

Laboratory Practical Manual of CROP IMPROVEMENT-I

Course Code: ASPG3104

Credits: 2(1+1)



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Exercise No. 1

EMASCULATION AND HYBRIDIZATION TECHNIQUES

Selfing and crossing are the essential procedures in crop improvement process which depends on the floral structure and pollination.

I. Selfing

In the selfing of cross-pollinated species, it is essential that the flowers are bagged or protected to prevent natural cross-pollination before flower opening and the breeder should acquaint him self with the flowering habit of the crop. In the case of self pollinated crops such as wheat, rice, barley, groundnut etc., the plant is permitted to have selfpollination and the seeds are harvested. If the extent of natural cross pollination is more, then the flowers should be protected by bagging. Cages are also used to prevent the insect pollination in certain legumes.

II. Emasculation

Removal of stamens or anthers or killing pollen of a flower without disturbing the female reproductive organ is known as emasculation. In bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious plants, male flowers are removed. (castor, coconut) or male inflorescence is removed (maize). In species with large flowers e.g. (bhindi, cotton, pulses) hand emasculation is accurate and it is adequate.

1. Hand Emasculation- In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed using forceps. Sometimes corolla maybe totally removed along with epipetalous stamens e.g. sesame. In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. Gynoecium should not be injured. An efficient emasculation technique should prevent self pollination and produce high percentage of seed set on cross pollination.

2. Suction Method- It is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck only the anthers from the flowers. Considerable self-pollination (upto 10%) is likely to occur here. Washing the stigma with a jet of water may help in reducing self-pollination, However self pollination cannot be eliminated in this method.

3. Hot Water Treatment- Pollen grains are more sensitive than female reproductive organs to both genetic and environmental factors. In case of hot water emasculation, the temperature of water and duration of treatment vary from crop to crop. It is determined for every species. For rice, 10 minutes treatments with 40-44°C is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

4. Alcohol Treatment-It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water.

5. Genetic Emasculation-Genetic/ cytoplasmic male sterility may be used to eliminate the process of emasculation. This is useful in the commercial production of hybrids in maize, sorghum pearl millet, onion, cotton, and rice, etc. In many species of self-incompatible cases, also emasculation is not necessary, because self-fertilization will not take place. Protogyny will also facilitate crossing without emasculation.

6. Use of Gametocide (Chemical hybridizing agents, CHA)-These are chemicals which selectively kills the male gamete without affecting the female gamete. eg. Ethrel, Sodium methyl arsenate, Zinc methyl arsenate in rice, Maleic hydrazide for cotton and wheat.

III. Bagging

Immediately after emasculation the flower or inflorescence are enclosed with suitable bags of appropriate size to prevent random cross-pollination.

IV. Crossing

The pollen grains collected from a desired male parent is transferred to the emasculated flower. This is normally done in the morning hours during anthesis. The flowers are bagged immediately after artificial crossing.

V. Tagging

The flowers are tagged just after bagging. They are attached to the inflorescence or to the flower with the help of a thread. The following is recorded on the tag with pencil.

1. Date of emasculation:
2. Date of pollination
3. Parentage:
4. No. of flowers emasculated:

Exercise No. 2

MAINTENANCE BREEDING & STUDY OF FIELD TECHNIQUES FOR DIFFERENT KHARIF CROPS

The ultimate aim of any plant breeding programme is to develop varieties superior to the existing ones in yielding ability and other desirable aspects. The strains thus developed have to be valuated with best existing variety (check variety) in yield trials. First evaluation is conducted at the originating station for 2-3years. Later evaluations are done at different research stations located in various agro climatic conditions as multi location trials. All these trials have to be laid out in appropriate experimental designs.

Planning of the experiment to obtain appropriate data with respect to any problem under investigation is known as Design of the experiment'. Objects of comparison which an experimenter has to try out in the field for assessing their values are known as treatments *eg.* varieties, manures, cultivation practices, cattle feeds, methods of seed treatment etc. Experimental material is the material on which the experiment is performed, *eg.*, a field, a herd of cows, seeds etc. Experimental material is divided into a number of ultimate units known as experimental units to which the treatments are applied, *eg.*, a plot of land, a cow, a batch of seeds etc.

Observations recorded in any experiment vary considerably. Variations include known variations' and unknown variations'. Variations due to treatments constitute known variations. Variations in the experimental material such as differences in soil fertility or variations in environmental conditions constitute unknown variations, also known as experimental error' Experimental error maybe either systematic or known in their mode of incidence. There are three basic principles of experimental design/field experimentation *viz.*, randomization, replication and local control which are devices to avoid systematic error and to control random error.

- a) Replication: repetition of treatments
- b) Randomization: unbiased allocation of treatments to the experimental units.
- c) Local control: minimizing the effect of heterogeneity of the experimental units.

Experimental designs:

1. Completely Randomized Design (CRD)
2. Randomized Block Design (RBD)- widely used design
- 3) Latin Square Design (LSD)

Lay Out of RBD

The experimental material(field) is first divided into blocks consisting of homogeneous(uniform) experimental units. Each block is divided into number of plots equal to the total number of treatments. Randomization is done within each block and the treatments are applied following the random number table.

Collection and analysis of data:

After the collection of data from the individual experimental units(treatments), tabulation and analysis is done. ANOVA (Analysis of Variance) table is formed . The significance of ANOVA table is that it indicates the sources of variation exhibited by the treatments, the magnitude of variation derived from different sources and their significance/ non significance.

Computation of Critical Difference (CD):

Critical Difference is the difference between the treatment means, which places the treatments statistically as well as significantly apart. Otherwise if the difference of two treatment means is less than the CD it can be concluded that both the treatments are on par.

Maintenance of records and registers:

Crop improvement programmes are of long duration, conducted at more than one research station and involve the participation of scientific and supporting staff of several disciplines. The plant breeder has to observe and evaluate thousands of plants in several replications and multi locational trials every year. There will be numerous strains under each species. A systematic record keeping of all breeding activities carried out over years and locations is necessary to provide individual identity to seedlots held in the store and the breeding plots in the field. Unless complete, simple, accurate and easily retrievable record sare maintained, evaluation of the breeding material is impossible.

Most of the records kept at various stations are concerned with origin and pedigree of seed which are described below.

Origin of seed:

Each seedlot of a breeding material has an origin. For example, an origin & depicted as Hyd.13K-4001 relates to the harvest of plot number4001 sown at Hyderabad station during Kharif season of year 2012. Standard abbreviations are used for each research station. Last two digits of the year denote the year. Crop seasons are *Kharif* (crop sown in April-July) *Rabi* (crop sown in October– December) and *Zaid*(spring) (Crops own in February– March) abbreviated to K, R &Z respectively. A set of plot numbers are used for each type of yield trial. Plot numbers used in one location in one crop season in a year are not duplicated elsewhere. These plot numbers of a plot, of a given location, become the origin of the following crop season. Seedlots harvested are tagged using this number.

Pedigree of seed

Pedigree of breeding materials describes its complete past breeding history. It has two major components

- i) Name of the parental variety in an abbreviated form
- ii) Detailed breeding programme executed in each generation it was grown.

Following the name of the parental variety, a specific research station code may be added to identify the station where the breeding programme was initiated. The pedigree also provides information on the number of generations of self pollination, sib pollination or selection carried out.

For example the pedigree of an inbred line in maize depicted as Cuba 11J–A46#-#-#* denotes the following. The inbred line was derived from Cuba 11J by self pollination at Delhi centre (Station code A). The 46th self-pollinated plant was selected which was later maintained by sib pollination for the next three generations.

Types of records:

1. Accession Register
2. Germplasm bank
3. Descriptive blank register
4. Cropping programme
5. Single plant selection register

Field Note books

6. Row test
7. Replicated row test
8. Preliminary/Initial yield evaluation trial
9. Comparative yield/yield evaluation trial
10. Multiplication I, II trials
11. Quality observations note book
12. Record of crosses
13. F₁ generation
14. F₂ segregation generation note book

There are different types of records such as accession record, project book, planting plan, planting list, record book for crosses and field book. The records have to be complete such that any new plant breeder on studying them can understand the entire breeding material available.

1. Accession record:

This is an important and continuing permanent record which provides information on all material received and tested. For every crop there is a separate crop-wise accession book. One line of each double page of the accession book is given to one variety. There are eight columns on each double page. These show accession number, name of variety, date of receiving material, source/origin of seed, source number and complete address of seed donor, pedigree record, seed description & remarks respectively.

Numbering or assigning an accession number starts with '1' every year. Last two figures of the year (eg.13 for the year 2013) in which the variety was first recorded is subscribed to the previous identifying number.

