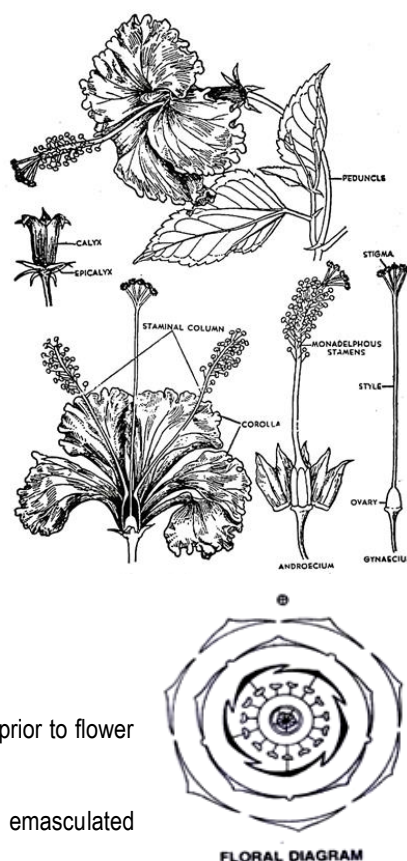
Gynoecium: Pentacarpellary, syncarpous, superior, pentalocular, axile placentation, many ovules in each loculus; style passing through the staminal tube; stigma 5 capitate.

Fruit: Capsule.

Floral formula: $\text{Epi}_{5-7} \text{K}_{(5)} \text{C}_5 \text{A}_{(\infty)} \overline{\text{G}}_{(5)}$

Emasculation and crossing techniques:

- Matured flower buds of the desired female parent are emasculated one day prior to flower opening and bagged with butter paper cover.
- The dehiscent pollen plant is also bagged.
- Pollen from male parent is smeared on the sticky stigmatic surface of the emasculated flower of the female parent, the next day.



- The crossed flower is bagged with butter cover to prevent natural cross pollination.
- The flower is properly labeled 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.

FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF MARIGOLD (*TAGETES* SPP).

Family: Asteraceae

Inflorescence: Capitulum surrounded by involucre.

Capitula large with two kinds of flowers:

- The peripheral flowers or ray florets, which are large, attractive and ligulate.
- 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: $Br \ \% \ Neuter \ or \ \text{♀} \ K \ pappus \ C_{(5)} \ A_0 \ \overline{G}_{(2)}$

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.

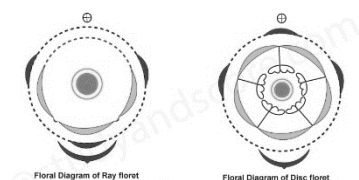
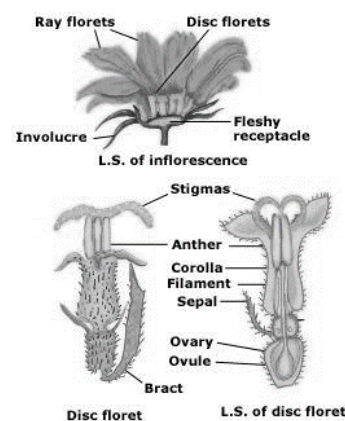
Gynoecium: Bicarpellary, syncarpous, unilocular, inferior, basal placentation, single basal ovule, style single long; stigma bifid.

Floral Formula: $Br \ \oplus \ \text{♀} \ K \ (pappus) \ C_{(5)} \ A_{(5)} \ \overline{G}_{(2)}$

Emasculation and crossing techniques: 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 prior 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.

Note: Floral biology, emasculation and pollination method of china aster (*Callistephus chinensis*) is almost same as Marigold (*Tagetes* sp).



FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF *PETUNIA*.

Inflorescence: Axillary or terminal condensed cymes.

Flower: Pedicellate, bracteate, hermaphrodite, complete, actinomorphic, pentamerous, hypogynous.

Calyx: Sepals 5, gamosepalous, campanulate, sepals free above and fused below, green, hairy, imbricate aestivation.

Corolla: Petals 5, gamopetalous, campanulate, tube hairy, twisted aestivation.

Androecium: Stamens 5, polyandrous, epipetalous, alternating with the petals, filaments long, anthers basifixed, dithecous, introrse.

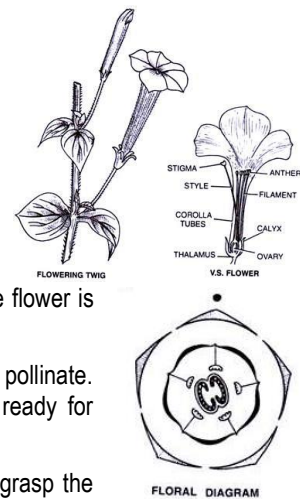
Gynoecium: Bicarpellary, syncarpous, ovary superior, obliquely placed, bilocular; swollen axile placentation; ovules many; style long, slightly, twisted, stigma capitate, bilobed.

Fruit: Capsule.

