CROP IMPROVEMENT-II (*RABI* CROPS)
(AGP-313) 2(1+1)

PRACTICAL MANUAL

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2020

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Syllabus Crop Improvement – II Rabi Crops AGP-313 2(1+1):

Floral biology, emasculation and hybridization techniques in different crop species namely Wheat, Oat, Barley, Chickpea, Lentil, Field pea, Rajma, Horse gram, Rapeseed Mustard, Sunflower, Safflower, Potato, Berseem. Sugarcane, Tomato, Chilli, Onion; Handling of germplasm and segregating populations by different methods like pedigree, bulk and single seed decent methods; Study of field techniques for seed production and hybrid seeds production in *Rabi* crops; Estimation of heterosis, inbreeding depression and heritability; Layout of field experiments; Study of quality characters, study of donor parents for different characters; Visit to seed production plots; Visit to AICRP plots of different field crops.

Name of Student .....

Roll No. .....

Batch .....

Session .....

Semester .....

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Date: Course Teacher

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Exercise No. 1

Objective: To study floral biology, emasculation and pollination techniques in wheat

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in wheat:

Procedure: ……

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Exercise No. 2

Objective: To study floral biology, emasculation and pollination techniques in oat

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in oat:

Procedure: ……

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Exercise No. 3:

Objective: To study floral biology, emasculation and pollination techniques in barley

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in barley:

Procedure: ……

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Exercise No. 4

Objective: To study floral biology, floral structure, emasculation and pollination techniques in chickpea

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in chickpea:

Procedure: ……

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Exercise No. 5

Objective: To study floral biology, floral structure, emasculation and pollination techniques in lentil

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in lentil:

Procedure: ……

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Exercise No. 6

Objective: To study floral biology, floral structure, emasculation and pollination techniques in field pea

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in field pea:

Procedure: ……

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Exercise No. 7

Objective: To study floral biology, floral structure, emasculation and pollination techniques in rajma

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in rajma:

Procedure: ……

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Exercise No. 8

Objective: To study floral biology, floral structure, emasculation and pollination techniques in horse gram

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in horse gram:

Procedure: ……

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Exercise No. 9

Objective: To study floral biology, emasculation and pollination techniques in rapeseed-mustard

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in rapeseed-mustard:

Procedure: ……

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Exercise No. 10

Objective: To study floral biology, emasculation and pollination techniques in sunflower

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in sunflower:

Procedure: ……

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Exercise No. 11

Objective: To study floral biology, emasculation and pollination techniques safflower

Materials required: ……..……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in safflower:

Procedure: ……..……

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Exercise No. 12

Objective: To study floral biology, emasculation and pollination techniques in potato

Materials required: …………………………………………………………………………………………...……

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Botanical name: …………………………….. **Chromosome no.: =**……

Family: ………………………………………

Floral biology and floral structure:

Emasculation and Pollination in potato:

Procedure: ……………………………………………………………………………………………...…………

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Exercise No. 13

Objective: To study floral biology, floral structure, emasculation and pollination techniques in berseem

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in berseem:

Procedure: ……

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Exercise No. 14

Objective: To study floral biology, floral structure, emasculation and pollination techniques in sugarcane

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in sugarcane:

Procedure: ……

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Exercise No. 15

Objective: To study floral biology, floral structure, emasculation and pollination techniques in tomato

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in tomato:

Procedure: ……

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Exercise No. 16

Objective: To study floral biology, floral structure, emasculation and pollination techniques in chilli

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in chilli:

Procedure: ……

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Exercise No. 17

Objective: To study floral biology, floral structure, emasculation and pollination techniques in onion

Materials required: ……

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Botanical name: …….. **Chromosome no.: =**…….

Family: ……

Floral biology and floral structure:

Emasculation and Pollination in onion:

Procedure: ……

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Exercise No. 18

Objective: Handling of segregating generations and maintenance of pedigree record

Materials required: .....

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Records:

Experiment details

1. Name of the experiment: .....
2. Season: .....
3. Crop: .....
4. Design of the experiment: .....
5. Replication: .....
6. Size of the plot: .....
7. Spacing: .....
8. Date of sowing/planting: .....
9. Date of harvest: .....
10. Comments: .....

Data recording in field notebook for a given crop.