Proforma for accession register:

1	Accession number
2	Name /variety
3	Date of receipt
4	Source of seed
5	Source number
6	Pedigree record
7	Description of the material
8	How disposed and to whom sent
9	Feedback information
10	Remark

2. Project book/Basic record:

Every project is separately numbered and its name, objective, plan and duration are written on a specific page of the project book. It records the purpose and procedure of the complete breeding programme.

3. Breeding book:

A separate breeding book is maintained in the research station for each crop season. It has planting plans or sowing plans for each experimental field giving location and other details. Planting plan is the elaborate plan of a breeding block or nursery which is made well in advance of the sowing time. Planting plan contains number of replications size of plots, row and planting distances, number of plants and location of check rows and check plots. Every row

or plot in the block can be identified by row or plot number. Breeding book, in addition to sowing plan, lists the pedigree and origin of seed as well.

4.Planting list:

Planting list or planting schedule contains the information regarding the location of varieties in different plots. It records sowing time, starting of germination etc. which is transferred to field note book afterwards.

5.Record diary for crosses:

The detailed information regarding each cross is separately recorded under different heads. Name of the cross, objective, number of female heads used number of F1 seeds obtained, number of seeds sown and plants harvested in F1,F2 and advanced generations etc. is given. The criterion of selection is also mentioned.

6.Field book:

Field books are permanent records made on standard notebooks. They are always taken to the field by the plant breeder for recording daily observations. For yield tests, printed sheets containing column headings with important characters row-wise may be bound together and used.

Numbering and labelling the material

For numbering selections, crosses, introductions and mutants notations

I,II,III and M respectively may be used.

I- 13-18

II- 13-1608

III- 3-4251

M- 13-5172

The first symbol denotes whether it is as selection, cross, mutant etc.The second number denotes the year in which selection, cross etc. were made. The third number indicates the number of the particular plant selected

After emasculation & artificial cross pollination, the tag labelled on the female parent must read as follows.

No. of crossing
Name of female parent x Name of male parent
Date of emasculation,
Date of pollination &Initials of breeder

Dendrobium crumenatum x *D. philippica*

31-01-2013, 1-02-2013

STM

A standard form of a field note book

Each field note book should contain the following information

A Yield trials

1. First page :

- a. Number and title of the project
- b. Season of raising the crop
- c. Unit under which the trial is being conducted

2. Second page:

- a. A full plan of the field showing the location of the trial with approach path b. North/East direction should be specified

3. Third page:

- a. Plan of the experiment
- b. Experiment details:
 1. Name of the experiment
 2. Season
 3. Number of varieties
 4. Design of the experiment
 5. Replication
 6. Size of the plot (Block/plot/row etc.,)
 7. Spacing (between rows and within the row in cm)
 8. Date of sowing

9. Date of harvest

10. Name of the principal investigator

4. Fourth page: Detail of cultural practices followed for the plot/field a. Dates of ploughing

b. Date of layout of the trial

c. Manurial schedule adopted

Basal:

Top dressing:

Irrigation schedules with date, from life irrigation onwards.

5. Fifth page onwards:

One page for each variant per replication is allotted. The following information have to be recorded in each page.

1. Date of germination

2. Date of gap filling

3. Initial stand on

4. Date of first flowering

5. Date of general flowering

6. Date of harvest

7. Final stand

8. Wet weight of grain

9. Wet weight of haulms/straw etc.

10. Dry weight of produce after cleaning

11. Yield per ha in kg

The page will also have additional information on observations about the variant, recorded by the breeder in relation to the object of the project.

The fifth page will also contain the following information and their modification depending upon the crop

eg. Rice –number of tillers, date of ear head emergence etc.,
Sesame-number of branches, days to first flowering etc.,

B. Generation study

This field note book will contain in addition to details as per A (i),(ii),(iii) and (iv) the third page will contain the following information.

a. Plan of the segregating generation

b. Details of the generation

1. Name of the generation

2. Number of crosses

3. Details of the cross number, details of parents, number of families, and number of seeds sown

4. Length of row

5. Spacing(cm)

6. Date of sowing

7. Dates of harvest & 8. Name of the principal investigator

Exercise No 3

Handling of segregating generation – Pedigree Methods

Mass selection and pure line selection cannot be applied to segregating population. E. g F₂, F₃ etc. The method is generally used for handling segregation generation may be grouped into three categories.

i) Pedigree Method

ii) Bulk Method

iii) Back Cross Method

The objectives of all these methods are to develop pure line varieties.

In pedigree method, individual plants are selected from F₂ and the subsequent generation and their progenies are tested. During the entire operation, a record of the entire parent's offspring relationship is kept, is known as pedigree record. The selection of individual plant is continued till the progenies show no segregation. At this stage, selection is done among the progenies, because there would be no genetic variation within progenies.

Pedigree method of plant breeding includes following steps.

Hybridization:

Crossing between selected parent plants is the first step in pedigree method.

F₁ generation:

Seeds obtained by hybridization (F₁ seeds) are planted with proper sowing distance. Seeds of about 20-30 plants are harvested in bulk and forwarded to grow F₂ generation.

F₂ generation:

Selection is the main process carried in this step. About 10,000 plants are grown from F₁ generation seeds (F₂ seeds). With application of selection process about 500 plants are selected and harvested separately.

F₃ generation :

About 30 or more progenies are raised from each of the selected plant of F₂ generation. About 100-400 superior plants (the number could be anything, preferably less than those selected in F₂ generation) are selected

F₄ generation:

Seeds from F3 generation are space planted. Plants with desirable characters are selected in number much less than those selected in F3 generation.

F5 generation:

Individual plant progenies planted in multi row (3 or more) plots so that superior plants (about 50 – 100) can be selected by comparison.

F6 generation:

Individual plant progenies planted in multi row (3 or more) plots. Plants are selected based on visual evaluation, progenies showing segregation can be eliminated.

F7 generation:

Preliminary yield trials with minimum 3 replications and a check. Quality tests are conducted.

F8 to F12 generation:

Multi-location yield trials with replications are conducted. Tests for quality and disease resistance are conducted.

F10 or F13 generation:

Seed multiplication for distribution.

Merits of pedigree method are listed below

Excellent method for improvement of easily observable, high heritability characters.

As pedigree record is maintained, information regarding inheritance pattern of characters can be obtained as and when required.

Each plant can be traced back to its parent plant.

Only those progeny lines which contain plants with desired characters are selected for next generation. So there is scope for plant breeder's skills.

Progeny tests are done, thus it is based on genotypic value rather than phenotypic value.

Increased breeding efficiency by early identification of superior heterogeneous populations

Scope for transgressive segregation to occur for the characters like yield. New variety development takes short period as compared to bulk method.

Demerits of pedigree method are as follows

Costly, Labour intensive, Requires skilled person as selection is practiced

Pedigree record maintenance is time consuming.

Selection for yield or other characters in F2 and F3 is ineffective.

One important to note is genetic variation available for selection gets decreased in later generations due to the individual plant selection carried out earlier.

Application of Pedigree Method:

- 1) Selection of desirable plants from the segregating population in self- pollinated crops.
- 2) This method is commonly used to correct some specific weaknesses of an established variety (Combination breeding).
- 3) It is also used in the selection of new superior recombinant type's i.e Transgressive breeding.
- 4) This method is suitable for improving specific characteristics such as disease resistant, plant height, maturity etc.

Experiment: 4

Handling of segregating generation - Bulk Population Method

Bulk population method of breeding in self –pollinated crop is also known as mass method or population method of breeding. It was first used by Nilsson Ehle in 1908. It refers to a species is grown in bulk plot (from F1 to F5) with or without selection, a part of the bulk seed is used to grow the next generation and individual plant selection is practised in F6 or later generation. In this method duration of bulking may vary from 6-7 to 30 generation.

Application of Bulk Population Method:

This method is suitable and most convenient for handling the segregating generation of cereals, smaller millet, grain legume and oilseeds. This may be used for three different purposes.

- i) Isolation of homozygous lines.
- ii) Waiting for the opportunity of selection.
- iii) Opportunity for natural selection to change the composition of the population.

Procedure of Bulk Population Method:

1) Hybridization:

Parents are selected according to the objective of the breeding programme and crossed.

2) F1 Generation:

The F1 generation (10 to 25 F1) is space planted and harvested in bulk.

3) F2-F6 – Generation:

F2 to F6 generations are planted at commercial seed rate and spacing. These generations are harvested in bulk. During these generations the population size should be as possible, preferably 30 to 50 thousand plants should be grown in each generation.

4) F7 Generation:

About 30 – 50 thousand plants are space planted and out of this only 1000 to 5000 plants with superior phenotypes are selected and their seeds harvested separately. Selection is made on the basis of phenotypes of plants, grain characteristics etc.

5) F8 Generation:

Individual plant progenies are grown in single or multi row plots. Most of the progenies would be homozygous and are harvested in bulk. Weak and inferior progenies are rejected and only 100- 300 individual plant progenies with desirable characters are selected.