Floral formula: $Br \oplus \text{♀} K_{(5)} \overset{\curvearrowright}{C}_{(5)} A_5 \underline{G}_{(2)}$

Emasculation and crossing techniques:

- Select a healthy, freshly bloomed petunia plant. Lightly swipe our finger nail over the pollen on the anthers inside one of the plant's flowers-if the pollen sticks to our nail, the flower is ready to pollinate other flowers.
- Look for the stigma that's sticking out in the centre of a petunia flower that we want to pollinate. Lightly touch it with our finger. If it is glistening and sticky to the touch, the flowers ready for pollinate.
- Hold on to the pollen parent flower with our non-dominant hand. Use the tweezers to grasp the base of the filament inside the flower. The filament is the thin stem that holds up the anther with pollen. Pull upward to remove the filament from the flower with the anther and pollen intact.
- Rub the anther over the top of the stigma of the receiving petunia flower so the pollen adheres to it. Pollinate as many as we like in this manner.



FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF PANSY (*VIOLA TRICOLOR*)

Family: Violaceae

Inflorescence: Solitary axillary.

Flower: Bracteate, two bracteoles, pedicellate, complete, hypogynous, hermaphrodite, zygomorphic, colour variable.

Calyx: 5 sepals, free, quincuncial, green, inferior

Corolla: 5 petals, polypetalous, imbricate, anterior petal produced into a spur, in which the honey produced by the spurs of the connectives of two anterior stamens is collected.

Androecium: Stamens 5, connate, forming a ring around the ovary, filaments small.

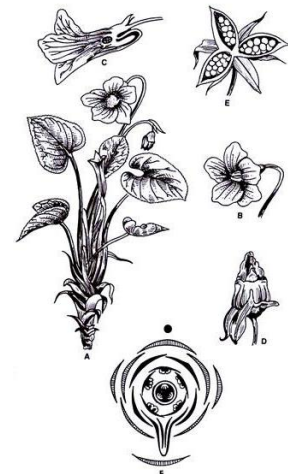
Gynoecium: Carpels 3, syncarpous; ovary superior, unilocular with numerous ovules attached to parietal placentae; style short, filiform; stigma swollen.

Floral formula: $Br \% \text{♀} K_5 C_5 A_{(5)} \underline{G}_{(3)}$

Floral formula:

Emasculation and crossing techniques: Emasculation is done one day ahead or at the time of opening of the flowers. Bud to open in an inflorescence is emasculated. The most advanced bud in the inflorescence can be identified. The buds selected is opened and the stamens are removed with fine pointed forceps. The emasculated flowers are covered with small butter paper bags to protect out crossing.

Pollination is made following morning upto 9.30 - 11AM stigma of the emasculated flower is lightly touched with a ripe anther and the pollen is dusted on the stigma. After pollination, the flowers are bagged.



FLORAL BIOLOGY, EMASCULATION AND POLLINATION OF SWEET PEA (*LATHYRUS ODORATUS*)

Family: Fabaceae

Inflorescence: solitary axillary.

Flower: Bracteate, pedicellate, complete, hermaphrodite, zygomorphic pentamerous, hypogynous.

Calyx: Sepals 5, gamosepalous, pentapartite, companulate, odd sepal anterior, imbricate aestivation, green, hairy.

Corolla: Petals 5, polypetalous, papilionaceous, consisting of a large posterior petal – the vexillum or standard, two lateral-alae or wings and two inner fused to form a boat shaped structure the keel or carnia, vexillary aestivation.

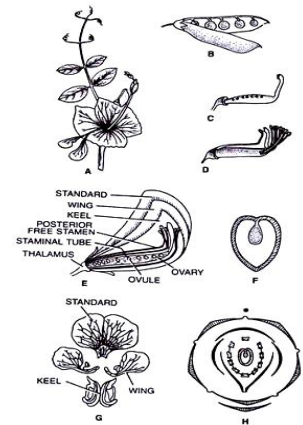
Androecium: Stamens 10, diadelphous, nine are fused by the lower halves of their filaments to form a tube round the ovary and tenth posterior one free, anthers basifixed, introrse, dithealous, enclosed in the keel.

Gynoecium: Monocarpellary, ovary superior, unilocular, hairy, elongated, laterally compressed, marginal placentation ovules many, style long, stigma hairy.

Fruit: Legume.

Floral $Br \ % \ \overset{\sigma}{\text{K}} \ \underset{\text{♀}}{\text{C}} \ (5) \ C_{1+2+(2)} \ A_{(9)+1} \ G_1$ **formula:**

Emasculation and crossing techniques: For emasculation, the flower bud chosen should have developed to the stage just before anther dehiscence, indicated by extension of petals beyond sepals. Flowers can be emasculated any time. The first step is emasculation is to tear away with the forceps the tip of the sepal from in front of the keel. The fore finger is positioned behind the flower and thumb in front and light pressure is applied. This spread the standard and wings to expose the keel. The exposed keel is slight open by tips of forceps. Pressure can be applied by the thumb and finger on keel for increased exposure of the pistil and stamens. The 10 stamens are pulled out.



Pollen can be obtained throughout the day, preferably from a freshly opened flower. For pollen collection, it is more convenient to pick the male flowers, remove the standard and wings, pull back the keel so that the style protrudes and use the pollen covered styler brush as an applicator to transfer the pollen to the stigma of the emasculated bud. Older flowers and other flower bud not used in crossing and removed the peduncle to increase the pod set after crossing.

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.