The Neotia University

A practical manual on Crop Improvement I (*Kharif* crops)

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CONTENT

PRACTICAL 1: EMASCULATION AND HYBRIDIZATION TECHNIQUES

https://www.youtube.com/watch?v=v3JKjRd2acE

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 / herself with the flowering habit of the crop. In the case of self-pollinated crops such as wheat, rice, barely, groundnut etc., the plant is permitted to have self-pollination 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 (upto10%) 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, 10minutes treatments with 40-44℃ 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-*

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. 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:

1. What is emasculation?

2. What are the precautions that you have taken during emasculation?

PRACTICAL 2: MAINTENANCE BREEDING & STUDY OF FIELD TECHNIQUES FOR DIFFERENT *KHARIF* CROPS

https://www.youtube.com/watch?v=xn6s5ceeXYk

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 been 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 inappropriate 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 may be 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 treatment
b) Randomization: unbiased allocation of treatments to the experimental units.
c) Local control: minimizing the effect of heterogeneity of the experimental units.
d) 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 seed lots 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 seed lot 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. Seed lots 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 Cuba11J by self-pollination at Delhi center (Station code A). The 46^{th} 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
6. Field Note books
7. Row test
8. Replicated row test
9. Preliminary/Initial yield evaluation trial
10. Comparative yield/yield evaluation trial
11. Multiplication I, II trials
12. Quality observations note book
13. Record of crosses
14. F_1 generation
15. F_2 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 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 a 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 F_1 seeds obtained, number of seeds sown and plants harvested in F_1, F_2 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 yield 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 election, 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

A standard form of a field note book

Each field note book should contain the following information

A. <u>Yield trials</u>

1. *<u>First Page:</u>*
    a. Number and title of the project
    b. Season of raising the crop
    c. Unit under which the trial is being conducted
2. *<u>Second page:</u>*
    a. A full plan of the field showing the location of the trial with approach path
    b. North /East direction should be specified
3. *<u>Third page:</u>*
    a. Plan of the experiment
    b. Experiment details
        - ✓ Name of the experiment
        - ✓ Season
        - ✓ Number of varieties
        - ✓ Design of the experiment
        - ✓ Replication
        - ✓ Size of the plot (Block/plot/row etc.,)
        - ✓ Spacing (between rows and within the row in cm)
        - ✓ Date of sowing
        - ✓ Date of harvest
        - ✓ Name of the investigator
4. *<u>Fourth page:</u>*
    a. Detail of cultural practices followed for the plot / field
    b. Dates of ploughing
    c. Date of layout of the trial

d. Date of layout of the trial
e. Fertilizer 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.

a) Date of germination
b) Date of gap filling
c) Initial stand on
d) Date of first flowering
e) Date of 50%l flowering
f) Date of harvest
g) Final stand
h) Wet weight of grain
i) Wet weight of haulms/straw etc.
j) Dry weight of produce after cleaning
k) 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

- ✓ Name of the generation
- ✓ Number of crosses
- ✓ Details of the cross number, details of parents, number of families, and number of seeds sown
- ✓ Length of row
- ✓ Spacing (cm)
- ✓ Date of sowing
- ✓ Date of harvest
- ✓ Name of the principle investigator

Exercise:

1. Prepare a standard field note book for maintenance breeding of *kharif* crops under your study.

PRACTICAL 3: HANDLING OF SEGREGATING GENERATIONS

https://www.youtube.com/watch?v=Ug7hHtlPmVs

Mass selection and pure line selection cannot be applied to segregating population. E. g. F_2, F_3 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. 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.

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 practiced 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.

6) 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.

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

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
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:

1. Prepare a field note book for handling a segregating population.

Practical 4. Emasculation and hybridization techniques in Rice

https://www.youtube.com/watch?v=gCfPsuoIftM

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Cyperales

Family: Gramineae

Genus: Oryza

Species: Sativa

Subspecies: Indica

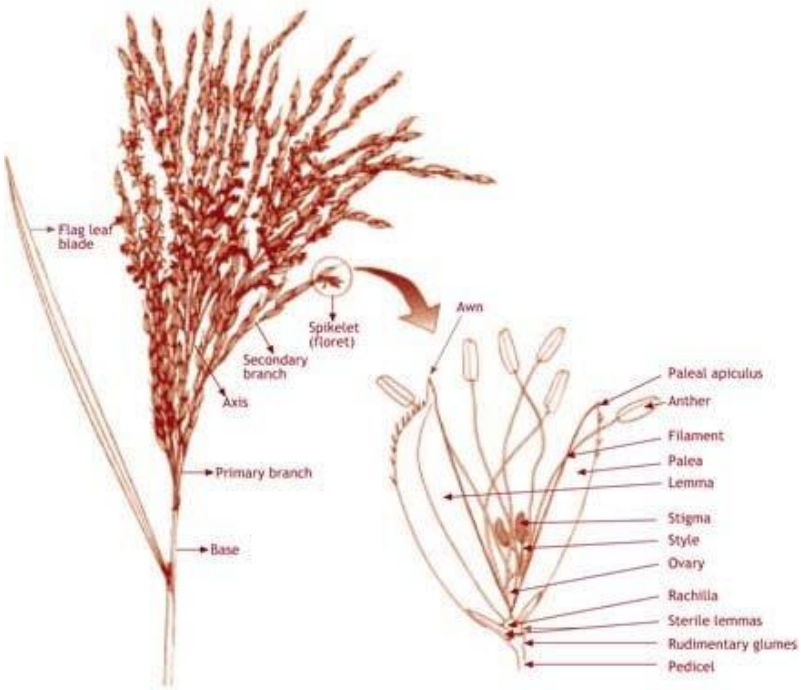
Common name: Paddy

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 anther is 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 tricarpellary, single celled, single ovule and with basal placentation.