6) F9 Generation:

Preliminary yield trial is conducted along with standard variety as check. The evaluation of progeny is done for important desirable characteristics. Quality test may be conducted to reject the undesirable progenies.

7) F10- F12 Generation:

Replicated yield trails are conducted at several locations using standard commercial varieties as check. The lines are evaluated for important agronomic characteristics. If lines are superior to the standard check, released as new varieties.

8) F13 Generation:

Seed multiplication of the newly released variety for distribution to the farmers.

Merits of Bulk Population Method:

- 1) This method simple, convenient and inexpensive.
- 2) Little work and attention is required in F2 and subsequent generation.
- 3) No pedigree record is to be kept.
- 4) It eliminates undesirable types and increases the frequency of desirable types by artificial selection.
- 5) It is suitable for studies on the survival of genes and genotypes in populations.
- 6) There are greater chances of isolation of Transgressive segregates than pedigree method.

Demerits Bulk Population Method:

- 1) It takes much longer to develop a new variety.
- 2) It provides little opportunity for the breeder to exercise his skill in selection.
- 3) A large number of progenies have to be selected at the end bulking period.
- 4) Information of inheritance of characters cannot be obtained like that of pedigree method.

Achievement:

This method has been used in Barley crop for developing some varieties from the crosses (Allas X Vaughn), like Arival, Beecher, Glacier, etc. In India only one variety "NarendraRai" has been developed in Brown Mustard. This method has a limited application in practical plant breeding.

Experiment: 5

Handling of segregating generation – Single seed descent Method

Another modification of the bulk method is the single-seed-descent method, which is becoming increasingly popular. In this method, a single seed from each of the one to two thousand F₂ plants is bulked to raise the F₃ generation. Similarly, in F₃ and the subsequent generations one random seed is selected from every plant present in the population and planted in bulk to raise the next generation. This procedure is followed till F₅ or F₆ when the plants would have become nearly homozygous. In F₅ or F₆, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families not showing segregation. Thus preliminary yield trials and quality tests begin in F₇ or F₈ and coordinated yield trials in F₈ or F₉.

The objective of single-seed-descent method is to rapidly advance the generations of crosses ; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. F₂ and the subsequent generations are grown at very high plant densities as vigour of individual plants is not important. In each year, 2-3 generations may be raised using off-season nurseries and greenhouse facilities. The important features of this scheme are : (1) lack of selection, natural or artificial, till F₅ or F₆ till the population is reasonably homozygous , and (2) raising of F₃ and later generations from a bulk of one seed from each F₂ and the subsequent generation plant in order to ensure that each F₂ plant is represented in the population. As a result of the speed and economy, the single-seed -descent scheme is becoming increasingly popular with the breeders. The single-seed-descent scheme (1) advances the generation with the maximum possible speed in a conventional breeding method; (2) requires very little space, effort and labour ; (3) Makes the best use of greenhouse and off-season nursery facilities; and (4) ensures that the plants retained in the end population are random sample from the F₂ population. However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, e.g., pulses, plant loss may be one of the most serious problems of the scheme.

Exercise No. 6

ESTIMATION OF HETEROISIS AND INBREEDING DEPRESSION

The term heterosis is coined by Shull in 1914. It refers to the superiority of F1 in one or more characters over its parents. It is also defined as increase in fitness and yield over its parental values. It is also called as hybrid vigour. The three main causes of heterosis are over dominance, dominance and epistasis, of this dominance is the widely accepted one. In crop plants there are three main ways for fixation of heterosis i.e. asexual reproduction, polyploidy and apomixis. Four different methods are used to estimate heterosis.

$$1) \text{ Relative heterosis-overmid parental value} = \frac{F1-MP}{MP} \times 100$$

$$2) \text{ Heterobeltiosis-overbetterparent} = \frac{F1-BP}{BP} \times 100$$

$$3) \text{ Economic/ useful heterosis-over commercial cultivar} = \frac{F1-CC}{CC} \times 100$$

$$4) \text{ Standard heterosis-overstandardhybrid variety} = \frac{F1-SH}{SH} \times 100$$

Among these standard heterosis and economic heterosis is useful to plant breeders.

Inbreeding depression is the phenomenon of decrease in fitness and vigour in F2 as a result of continuous inbreeding. Inbreeding depression is estimated when F1 and F2 progenies of the same cross are available and is calculated as,

$$\text{Inbreeding depression} = \frac{F1 - F2}{F2} \times 100$$

Exercise No. 7

EMASCULATION AND HYBRIDIZATION METHODS IN RICE

1. RICE (*Oryza sativa*) (2n = 24) (Family – Poaceae)

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Cyperales

Family: Gramineae

Genus: *Oryza*

Species: *Sativa*

Subspecies: *Indica*

Common name: Paddy in English, Nel in Tamil, Nellu in Malayalam

Panicle:

- The terminal component of the rice tiller is an inflorescence called as **panicle**. The inflorescence or panicle is borne on the uppermost internode of the culm. The panicle bears rice spikelets, which develop into grains.
- The panicle base often appears as a hair like ring and is used as a dividing point in measuring culm and panicle length. The panicle base is often called the **neck**.
- The **panicle axis** is continuous and hollow except at the nodes where branches are borne.
- The swellings at the panicle axis where the branches are borne are referred to as the **panicle pulvinus**.
- Each node on the main panicle axis gives rise to primary branches which in turn bears secondary branches. Primary branches may be arranged singly or in pairs.
- The panicles bear spikelets, most of which develop into grains. These spikelets are borne on the primary and secondary branches. The spikelet is the basic unit of the inflorescence and panicle. It consists of the **pedicel** and the **floret**.
- The floret is borne on the pedicel.
- The **rudimentary glumes** are the laterally enlarged, cup like apex of the pedicel. The rudimentary glumes are the lowermost parts of the spikelet. During threshing, the rudimentary glumes are separated from the rest of the spikelet.

Spikelets:

- The sterile lemmas are small, bract like projections attached to the floret. The **rachillae** is a small axis that bears the single floret. It is between the sterile lemmas and the floret
- The spikelets are carried on small rachillae at the ends of the branches of the panicle.
- The spikelet in rice is single flowered enclosed by the lemma and palea.
- There are six stamens, in two whorls of three each, the filaments being very slender and delicate and having sudden exertion of growth at the time of anthesis.
- The anthers are linear. The pistil has a single ovary, two styles each with a plumose and laterally exerted stigma ovary, two styles each with a plumose and laterally exerted stigma of different shades of purple.
- The ovary is tricarpeal, single celled, single ovuled and with basal placentation.

- In most cases the plumose stigmas are dusted with pollen when they emerge out as the dehiscence of the anthers takes place in many varieties just at the time of opening.
- Though self-pollination is the rule, natural cross-pollination also occurs, varying from 0.1 to 4.0 percent.

Grain:

- The ovule after fertilization develops into the seed with its coats completely fused together with the developing ovary wall or pericarp.
- **Pericarp of fruit coat:** The pericarp is made up of distinct layers of quadrangular cells which form the **Epicarp**. These cells have slight thickening and are followed by cells which are much compressed and form the **Mesocarp** consisting of two or three layers. The **Endocarp** is a single layer of tube cells. The color in the rice grain is found in the pericarp layer in the mature stage.
- **The seed coats:** Due to the pressure brought out by the developing seed on the pericarp, the testa and tegmen become much pressed down and out of shape. A few layers of such cells below the pericarp can be diagnosed as the integuments of seed coats.
- **The aleurone layer:** A prominent layer of rectangular cells which contain protein lies next to the seed coats. This layer is known as the aleurone layer. The layer in rice is not coloured unlike in case of maize.
- **The endosperm:** the entire mass of tissue below the aleurone layer is made up of cells which contain plenty of starch grains and these form the endosperm.
- **The embryo:** the scutellum has an upper free part which has a fleshy projection known as the **ventral scale**. Below this upper ventral scale and almost at the middle of the free part there is another outgrowth which can be called as the **inner ventral scale** and this inner ventral scale is peculiar to rice embryos only.

In rice anthesis commences shortly after emergence of panicle. Spikelets at the tip bloom first and proceed downwards. Anthesis time 8-10 am. Each spikelet remain open 30 minutes and then closes. The anther dehiscence takes place immediately after the opening of the spikelets. Receptivity remains for one day.

Emasculation and Crossing techniques

Emasculation is necessarily followed by controlled pollination. Emasculation is done during early morning between 6 and 8 AM in spikelets, due to open on the same day. Emasculation should be over well ahead of the time of anthesis. Crossing techniques in rice differ based on the method of emasculation. Since maximum number of spikelets open on the 3rd or 4th day of anthesis, panicles of that stage are selected for emasculation. The following methods are widely used for hybridization in rice.