Pedigree .....

Data on various characters of .....

S. No.	Strain	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	Ch7	Ch8	Csh9	Ch10
1											
2											
3											
4											
5											
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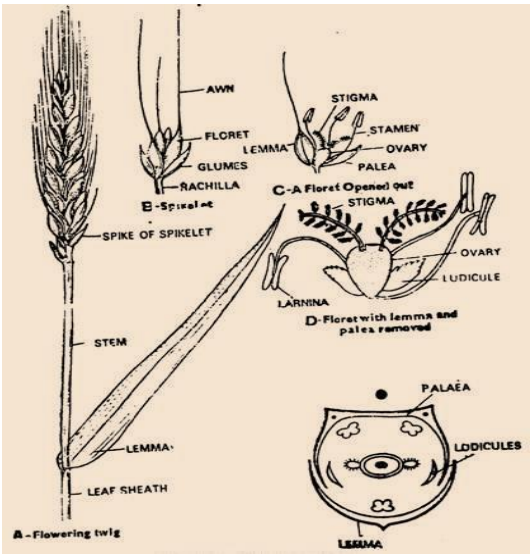
WHEAT

Botanical name: *Triticum aestivum* L.
Chromosome number: 2n = 42
Family: Gramineae
Inflorescence: Spike of spikelets
Flower: Bracteate, sessile, hermaphrodite, zygomorphic, incomplete
Perianth: 2 membranous scales – the lodicules
Androecium: Stamens (3), polyandrous, filament long, anthers dorsifixed when young and versatile when mature.
Gynoecium: Monocarpellary, theoretically tricarpellary, ovary superior, unilocular, single ovule, basal placentation, style short; stigma (2), feathery
Floral formula: $P_{2\ (lodicules)}$, A_3 $G_{\underline{1}}$
Emasculation & crossing technique: The spike enclosed in leaf sheath or partially emerged is selected for emasculation. The awns and tips of spikelets are cut off to avoid obstacle in the process of emasculation and pollination, similarly the central sterile flower is also removed with the forceps. The requisite numbers of spikelet are kept on the spike and with the help of forceps the glumes are separated, and three young immature greenish yellow anthers are removed from each flower and the spike is bagged. On the next morning between 9.00 to 11.00 a.m. the pollen grain is collected from the desired plant in Petri dish and dusted on stigma of emasculated flower with the help of hair-brush. The spike is covered with bag after pollination and labeled.



OAT

Botanical name: *Avena sativa* L. **Chromosome number:** 2n = 14
Family: Poaceae
Inflorescence: Spike of spikelets
Flower: Bracteate
Perianth: Represented by two-minute scary lodicules borne on a short axis within the two paleae
Androecium: Stamens (3), polyandrous
Gynoecium: Monocarpellary, Ovary superior with single basal ovule, Placentation: basal, Style: short, Stigma: two, feathery
Floral Formula: $P_{2\ (lodicules)}$, A_3 $G_{\underline{1}}$
Emasculation & crossing technique: Emasculation is done by removing the anthers with forceps. It is done preferably in the morning.

1. Select the spike for emasculation which is still enclosed by the flag leaf sheath.
2. Open the flag leaf sheath by forcing the leaf edges with the help of forceps.
3. Cut the sheath and flag leaf at the level of the first node of the rachis.
4. Remove lateral florets and tip of the spike.
5. Remove all the anthers with the help of forceps from all the florets.
6. All the florets on the spike must be emasculated to prevent self-fertilization.
7. Bag the emasculated spikes to prevent contamination from foreign pollen.

Pollen remains viable from early morning to mid-morning. Stigma is receptive at all the time of day.