1) Clipping method

In the previous day evening, top 1/3rd and bottom 1/3rd portions in the panicle of the desired female parent are clipped off by using scissors leaving the middle spikelets. With the help of scissors again, top 1/3 portion in each spikelet is clipped-off in a slanting position. The six anthers present in each spikelet are removed with the help of the needle (Emasculation). Care must be taken during emasculation for not to damage the gynoecium. Then to prevent contamination from the foreign pollen, the emasculated spikelets are covered with a butter

paper bag. In the next day morning (usually at 9.00AM), the bloomed panicle from the desired male parent is taken. The top portion of the butter paper bag which was originally inserted in the emasculated female parent is now cut to expose the panicle. The male parent panicle is inserted in an inverted position into the butter paper bag and turned in both ways in order to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened butter paper bag is closed using a pin. Coloured thread may be tied at the base of the panicle to identify the crossed ones. After ensuring pollination, the bag may be removed.

2) Hot water method

A method of hot water emasculation is used to about the same extent as the clipping method. Panicles in 3rd (or) 4th day of blooming are chosen as female parents. An hour or so before blooming (i.e. normally at 7. A.M.), the panicle is selected and under developed and opened spikelets are removed. Now, the tiller is bent over (carefully to avoid breaking) and the selected panicle is immersed in hot water contained in a thermos bottle at 40-44°C for a period of 5 to 10 minutes. This treatment causes the florets to open in a normal manner and avoids injury. Then, emasculation is done by removing the six stamens by fine forceps or needles and then dusting should be done.

3) Dr. Ramiah method

Panicles on the 3rd or 4th day of its blooming are selected; top and lower spikelets are removed leaving only the middle. It is covered with a wet cloth and air is blown from mouth. This facilitates opening of spikelets. After 2-3 minutes, wet cloth is removed and spikelets are found to be open. Then, the six anthers are removed.

4) Vacuum emasculation method

This works on the principle of suction pressure. The spikelets are clipped off prior to operation. The minute pipette is to be shown at the point of clipping and pollen is sucked in. Six panicles can be emasculated at a time. By hand emasculation, 100 flowers can be emasculated by a person. With the vacuum emasculator, six persons can operate and emasculate 3000 to 3600 florets/hour.

5) Cuttack Method

The technique was developed by CRRI, Cuttack. The panicle to be emasculated is inserted into hollow piece of bamboo closed at one end and plugged with cotton wool and split cork at the other end. The flowers thus enclosed will open within 5-10 minutes. The anthers are removed.

6) Brown paper method

The panicles are enclosed in a Brown paper cover before a couple of hours of blooming. Heat develops inside due to which the anthers extrude, but do not dehisce. This happens in 15-30 minute then the anthers are easily clipped off. Stigmatic surface is then dusted with pollen grains collected from the chosen male parent. The crossed panicle is then properly tagged and protected with paper cover which is retained in a position for 7 – 10 days.

7) Rhind's method

In this method hot water is kept in the flask and it is poured outside . After pouring out the water inside of the flask will be warm and humid. The panicle to be emasculated will be inserted into the flask and kept for some time. Due to high temperature and humidity the spikelets will get opened and the anthers are exposed which can be removed with the help of forceps.

EMASCULATION AND HYBRIDIZATION METHODS IN MAIZE

MAIZE (*Zea mays*) ($2n = 20$), Family : Poaceae

Maize is predominantly cross pollinated. Wind pollination (Anemophily) is the general rule. Pollination by insects also takes place to certain extent. The following are the adaptations for cross pollination, *i.e.*, Monoecious inflorescence, unisexual flower, differences in the time of maturity of the male and female inflorescences, silk receptive on entire length and abundant pollen production. It has protoandry and the tassel anthesis extends 2-14 days. Pollen viability remains for 24 hours. Anthesis of female spikelets starts after the completion of tassel opening and extends up to 2-5 days. The stigma is receptive throughout its length for 14 days.

Floral morphology: Male inflorescence consists of many spikelets. The spikelets occur in pairs—the lower one is sessile and the upper one is stalked. Each spikelet is two-flowered. They have four glumes. The first and the second glumes located at the base, are sterile, and the third one, called flowering glume, and the last one, the two-nerved palea, enclose a flower. Flowers are obviously unisexual. Perianth is represented by a pair of fleshy scale-like bodies called lodicules. Androecium is composed of three free stamens with long prominent linear anthers

Female inflorescence is a spadix arising from the axil of a lower leaf. A large number of spikelets are arranged closely on the fleshy axis, forming what is known as cob. It remains surrounded by a few large hyaline bracts called spathes.

Each spikelet, like the male ones, is two-flowered and has protective glumes. Of the two, usually the upper flower is fertile and the lower one aborts. Lodicules are absent.

Gynoecium is monocarpellary. The ovary is one-chambered, superior, ovoid in shape with a single anatropous ovule. The styles are long and silky. Persistent styles hang out in tufts from the apex of the cob. Stigma is long and feathery.

Flowers are wind-pollinated. Small dusty pollen grains are easily caught by the feathery stigma of the carpel. Fertilization takes place by normal process. Fruit is a caryopsis with a single closely fitted seed where fruit wall and seed-coat are inseparably united.

A large number of fruits, called grains, remain densely crowded on the spongy axis. Seeds are albuminous. The single cotyledon, scutellum, serves as the absorbing organ. Germination is hypogeal.

Selfing

1. Bag the tassel before anthesis with a paper cover. Bagging of tassels should be done in the previous day evening to avoid contamination from foreign pollen.
2. Cut the tip of the cob before the silks emerge and cover with a paper cover.
3. After 3-4 days, the silks will emerge in the form of a 'saving brush' in which the silks will be of same height and stand erect.
4. Remove the cover of the tassel containing pollen and insert it over the cob after removing the cob-cover. The inserted cover is then tied.

Crossing technique:

Female parent

- a. Detassel
- b. Cut the tip of the cob before the silks emerge and cover with a butter paper cover.

Male parent

- a. Cover the tassel before anthesis begins or as soon as the tassel emerges.

When the silks emerges in the female parent in the form of a brush, pollination is done by transferring the freshly shed pollen cover form the male parent and inserting it over the cob of the female parent after removing the cover from the cob.

The details like date of pollination, parentage and breeding programme to be carried out are clearly written by water proof pencil. The date or pollination will be one day later than the date of tasselling. Pollination should be completed within one week of silk emergence. Isolation distance for maize = 400M.

Exercise No. 9

EMASCULATION AND HYBRIDIZATION METHODS IN SORGHUM

SORGHUM (*Sorghum bicolor*) (2n = 20) Family – Poaceae

Sorghum is normally self-pollinated but some florets are *protogyny* resulting in cross pollination averaging about 6%. So, it is classified as often cross-pollinated. The amount of natural cross pollination varies from 0.6 to 50 per cent in different varieties and places. The cross pollination is more in loose panicles than in compact ones. Anthesis starts from tip to downwards at the rate of 2-5 cm per day and completes within 7-10 days. Anthesis time 3-6 am. The pollen grains are viable only for short period and stigma is receptive for 8-16 hours.

Floral morphology:

S. bicolor is predominantly self-pollinating, but under specific conditions wind-mediated cross-pollination can occur over 60%, depending on the genotype, and average about 6%. As a result of self-pollination and outcrossing, most sorghum landraces grown by subsistence farmers are mixtures of inbred and partially inbred lines. The level of outcrossing varies and is influenced by the panicle type of the cultivar; typically outcrossing is higher in loose-panicled grassy sorghum and lower in compact-panicled domesticated sorghum. The estimated outcrossing rate in domesticated sorghum under field conditions ranges from 5% to over 40%. Several pollinator species have been observed consecutively visiting domesticated sorghum flowers.

Inflorescence development begins when a floral initial forms 30 to 40 days after germination. Domesticated sorghum normally flowers 55 to 70 days after germination in warm climates, but depending on the genotype, flowering may occur 30 to 100 days after germination. Wet and cool weather can also delay flowering. Flowers begin to open two days after the emergence of the inflorescence from the boot. Flowering starts in the sessile spikelets (multiflowered subdivisions of the inflorescence) at the tip of the inflorescence and progresses downwards over 4 to 5 days. A single panicle may have up to 6,000 florets. All heads do not flower at the same time in a field, so pollen is usually available for 10 to 15 days. Flowering time varies based on the genotype and climate, usually occurring from midnight to mid-morning and peaking around sunrise. The swelling of the lodicules facilitates flower-opening. When the stigma becomes visible, the stamen filaments elongate and the anthers become pendent. When the anthers are dry they dehisce and pollen is shed through the apical pore. Most pollen from a head fertilizes eggs on the same head. Cross-pollination can occur if pollen is blown into the air. The stigma is pollinated before the emergence of the anthers from the spikelets. Pollen grains drift to the stigma and germinate. A pollen tube develops with two nuclei and grows down the style to fertilize the egg. A sperm nucleus fertilizes the egg to form a 2n embryo and the other nucleus fuses with the polar nuclei to form a 3n endosperm. After pollination, the glumes close and the empty anthers and stigmas usually protrude. Some long-glumed varieties are cleistogamous (the florets do not open for fertilization). Unpollinated stigmas remain receptive for up to 16 days. After fertilization, organ differentiation occurs over approximately 12 days. Seeds pass

through three development stages: milk, early dough, and late dough, and reach maturity after about 30 days. *S. bicolor* reproduces through seeds.

Selfing

Head bagging becomes efficient for selfing the ear heads. Once the decision to bag heads has been made, all heads in a row should be covered. If a head has already begun to flower, the flowering portion should be cut off. During head bagging, boot leaf of the plant is usually removed prior to placing the bag.

Emasculation

1) Hand emasculation

Only a part of the panicle is emasculated and the remaining panicle is clipped away. During clipping, flowered tip and the lower panicle branches are removed. About 50 florets which would normally flower the following day are selected for emasculation. The needle is inserted at the middle of the floret and moved across the glumes. The needle is rotated at 90° and three anthers are lifted out. The emasculated panicle is covered by a suitable paper bag.

2) Hot water method

In this method, in the panicle flowered tip and lower panicle branches are removed. About 50 florets (in clusters of two or three) are immersed in hot water at 48°C for 10 minutes.

3) Plastic bag/ mass emasculation technique

In this method, sorghum panicle is covered with plastic bag. This creates high humidity inside the bag. Under such a humidity, the florets open, the anthers emerge but shed no pollen. The anthers are knocked free of head by tapping. In this method, some selfing occurs. Therefore, marker genes are needed to identify the plants arising from selfed seed.

On a dry morning when pollen shedding is occurring between 6 and 7 A.M., the hand pollination may begin around 9.30 A.M. In rainy days, the operation may be started at 11.30 – 12.30 A.M. The pollen is collected in paper bags. Sorghum pollen kept in bags is viable for 10-20 minutes. For collection, appropriate heads may be selected and bagged in the previous night itself.

The selected male parent panicle will be covered with brown paper bag the previous day evening before dehiscence of anthers. Next day the pollen will be collected by tapping the bag. The collected pollen will be dusted on to the emasculated head and covered with butter paper bag labeled properly. Dusting of pollen is done for two to three days continuously.

EMASCULATION AND HYBRIDIZATION METHODS IN PEARL MILLET

Cumbu / PEARL MILLET (*Pennisetumglacum*) (2n = 14) Family – Poaceae

Cumbu (Bajra) is naturally cross pollinated (Allogamous). Wind is the chief agent of pollination (anemophily). Adaptations for cross pollination is Protogyny. Anthesis commence from 1/3rd of the apex of spike and proceeds both ways. Stigma emerges first and anthesis is over within 2-3 days. This is followed by the first male phase in which the anthers from the perfect florets emerge out. On the fifth day of anthesis the 2nd male phase begins in which anthers from the staminate florets emerge. Anthesis time 8 pm -2 am.

Inflorescence: Pearl millet inflorescence is a compound terminal spike called panicle and its length generally varies between 20-25 cm with a circumference of 7-9 cm (see photo 16). The panicle shape varies considerably as shown in Fig. 6, but, the common shapes are either cylindrical or conical. Inflorescence consists of a central rachis covered with soft/short hairs and bears fascicles on rachillae. The density of fascicles and the length of rachillae determine the degree of compaction of the panicle. Each fascicle contains spikelets surrounded by a wall of bristles (i.e., involucre) (Fig. 7). The prolongation of the fascicle axis determines the length of bristles (Photo 21)

A spikelet may contain 2-4 flowers or florets, but generally two. The lower floret is staminate and the upper floret is bisexual or hermaphrodite.

Pearl millet is a protogynous species. The styles start protruding two to three days after the emergence of the panicle. The stylar branches protrude first from the florets in the upper middle region of the panicle and then proceed both up and downwards. In the hermaphrodite flowers, the stigmas emerge earlier than the anthers and hence stigmas receive pollen from inflorescence of other plants. The time required for complete stigma emergence varies from 2 to 3 days and they remain receptive for next two to three days.

By the time anther emergence commences, the stigma would have emerged and also pollinated, avoiding selfing. The emergence of the first anther usually begins about three to four days after the first stigma has emerged. The anther emergence occurs in two phases. The first phase involves solely the hermaphrodite flowers, and the second phase includes the staminate flowers. When the first phase of the emergence of anthers has reached the basal spikelets, the second phase begins when the staminate flowers are functional from the upper part of the panicle (Photo 31-34). A panicle continues shedding pollen for about 3 days. The anther emergence continues throughout the day and night. The anthesis occurs between 8 am and 2 pm with a peak at about 10 am. The increase in humidity and a decrease in temperature have been noted to reduce anther emergence, while lowering of humidity and a rise in temperature enhances up the anthesis.

Selfing

To ensure selfing, spikes may be bagged before emergence of the stigmas. As the spike elongates it may be necessary to adjust the bag to cover the lower most spikelets. Another procedure is to enclose within a bag two full spikes from the same plant, one day (or) 2 days

older than the other and ready to shed pollen as the stigmas are emerging from the younger spike.

Crossing

Emasculation in Cumbu is laborious and difficult due to the small size of the flowers and the late maturity of the anthers when compared to the stigma. About four-fifths of the upper portion of the spike is removed and the rest is bagged before the styles appear to prevent contamination. Flowers are pollinated by dusting them with fresh pollen obtained from the desired male plant or by shaking a spike which is shedding pollen over the exposed stigmas.

Controlled cross pollination

Pearl millet does not require emasculation for making crosses. The female line will be covered before stigma emergence with butter paper bag. Without removing butter paper bag we can see emergence of stigma. After most of the stigma have emerged. Pollen from desired male parent is collected and dusted on to the female line. Pollination is usually made in the morning. Care should be taken to cover pollen parent previous day with butter paper bag. The crossed heads are labeled.

Another method is instead of removing the selfing bag of female and dusting, the top of the cover clipped of desired male parent inflorescence in the process of pollen bursting is inserted to brush the stigma. Then the clipped top of the bag is folded and stapled. The crossed heads can be collected after 30-35 days.

Exercise No.11

EMASCULATION AND HYBRIDIZATION METHODS IN RAGI

Ragi/ Finger millet (*Eleusinecoracana*) (2n:36) Family: Poaceae

In this crop self pollination is the general rule. The inflorescence takes 7-8 days to complete anthesis. Time of anthesis 1 am – 5am. In each spike the order of opening is from the top to bottom. In each spikelet the opening of the floret is from the base to top and one floret in each spikelet opens a day.

Floral structure: Terminal whorl bearing 2 to 10, but averaging 5 or 6 spikes arranged like a birds foot at the top of the peduncle. The lowest spike is separated by 2 to 5 cm from the other spikes and which arise from the same point at the end of the stem. In each finger there are about 70spikelet's, each spikelet having five to seven complete flowers. In the spikelet the flowering proceeds from bottom to top and in a finger the order of flowering is from the top spikelet downward. An ear head contains 1,500 to 3,000 flowers, and the flowering period varies from six or seven to ten days.

Inflorescence types: TOP INCURVED OPEN TYPE INCURVED TYPE

Floral biology: Anthesis commences from top spikelet and progress downwards. Each spikelet contains 4 to 6 flowers, the opening of the florets starts from bottom to top. One floret in the spikelet opens per day. Flowering takes place simultaneously in all fingers. Complete emergence of the inflorescence requires 7 -8 days. Depending upon the ear shape Flower opening period will vary; Compact : 2-3 a.m. Fisty : 3-5 a.m. Open : 1-2 a.m. Pollen viability is very short, 10 – 15 min.

Anthers require about 45 minutes for dehiscence after emergence The stigma is receptive for about five minutes after emergence from the glumes Self pollination is the general rule because the period of anthesis is very short. Cross fertilization by wind and insects is less than 1 per cent.

Selfing, emasculation and pollination techniques

Selfing

The panicle before commencing anthesis is covered with paper cover and retained till the blooming is over.

Crossing

Emasculation and crossing are tedious. However, both hand emasculation and hot water treatments are followed. Hand emasculation is done in the evening and pollination is done very early in the morning i.e., before 6 a.m. Hot water technique of emasculation of florets is also successful. Hot water treatment at 520C for 2 minutes was the best as judged from the percentage of hybrid seed-set. Then the spikelets are pollinated early in the morning.

Approach Method or contact method

The inflorescence to be opened will be selected and cut with long stalk from the male parent. This is brought to the emasculated flower. The male flower as a whole will be tied round with female flower. Then they are covered with butter paper bag. The cut end of the male inflorescence will be immersed in water kept in a bottle. Natural cross pollination takes place in 2 to 5 days. Marker genes are utilized for identifying the hybrid seedlings in the nursery plot. 60-90% seed set is recorded in both methods.