1. For pollination, spike is selected in which the anthers have begun to dehisce.
2. Cut the top one third of the spikelet to expose the anthers.
3. When pollen become visible, dust the spike on emasculated one

BARLEY

Botanical name: *Hordeum vulgare* **Chromosome number:** 2n = 14
Family: Poaceae
Inflorescence: Spike of spikelets
Spikelet: Two long narrow bracts, almost parallel to each other at the base of the spikelet. These bracts may be considered as glumes. Behind these glumes the solitary flower is inserted, enveloped in lemma and palea.
Flower: Sessile, zygomorphic, irregular, hermaphrodite, incomplete

Perianth: The perianth is represented by two small membranous lodicules, arranged opposite the palea.
Androecium: Three stamens; filaments long, slender, free
Gynoecium: Carpel one (monocarpellary); ovary superior, unilocular, single basal ovule, two feathery stigmas
Fruit: A caryopsis with pointed ends, usually invested by lemma and palea.
Floral Formula: $P_{2\ (lodicules)}$, A_3 $\underline{G_1}$
Emasculation & crossing technique:

1. Choose the female parent and clip off the spike near the tip of the last spikelet. Then remove the flag leaf and the upper part of the awns.
2. Expose the female spike by unrolling the flag leaf sheath.
3. Clip individual spikelets just above the anthers.
4. Use fine-pointed tweezers/forceps to remove the anthers (3) from each spikelet.
5. Clipping of the spike is complete when the awn and the upper half of each spikelet are removed.

Pollination:

1. For pollination, male parent is selected in which the anthers are pale yellow and are still at the base of each spikelet that has not shed pollen.
2. Clip the spikelets.
3. One to five minutes after clipping the spikelets of the male, the anthers start to enlarge (puff up). Shortly thereafter, the filaments start elongating and force the anthers upward.
4. When the anthers start to enlarge, they will shed pollen and can be used for crossing.
5. Remove one anther from the male spikelet for pollination.
6. Place the anther(s) above the open female spikelet (flower) and break the anther.

CHICKPEA

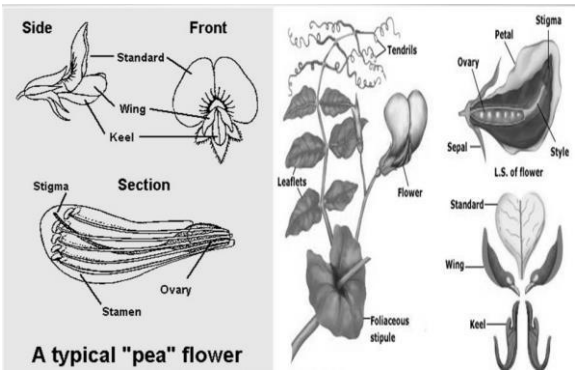
Botanical name: *Cicer arietinum* L. **Chromosome number:** 2n = 16
Family: Fabaceae
Inflorescence: The solitary flowers are borne in an axillary raceme.
Flower: Chickpea flowers are complete and bisexual.
Calyx: Five sepals
Corolla: Chickpea flowers have five petals which are generally purplish red or light pink color. The petals are polypetalous i.e., consisting of standard, wings and keel.
Androecium: There are 10 stamens in diadelphous (9) +1 condition.
Gynoecium: The ovary is monocarpellary. It is ovate with a pubescent (glandular hairs predominate) surface. The ovary is 2-3 mm long and 1-15 mm wide.
Floral Formula: $K_{(5)}$ C_{2+2+1} $A_{(9+1)}$ $\underline{G_1}$
Emasculation & crossing technique: Buds should be selected one or two days before anthesis. a. Remove frontal sepal b. Open keel c. Remove anther d. Place the desired pollen. The time of active blooming is 9:00 to 10:00 a.m. Half opened flowers are selected in male flower for pollen collection.

LENTIL

Botanical name: *Lens culinaris* Medic. **Chromosome number:** 2n = 14
Family: Fabaceae
Inflorescence: The solitary flowers are borne in an axillary raceme.
Flower: Flowers are small, pale blue, purple, white or pink, in axillary 1-4-flowered racemes; 1-4 flowers are borne on a single peduncle and a single plant can produce upto 10-150 peduncles each being 2.5-5 cm long. Flowering proceeds acropetally.
Calyx: Five sepals
Corolla: Flowers have five petals. The petals are polypetalous i.e., consisting of standard, wings and keel.
Androecium: There are 10 stamens in diadelphous (9) +1 condition. The filaments of nine of the stamens are fused, forming an androecial sheath; the tenth stamen is free.
Gynoecium: The ovary is monocarpellary.
Floral Formula: $K_{(5)}$ C_{2+2+1} $A_{(9+1)}$ $\underline{G_1}$
Emasculation & crossing technique: Buds should be selected one or two days before anthesis. a. Remove frontal sepal b. Open keel c. Remove anther d. Place the desired pollen. The time of active blooming is 9:00 to 10:00 a.m. Half opened flowers are selected in male flower for pollen collection.