Exercise No.12

EMASCULATION AND HYBRIDIZATION METHODS IN PIGEONPEA

RED GRAM (*Cajanuscajan*) ($2n = 22$) Family – Fabaceae

Self pollination is the rule in Red gram and natural crossing extents up to 65 per cent. Therefore it is also known as often cross pollinated crop.

Floral Morphology: Racemes axillary, rarely branching, peduncle erect with 1 – 3 additional nodes, usually slightly shorter than the leaves, mostly with 2-6 flowers at the tip; bracts about 5 mm long; pedicel to about 9 mm long; calyx campanulate, lobes triangular or lanceolate, tube glandular and pubescent, the upper lobe bifid, the lower lobe longest; vexillary petal basally inflexed, biauriculate, mostly with yellow to reddish striate, bicallose in the target area, glabrous, with a claw, wings slightly obovate, with short auricle; keel apex obtuse, slightly inflexed. Stamens 9+1, 15 – 18 mm in length, tapering towards the top; anthers ellipsoid, about 1 mm long, dorsifixed, yellowish, slit longitudinal; pollen 3-colporate, ora-circular, exine areolate; ovary 1 chambered; ovary hairy; style long; stigma terminal, simple, Pods linear-oblong, compressed, bi-valved, depressed between the seeds,; upper suture swollen, the lower indistinct; beak down curved; seeds 1 - 5, compressed, subspherical about 6 x 4 x 1.5 mm, of various colours, hilum linear to oblong to somewhat elliptic, about 3 mm long

Adaptations for self pollination:

1. Bisexual
2. Close proximity of anthers and stigma
3. Simultaneous maturity of anthers and stigma.

Selfing, emasculation and pollination techniques in Red gram

Selfing

Mature flower buds are to be covered with paper bags for one or two days.

Crossing

Hand emasculation followed by artificial cross pollination is essential. Emasculation should be done in the previous day evening and the emasculated buds are protected by covers. Early morning on the next day, pollination is done using pollen collected from the protected flowers of the selected male parents.

Exercise No.13

EMASCULATION AND HYBRIDIZATION METHODS IN BLACKGRAM

BLACK GRAM (*Vignamungo*) (Diploid, $2n = 22 \text{ \& } 24$) Family – Fabaceae

Floral morphology: Flowers are bisexual, papilionaceous, small; bracteoles are linear to lanceolate, exceeding the calyx; calyx : is a campanulate it includes 5 sepals; corolla: is a yellow in colour (5 petals), standard petal is a 12–16 mm wide, 2 wings petal is about as long as standard, 2 keel petals spirally coiled with a terminal horn-like appendage; stamens: are 10 in number, 9 united and 1 is free (Diadelphous)

Flower morphology: Flowers begin to open between 6 to 7 am. Flower is continues for an hour. Flower is remain open till noon & gradually close, being completely closed by 2 to 4 pm. Pollination occurs at bud stage • Anthers dehisce between 9 pm to 3 am • Petals will shed in the following morning

Selfing techniques: (1) It is a self pollinated crop, the occurrence of natural cross pollination is negligible i.e. less than 5% (2) Natural cross pollination is mainly by insects (3) In order to ensure 100% selfing, bag the flower before anthesis

Black gram is essentially a self pollinated crop, there are two methods involved in crossing of black gram (1) emasculation (2) pollination

(1) Selection of flower bud (2) Flower bud should held between fore and thumb finger (3) Dissecting needle is inserted just under the standard obliquely along the top of the bud (4) The left side standard & left wing are pushed outward away from the bud (5) The left keel is removed in pieces (6) Exposed anthers are removed

Pollination Take out stamen from freshly opened flowers Rubbed anthers again set the stigma of the emasculated bud Pollination should be immediately after emasculation gives good pod set

Self pollination is the rule. Here pollination occurs before flower opening (cleistogamous) in night. Anthesis time 1 am – 4 am. The flower opens in the morning at 7 am. The interval between pollination and opening of flower is 4 hours. This ensures self fertilization.

Selfing, emasculation and pollination techniques in Black gram

Selfing : As in red gram, bagging is done to avoid insect contact.

Crossing : Young unopened bud is kept between thumb and fore fingers of the left hand. The point of dissecting needle is inserted just under the standard petal in an oblique position along the top of the bud. The left side of the standard and wing petal are pushed outward and held with thumb and left hand. The left side of the keel petal is removed with the forceps. The pistil and stigma are then exposed and the anthers are removed with the forceps. Evening emasculation followed by morning pollination gives best results. Pollination is done by

gently rubbing anther of male, inserting the staminal column and closing it with standard and wing petal. Since flower shedding is common, putting better paper bag is avoided. The emasculated flowers are identified with thread wound round. The crossed pod will be smaller in size with two or three seeds only.

Exercise No. 14

EMASCULATION AND HYBRIDIZATION METHODS OF MUNGBEAN

Morphology

Mungbean belongs to family Leguminosae and sub-family papilionaceae.

It is an erect sub-erect deep rooted, much branched, somewhat hairy annual herb with the height ranging from 30-130 cm.

Leaves are alternate, trifoliolate, petiole long, stipules ovate, leaflets ovate upto 12x10 cm.

Flowers are in axillary racemes, peduncle up to 13 cm in length with clusters of 10-12 flowers, corolla yellow in colour sometimes curved, 5-10 cm long.

Number of seeds per pod ranges 10-15. The seeds are oblong, green or olive green in colour; sometimes yellow, brown or blackish also.

Inflorescence: axillary or terminal raceme with 10-20 flowers crowded on long peduncle.

Flower: hermaphrodite, zygomorphic, either lighter yellowish olive/olive yellow

Pods: Immature pods are usually green, mature pods are iron gray/olive gray/snuff brown color, round slender with short & moderate pubescence. Dehisces by both (dorsal & ventral) sutures into two halves. It contains 9-16 seeds

Seeds: Globular, green, surface has fine wavy ridges. Hilum is white, more or less flat
Germination is epigeal

Anthesis: Self pollinated, sometime cleistogamy is prevalent Cross pollination is 0.5-3%
Flower open between 6.00-8.00am, remain till about 11.00am. Close between 2.00-4.00pm.

Emasculation 4.00-6.00pm- For emasculation the young bud is kept between thumb & forefinger. Point of dissecting needle is inserted, just under the standard in an oblique position along the top of the bud. The left side of standard & wing petal are pushed outwards & held with thumb. The left hand of keel is removed in pieces with forceps. Pistil & stigma are then exposed & removed with forceps.

Pollination done in morning (8-11am): collect mature anthers from open flowers & gently pressing the ripe anthers against stigma.

Flower may be bagged after pollination until pods are matured. % of flower shed is very high -69%

Exercise No. 15

EMASCULATION AND HYBRIDIZATION METHODS IN SOYABEAN

SOYBEAN *Glycine max* (2n = 40), Family – Fabaceae

FLORAL MORPHOLOGY OF SOYBEAN: Soybean plants enter into reproductive stages following vegetative growth . Axillary buds develop into clusters of flowers. From 20 to 80% of the flowers, developed early, abscise and produce initially few pods. Generally, the earliest and latest flowers produced abort most often. Soybean has a typical papilionaceous flower with a tubular calyx of five unequal sepals and a five-part corolla. The corolla consists of a standard (posterior banner petal), two lateral wings and two anterior keel petals contacting with each other, but not fused. The stamens are clustered around the stigma, ensuring self-pollination The gynoecium consists of an ovary, style, and stigma. As many as four ovules develop in the ovary. Nine stamens are arranged in two whorls; the outer whorl contains five stamens, and inner whorl contains four stamens. The two whorls of nine anthers align themselves into a single whorl on a staminal tube. The larger and older anthers alternate with the smaller and younger anthers in sequence around the developing gynoecium. The single free stamen the last to appear. Soybean is highly self-pollinated with natural crossing usually below 1% because the stamens are elevated so that the anthers form a ring around the stigma. Thus, pollen is shed directly onto the stigma surface ensuring self-pollination.

Flower open early in the morning. The pollen is shed normally shortly before or after the flower opens. But pollen shedding may occur sometimes with in the bud itself. Normally cross pollination does not exceed 1 percent.

Emasculation and crossing

Hand emasculation is the method followed for crop breeding which is tedious since the floral parts are so small and seed set is also less. Emasculation is done in the evening and pollination is done in the morning hours.

Exercise No. 16

EMASCULATION AND HYBRIDIZATION METHODS OF GROUNDNUT

Inflorescence: The inflorescence of the groundnut appears as a cluster of flowers in the leaf axils and is a reduced monopodium, either simple or compound. It consists of three or more flowers, is spike-like and always occurs in the axils of cataphylls or foliage.

Flower Flowers are enclosed in between 2 bracts. One of them is simple, subtending a short peduncle and the other bifid, subtending the pedicel. The flower is sessile but appears stalked after the growth of a tubular hypanthium just before anthesis.

The calyx has 5 lobes. The typical papilionoid corolla is inserted on the top of the hypanthium and surrounds the staminal column. The stamens are 10, monadelphous with the staminal column surrounding the ovary.

The pistil consists of a single ovary surrounded by the base of the hypanthium. The stigma is club shaped or clavate, usually at anther level or protruding slightly above.

The Peg

Fertilization is normally completed before midday. After that the flower droops, the corolla closes, the calyx tube bends and the flower withers. During the early development of the young embryo, the ovary at the base of the calyx tube becomes mobilized for growth within a week. By then an intercalary meristem below the ovary is activated.

The green ovary turns purplish from the tip downwards. The developing ovary pierces through the floral parts by the activity of the meristem to reveal an elongating peg or carpophore. The peg is a stalk-like structure that bears the fertilized ovules at its tip. Its growth is positively geotropic, until it has penetrated the soil to some depth. The tip then becomes diageotropic.

After some initial development, the ovary shows no apparent change until it is diageotropically positioned in the soil. It is then that the ovary starts developing into a fruit.

Flowering: In bunch types flowering commenced from 26 to 34 days after sowing. First flower opened generally 7 to 10 days later in runner types than bunch types. Onset of flowering was gradual, flower production began to accelerate 3 weeks later in Virginia types and 2 weeks later in the bunch types. During the 5 weeks after the first flower appeared, 66% of the total flowers were produced in bunch types and 79% in Virginia types. The number of flowers produced per plant ranges from 40 to 250 in spreading types and 98 to 137 in bunch types. Fruiting efficiency depends on the pattern of flowering (number of flowers at different period of flowering), which is more important than total number of flowers per plant. The flower production increased rapidly immediately after the commencement and reached the peak in about a week, and later the number of flowers produced per day declined. Maintained

a lower rate of flower production for about 10 days. A second spell of increased flower production of lesser intensity than first occurred, and finally, there was a gradual decrease until cessation, 75 days after sowing. The cyclic flowering is inherent in the developmental process of groundnut and is not directly controlled by variation in environmental factors. Flowering gets reduced as pegging and fruiting progress. Daily minimum mean temperature recorded 0-3 days after flowering were positively correlated with flower production. Maximum temperature had a negative effect on production of flowers and high light intensity reduced it. Flowering stopped when the soil moisture dropped to wilting point but continuation of fruiting depended on the length of the drought. Relative humidity had a positive effect on the daily production of flowers.

Exercise No. 17

EMASCULATION AND HYBRIDIZATION METHODS OF SESAME

Scientific name : *Sesamum indicum*; Family: Pedaliaceae Chromosome number : $2n=2x=26$

Other names : Til, Gingelly, Tila, Ellu

FLORAL MORPHOLOGY: Flowers are solitary or in group, axillary, shortly, pedicellate. Calyx : persistent or deciduous, gamosepalous of 5 lobes. Corolla : tubular, obliquely campanulate, gamopetalous 2 lipped & ring of hairs towards base of stamens, variously coloured. Androecium : stamens are epipetalous & didynamous, filaments 4, slender, anther sacs are dorsifixed & dehisce longitudinally. Gynoecium : ovary superior, bicarpellary, 2 celled separated by false septum, 2 chambered, Ovules numerous & style is forked at the tip with stigmatic hairy lobes.

Fruit : Oblong or ovoid capsule, with albuminous seeds. ovary Style stigma corolla

Anthesis & stigma receptivity: Sesame crop comes to flowering 3-5 weeks after sowing.

At a time 2-3 flowers open in acropetal succession. Flower opening is between 5-8 am .

Anthers while growing starts bursting & start to dehisce between 2-4am.

Stigma become receptive at the same time as anthers dehisce & remains receptive till 8 am.

Pollen viability for few hours.

Pollination: Sesame is predominantly self pollinated crop(95%). Natural cross pollination also takes place to the extent of 4.6-5%. Experiments shows cross pollination up to 65% (Richharia-1940). It varies with location & other factors.

Selfing techniques: Bagging : bag the flowers for selfing to avoid natural crossing by insects. Tie a small soft cotton thread at the tip of the flower buds about 12-16 hrs prior to opening of the flowers.

Crossing techniques: 1. Emasculation & crossing : It is done in the previous evening to the day of flowering between 5-6pm . It is done by just pulling out the corolla as such by holding it in the tip. Emasculated flowers should be protected by covering it with paper bag. Artificial pollination is done in next morning at 5- 7am with a fine brush.

2. Soda Straw method: Remove corolla tube by hand in the previous evening leaving pistil intact. A section of soda straw is slipped on the pistil & folded tightly at the free end . Next morning the ripe anthers are rubbed over the stigma & soda straw is again inserted over the pollinated pistil.

Exercise No. 18

EMASCULATION AND HYBRIDIZATION METHODS OF CASTOR;

Tall glabrous annual some times shrubby or sub arboreous, leaves alternate, broad palmately Seven to many lobed, serrate, flowers large, in terminal sub panicle racemes, monoecious, apetalous, upper male crowded lower female .

Chromosome no: $2n=20$

Castor is a cross pollinated crop, protogynous and wind pollinated. Leaves are large palmately lobed. Inflorescences are borne terminally on the main and lateral branches.

INFLORESCENCE: The main stem ends in raceme, which is the first or primary raceme. After the first raceme appears, 2 or 3 branches arise at the nodes immediately below it. Each of these branches terminates in racemes after 4 or more nodes have formed which are known as secondary racemes. Branches arise from the nodes just beneath secondary racemes, ultimately terminating in tertiary racemes. This sequence of development (indeterminate growth habit) continues.

The racemes of castor are monoecious with the pistillate flowers on the upper 30-50% and staminate flowers on the lower part of the inflorescence. The proportion of pistillate and staminate flowers among the racemes varies a great deal both within and among genotypes. It is influenced by the environment of the plant, genotypes & nutrition.

FEMALE FLOWERS MALE FLOWER:

The spiny, globose seed capsule (left) dries and splits into 3 sections called carpels (centre). Each carpel (right) splits open and forcibly ejects a large seed. The shiny seeds of castor plants are of intricate designs. At one end is a small, spongy structure called the caruncle, which aids in the absorption of water when the seeds are planted.

POLLINATION:

Male flowers senesce shortly after shedding their pollen. The opening is between 4.30 and 5.00 A.M. Pollen grains are viable for 2 days & stigma is receptive for 3 days. The best environmental conditions for pollen dispersal are at a temperature between 26 °C to 29 °C and relative humidity of 60%, which may vary according to the cultivar. Each candle takes 10-12 days to complete flowering. Pollination is generally by wind and insects. Stigma remain receptive after anthesis, for a period of 5- 10 days.

Emasculation: It can be achieved by removing or rubbing off the staminate flowers by finger and thumb.

Crossing: Pollen grains are collected from the desired male parent and are dusted on the stigma of the female parent. Again the inflorescence is covered.

Unriped Copsuls FULLY MATURED COPSULS DRY SEEDS

MALE STERILITY: Absence or non-function of pollen grain in plant or incapability of plants to produce or release functional pollen grains. Genetic Male Sterility: pollen sterility, which is caused by nuclear genes, is termed as genic or genetic male sterility. It is usually governed by a single recessive gene *ms* or 's' with monogenic inheritance.

Progeny from crosses (*msms* X *Msms*) are used as a female and are inter planted with homozygous male fertile (*MsMs*) pollinator. The female line would contain both male sterile and male fertile and male fertile plants, the later must be identified and removed before pollen shedding. This is done by identifying the male fertile plants in seeding stage either due to the pleiotrophic effect of *ms* gene or due to phenotypic effect of closely lined genes. In USA used in castor PROS: In this rouging of male fertile plant from the female is costly operation and due to this cost of hybrid seed is higher.

Exercise No. 19

EMASCULATION AND HYBRIDIZATION METHODS IN COTTON

FLORAL MORPHOLOGY OF COTTON:

The petals of the cotton flower are a creamy white to yellow when the flower first opens. They are narrow at the base and broad at the tip. The petals darken, usually to a dark pink at the end of the first day. Below is a cross section through a petal.

The flower has three bracts, usually closed about the square. It also has a calyx, which surrounds the developing bud. Both the bracts and the calyx serve to protect the flower.

In the center of the flower is a five lobed pistil, surrounded by many stamens. Each of the stamens has a two lobed anther that releases pollen. Each pollen grain is viable for about 12 hours after release.