FIELD PEA

Botanical name: *Pisum sativum* L. **Chromosome number:** 2n = 14
Family: Fabaceae
Inflorescence: Axillary raceme
Flower: Flowers are borne on axillary racemes of 1-3 flowers, each 1.5-3.5 cm long.
Calyx: Sepal (5), gamosepalous, campanulate, calyx lobes un-equal, aestivation imbricate.
Corolla: Petal (5), one standard, two wing and two keel, aestivation vexillary.
Androecium: Stamen (10), diadelphous (9+1).
Gynoecium: Carpel one, ovary superior, placentation marginal.
Floral Formula: $K_{(5)}\ C_{1+2+(2)}\ A_{(9)+1}\ \underline{G}_1$
Emasculation & crossing technique: Flowers can be emasculated at any time. The first step in emasculation is to tear away with the forceps the tip of the sepal in front of the keel. Position the forefinger behind the flower and thumb in front and apply a light pressure. This will spread the standard and wings to expose the keel. Open the exposed keel by tips of forceps. Pressure can be applied by the thumb and finger on keel for increased exposure of the pistil and stamens. The ten stamens are pulled out with the help of forceps.



Pollination: Pollen can be obtained throughout the day, preferably from a freshly opened flower. For pollen collection, the standard and wings are removed from the male flowers and the keel is pulled back so that the style protrudes. The brush is used as an applicator to transfer the pollen to the stigma of the emasculated bud. To increase the pod set after crossing, older flowers and other flower buds which are not used in crossing are removed.

RAJMA

Botanical name: *Phaseolus vulgaris* L.**Chromosome number:** 2n = 22
Family: Fabaceae
Inflorescence: Axillary raceme bearing few flowers (2-3)
Flower: Flower is zygomorphic, bisexual, perigynous and white in colour.
Calyx: Sepals (5), Calyx-bracts nearly equalling or exceeding calyx tube. Calyx is green in colour, tubular, hairy and gamosepalous.
Corolla: Corolla is white in colour, typically papilionaceous with 5 petals being descending imbricate, the posterior petal is outermost (standard or vaxillum), the two lateral petals with long claw (wings) and the two anterior united petals (keel). The keel is somewhat coiled.
Androecium: Stamens are enclosed within the keel, diadelphous (9) +1, the posterior stamen is free and filaments of the rest nine are fused to form a tube-like structure.
Gynoecium: Gynoecium is comprised of one carpel. The ovary is superior, elongated, green in colour and unilocular. Ovules numerous and placentation is marginal.
Floral Formula: $K_{(5)}\ C_{1+2+(2)}\ A_{(9)+1}\ \underline{G}_1$
Emasculation & crossing technique: For emasculation an unopened flower bud is selected. The standard is detached from below with a pair of forceps and bent backward. Open the exposed keel by tips of forceps. Care should be taken to turn in the same direction as the spiral winding, otherwise the style may break. After pulling off keel, the stamens are removed. Freshly opened flowers are collected for pollen. The thickly pollinated stigmas emerge as soon as the wings are pressed downward. This stigma of male flower is rubbed against the stigma of the emasculated bud.

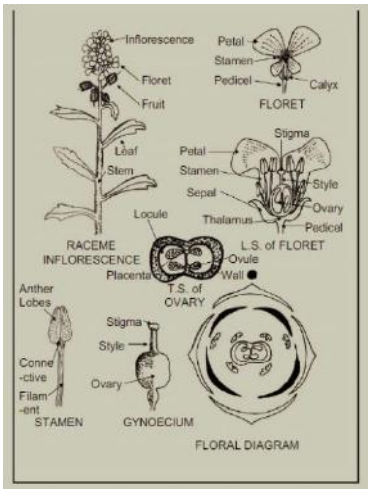
HORSE GRAM

Botanical name: *Macrotyloma uniflorum* L. **Chromosome number:** 2n = 20, 22, 24
Family: Fabaceae
Inflorescence: Axillary raceme
Flower: Flowers bisexual, papilionaceous; calyx: pubescent, lobes: triangular-lanceolate, upper pair entirely fused corolla with cream, yellow or greenish yellow standard, often with a small purple blotch inside, obovate-oblong, wings and keel greenish yellow, stamens 10 (9 fused and 1 free), ovary superior.
Floral Formula: $K_{(5)}\ C_{1+2+(2)}\ A_{(9)+1}\ \underline{G}_1$