The flower has a superior ovary, consisting of two or more *locules*, or seed chambers. Depending upon the species of cotton, each seed chamber can have 8-12 ovules, 5-9 of which usually mature.

Hybridization:

Normally pollen shed directly on the stigma when anthers open. Pollen is rarely wind born, as it is heavy and sticky. Cross – pollination to the extent of 5 to 30 % is possible by insects, mostly honey bees.

For hybridization emasculation is done one day earlier of the flower opening. Corolla is removed by hand or cut away with scissors. The stamens are removed with forceps. Ripe anthers are collected from pollen parent in straw tube and slipped over emasculated stigma and stigma immediately enclosed with bract by wire. Similarly, ripe anthers can be rubbed on the stigma of the emasculated flower. The pollination is usually done a day after emasculation. The emasculation may be done

1) By taking circular cut at the base and piercing needle through staminal tube or 2) Removed of anthers by pointed forceps. It is also be done by thumb and nail method and instead of bagging small piece of straw tube inserted over stigma of emasculated flower and tied along with bracts with thread.

Exercise No. 20

EMASCULATION AND HYBRIDIZATION METHODS IN COWPEA

COWPEA (*Vigna unguiculata*), Family – Fabaceae (Diploid $2n = 22$ and 24)

FLORAL MORPHOLOGY OF COWPEA: Cowpea is a self pollinated crop which is encouraged by the arrangements of the floral parts. However, Insects visiting cowpea flowers have been implicated in the movement of pollen from one cowpea plant to another. Flower opening of cowpea begins between 6:00am and 6:30am and closes between 11:30am and 12:00pm. Pollen analysis showed similarities in the pollen morphology of the cowpea varieties. Moreover Var. Drum produced the highest number of pollen grains per flower in terms of pollen productivity. Cowpea is self pollinating crop and usually pollination occurs in cowpea flower before they open. The enclosure of the pistil and stamen within the keel enhances self pollination. Pollen grains are transferred from the anther to the stigma and thus pollination occurs. However, Cowpea flowers produce nectars which attract insects to them. Various insects such as Butterflies, Houseflies (*Musca domestica*), Honeybees (*Apis mellifera*) were seen around the cowpea flower. Honeybee was observed to be the most prominent out of the various insects observed.

Pollination

Highly self pollinated because of Cleistogamy, Close proximity of the anthers and stigma and Simultaneous maturity of anthers and stigma

Selfing

Keeping the plants in insect proof cages will lead to selfing. Covering of individual flower buds will lead to poor pod setting.

Crossing

Select young buds, in an inflorescence and remove all immature buds. Split open the keel petals and remove the stamens one by one holding the filaments. Bring corolla back to position and cover the bud with a folded leaflet. Protection is given by keeping the plants in insect proof cages. Pollination is done on the next day morning by exposing the stigma from the keel petal and brushing it with the pollen collected from the male parent.

Exercise No. 21

EMASCULATION AND HYBRIDIZATION METHODS OF TOBACCO

FLORAL MORPHOLOGY OF TOBACCO:

Tobacco is normally self-pollinated, although as much as 4 to 10 per cent of cross-pollination occurs from pollen carried by insects. Most of the tobacco varieties flower between 55-80 days after planting.

The inflorescence of *N. tabacum* is terminal raceme, which may bear as many as 150 flowers. Tobacco flower is perfect and consists of a corolla, with a pistil and stamens enclosed within it. The corolla contains five petals which are fused into a long tube and which terminate in five expanded lobes at the top. Corolla outer surface is pale greenish cream in colour, upper half similar in colour or pink to red. The flower bears five stamens, which are fused to the corolla tube, and a pistil with a long slender style and a blunt two-lobed stigma. The stigma is generally sticky and pollen adheres to it readily. Capsule is narrow, elliptic, ovoid or orbicular, acute or blunt and 15-18 mm long. Seed is nearly spherical or elliptic and light brown.

In *N. rustica*, the inflorescence is usually a compact thyrse represented by a thickened central axis, shortened branches and monopodial-sympodial development of peripheral flowers. Corolla is yellow to greenish yellow in colour. Throat is broadly obconic with contraction near the mouth. Stamens are white, with four of them long and one short. Capsule is elliptic, ovoid or sub-globose, 7-15 mm long. Seeds are elliptic, oval and dusky brown.

The seeds of tobacco are extremely small and are usually borne in a two-valved capsule. A single *tabacum* capsule may yield from 2,000 to 5,000 very small seeds and hence, a single plant may produce several hundred thousand seeds. Plants of *rustica* yield about one-fourth of this number of seeds.

The flowers are pedicellate and hermaphrodite. Calyx contain five sepals. Corolla contain five petal. The stamens are five in number. It has superior ovary. It is self-pollinated crop but 4-10% of cross pollination occurs due to insects. therefore it is Grouped into often cross pollinated crop.

Pollens are viable for 24hrs. Stigma is receptive one day before and after flower opening.

Selfing technique : Covering entire inflorescence with paper bag will ensures the selfing technique 9

Crossing technique: It involves emasculation followed by pollination. For emasculation select the unopened flowers with pink colour tip and anthers are removed with five pointed forceps after tearing petals. Collect the pollen grain from matured flowers and dust it on the emasculated flower. Bag the pollinated flower and then tag it.

IMPORTANT VARITIES: Godavari,swarna,krishna,thangam, Sona, maragandam

Exercise No-22

STUDY OF QUALITY CHARACTERS, DONOR PARENTS FOR DIFFERENT CHARACTERS

COMMON NAME	SCIENTIFIC NAME	CHARACTER NO.	QUALITY CHARACTERS
Rice	<i>Oryza sativa</i>	2n=24	<ul style="list-style-type: none"> • Grain size and shape • Texture of endosperm • Quality of starch in endosperm • Aroma and cooling quality • Milling out form
Wheat	<i>Triticum aestivum</i>	2n=14 2n=28 2n=24	<ul style="list-style-type: none"> • Glutamin content • Dough • Flour quality • Water holding capacity of humus
Maize	<i>Zea mays</i>	2n=20	<ul style="list-style-type: none"> • Protein content • Protein in grain is 20% balanced one
Pegion pea	<i>Cajanus caja</i>	2n=22	<ul style="list-style-type: none"> • Protein content(23%)
Soyabean	<i>Glycine max</i>	2n=20	<ul style="list-style-type: none"> • Protein content(42-45%)
Green gram	<i>Vigna radiata</i>	2n=22	<ul style="list-style-type: none"> • Methionine (high)
Black gram	<i>Vigna mungo</i>	2n=22	<ul style="list-style-type: none"> • Protein content (24-27%) • Methionine
Ground nut	<i>Arachis hypogaeae</i>	2n=20	<ul style="list-style-type: none"> • Protrin content(45-55%) • High oil content • Kernel • High selfing
Sesamum	<i>Sesamum indicum</i>	2n=42	<ul style="list-style-type: none"> • High oil content
Sunflower	<i>Helianthus annus</i>	2n=34	<ul style="list-style-type: none"> • High oil content (46-54%)
Mustard	<i>Brassica compestris</i>	2n=20	<ul style="list-style-type: none"> • High oil content (40-45%) • Protein content (30-41%)
Chili	<i>Capsicum annum</i>	2n=24	<ul style="list-style-type: none"> • Fruit length • Ascorbic acid content • Capsacino content
Okra	<i>Abmoscnus esculentus</i>	2n=130	<ul style="list-style-type: none"> • Pod length, seed / pod test weight
Cucumber	<i>Cucumis sativa</i>	2n=14	<ul style="list-style-type: none"> • Shape of fruit firmness of fruits
Chrysanthemum	<i>Chrysanthemum monifolium</i>	2n=13	<ul style="list-style-type: none"> • Flowering quality, average flower weight, flower diameter

Gerbera	<i>Gerbera</i>	2n=50	<ul style="list-style-type: none"> • Colour of flower leaf and leaf area no. of flowers/plant shelf life of flowers buckers production capacity
Mango	<i>Mangifera indica</i>	2n=40	<ul style="list-style-type: none"> • Self life of fruit high ascorbic acid %
Guava	<i>Psidium guajava</i>	2n=22	<ul style="list-style-type: none"> • Colour, flower, texture, taste of fruit
Rose	<i>Rosa sp</i>	2n=14	<ul style="list-style-type: none"> • Large flower bud maximum bud diameter, no. of petals/flowers
Papaya	<i>Carica papaya</i>	2n=18	<ul style="list-style-type: none"> • TSS, pH, ascorbic acid
Banana	<i>Musa sp</i>	2n=22	<ul style="list-style-type: none"> • Fresh appearance of ----- fruit self like, shape of fruit at---- pedicel
Tomato	<i>Solanum lycopersicum</i> <i>Lycopersicum esculantum</i>	2n=24	<ul style="list-style-type: none"> • Fruit uniformity