RAPESEED-MUSTARD

Botanical name: *Brassica* species **Family:** Brassicaceae
Floral biology: Flowers of both the species have 4 sepals and 4 petals of deep yellow to pale yellow colour. Four flower has 6 stamens; 4 with long and 2 with short filaments. The pistil is compound and the ovary mature into 2 celled fruits.
Flowers: Regular and cruciform, bisexual and complete, hypogynous, ebracteate.
Calyx: Sepals (2 + 2), free, in two whorls.
Corolla: Petals (4), free, in one whorl, valvate, cruciform with distinct limb and claw, imbricate.
Androecium: Stamens (6), in two whorls-2 outer short and 4 inner long, tetradynamous.
Gynoecium: Carpels 2, syncarpous, ovary superior.
Inflorescence: Long racemose
Floral formula: $K_{2+2}\, C_4\, A_{2+4}\, \underline{G}_2$
Emasculation and pollination technique:

1. Select the flower buds which are expected to open next morning.
2. Remove the remaining floral buds, which are either very small or opened.
3. Remove the petals as well as stamens with the help of pointed forceps.
4. Bag the inflorescence after pollination.
5. Pollination is done in the morning hours by dusting pollens of the desired male parent over the stigmatic surface of the emasculated bud.
6. Cover the flowers after pollination to avoid natural crossing by bees.



SUNFLOWER

Botanical name: *Helianthus annus* L. **Chromosome number:** 2n = 34
Family: Compositeae
Floral biology: Sunflower is highly cross-pollinated crop. The flower opening in sunflower starts from outer side of the head and proceed towards the center of the head bloom within 5 to 10 days, depending upon size and season. Anthesis occurs from 5:00 to 8:00 a.m. The pollen remains viable for 12 hours and stigma remains receptive for 2 to 3 days.

SAFFLOWER

Botanical name: *Carthemus tinctorius* **Chromosome number**: 2n = 24
Family: Compositae
Floral Biology: Inflorescence is called as capitulum/head. It consists of 20 to 100 florets collected closely together on a circular receptacle. Stigma is well covered with florets own pollen ensuring self-pollination.
Emasculation and pollination technique: On main branch flower blooms earlier than on secondary branches. Within a capitulum blooming begins in the outer circle of floret and progresses centripetally. Flower should be emasculated before anthesis i.e. stamens are removed without damaging stigma. In the next morning freshly collected pollens are dusted and head is covered with paper cover.

POTATO

Botanical name: *Solanum tuberosum* **Chromosome number:** 2n = 48
Family: Solanaceae
The flowers are bisexual, consist of four basic whorls (calyx, corolla, stamens and pistil). The calyx consists of five sepals that are partly joined at their base forming a bell-shaped structure below the corolla. The corolla consists of five petals which are joined at the base by a short corolla tube, each lobe ends in a triangular point. The androecium consists of five stamens that are alternate with the petals. Anthers are generally fused in a conical column enclosing the pistils. The gynoecium of the flower consists of a single pistil which is composed of the ovary, style and stigma. The ovary is superior, that is the sepal, petal and stamens are attached to the receptacle just below the ovary. The inflorescence of potato is cymose.

BERSEEM

Botanical name: *Trifolium alexandrinum* L. **Chromosome number:** 2n = 2x = 16
Family: Fabaceae
Inflorescence: Axillary raceme
Flower: In berseem white coloured flowers are produced in cluster which are hermaphrodite in nature with five fused sepals and five free petals. The stamens are always ten in number and their filaments are fused in a group of (9) +1.
Floral Formula: $K_{(5)}\, C_{1+2+(2)}\, A_{(9)+1}\, \underline{G}_1$

SUGARCANE

Botanical name: *Saccharum* sp. **Chromosome number:** 2n = 40-128
Family: Poaceae
Floral biology: Cross pollination is the rule in sugarcane. Maximum anthesis takes place between 6:00 a.m. and 8:00 a.m. Stigma protrudes out first and anthers dehisce afterwards. Flower opening is from top to downwards. Selfing is done by covering the arrow with a bamboo framework or cage which is covered with muslin cloth or polythene paper. Such a cover is commonly called lantern. It prevents accidental cross pollination. The lantern must be supported by bamboo poles. The lantern must be opened once in a day to reduce the temperature that might build up inside during the daytime. This is done preferably during afternoon i.e. between 12:00 p.m. and 4:00 p.m.

TOMATO

Botanical name: *Solanum lycopersicon* **Chromosome number:** 2n = 24
Family: Solanaceae
Newly formed, unopened flower buds which are expected to open within the next 1–2 days should be selected. Emasculate the flower by removing the anthers from the flowers using either fingers or forceps. Anthers are removed as a group with or without the surrounding corolla, by inserting forceps between the sepals to grip the base of the anthers and / or petals which are then removed by a firm but steady pull. Select open, dark yellow flowers from the male plant for pollen. Collect the pollen by using a dissecting needle or scalpel to cut a slit in the anther cone and using the tip of the needle or scalpel to remove the pollen. Pollination is performed 24 to 72 hours after emasculation, usually early in the morning. The pollen is applied by dipping the exposed stigma into the pollen or with a small paint brush or a dissecting needle. Pollinated flowers are wrapped with cotton or small pollination bags for preventing any contamination. Pollen from a single flower from the male plant can pollinate 12–18 flowers.

CHILLI

Botanical name: *Capsicum annum* L. **Chromosome number:** 2n = 24
Family: Solanaceae
Flowers are emasculated at bud stage. Pollen is transferred to the stigma either from mature undehisced anthers by scooping it out through the lateral sutures with the needle or by touching a freshly dehisced anther to the stigma with the help of forceps. Hands and tools (a pair of sharp-pointed forceps, a needle, a pair of scissors) are washed with 95% ethyl alcohol. Pollinated flowers are identified by loosely tying coloured thread around the delicate pedicels, preferably enclosing a leaf petiole for protection. Pollinated flowers are protected from bees by a double layer of cheese cloth, loosely wrapped around the branch, enclosing leaves and flowers, and securely fastened. Pollinated flowers are periodically checked, and the cheese clothes are removed in 4-6 days. Fruits normally mature in about 45 days.

ONION

Botanical name: *Allium cepa* **Chromosome number:** 2n = 16
Family: Liliaceae
Inflorescence: The terminal inflorescence develops from the ring-like apical meristem.
Flower: Bracteate, 2-3 membranous spathe like bracts enclosing the flower during young stage, actinomorphic, trimerous, hypogynous, small and white.
Androecium: Six stamens in two whorls of three each.
Gynoecium: Tricarpellary, syncarpous. Ovary – superior, trilocular with 2 ovules in each locules**.** Anther dehisce between 07:00 a.m. and 05:00 p.m. Pollen fertility is highest on the days of anthesis. Stigma receptivity is also high on the day of anthesis. The duration of anthesis is approximately 4 weeks on individual umbel. The anthesis begins from outer flowers and goes centrally in succession. The flower is protandrous in nature and stigma becomes receptive when shedding of pollen is over.

HANDLING OF SEGREGATING GENERATIONS

The method of planting F_2 depends upon the procedure of handling this generation and the following generations. In case of pedigree method, F_2 seeds are grown in widely spaced rows with an appropriate distance from plant to plant, so that each F_2 plant can be evaluated and selected or rejected. In bulk method, F_2 is planted just like a commercial crop and no selection is practiced. Parents of the cross as well as standard check varieties, upon which improvement is sought are also planted along with F_2 to aid in selection of desirable plants of different crosses.

In the breeding material and trials comprising germplasm, strains, varieties and segregating populations of various crosses, data are recorded on the qualitative and quantitative characters.

Data recorded on these traits are used for making comparisons among different experimental strains in various trials and are also used in inheritance studies. A record is maintained of a complete history or pedigree of the various strains from the time of their introduction into a breeding programme up to maturity. Accurate record keeping enhances the efficiency of a breeder and way for effective selection.