<u>Rice *Oryza sativa* (2x = 2n =24)</u>

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 form the foreign pollen, the emasculated spikelets are covered with a butter paper big. 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 40-440C 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 splikelets 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 hallow 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.

Exercise

1. **Prepare the plant material for hybridization in rice. Make labelled diagrams showing the procedures.**

Practical 5: Emasculation and hybridization methods in Maize

https://www.youtube.com/watch?v=F6Fb2X4kYDA

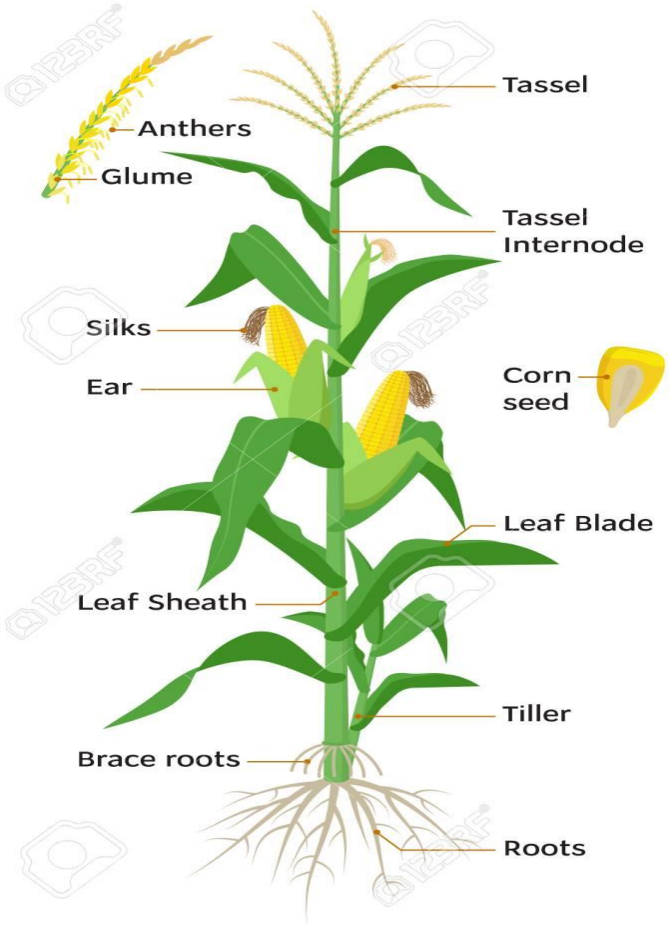
Maize (*Zea mays*) (2n=20)

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 anthsis extends 2-14 days. Pollen viability remains for 24 hours. Anthesis of female spekelets 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.



Emasculation and Pollination

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

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.

b) When the silks emerge 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.

c) 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

1. **Attempt controlled self-pollination and crossing in maize. Make labelled diagrams showing the procedures.**

Practical 6. Emasculation and hybridization techniques in Sorghum

https://www.youtube.com/watch?v=SU6jiDk9ax8

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.

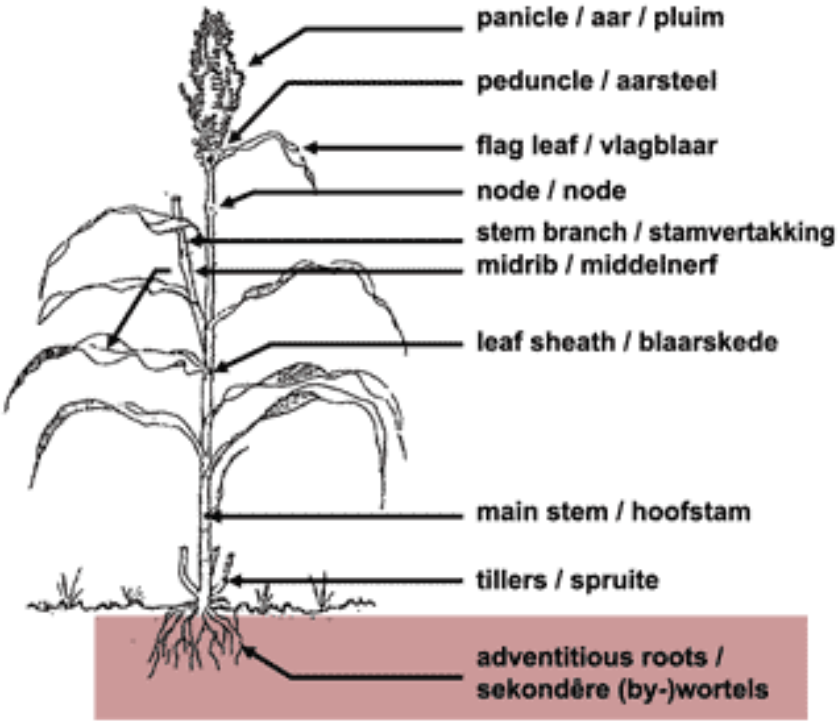
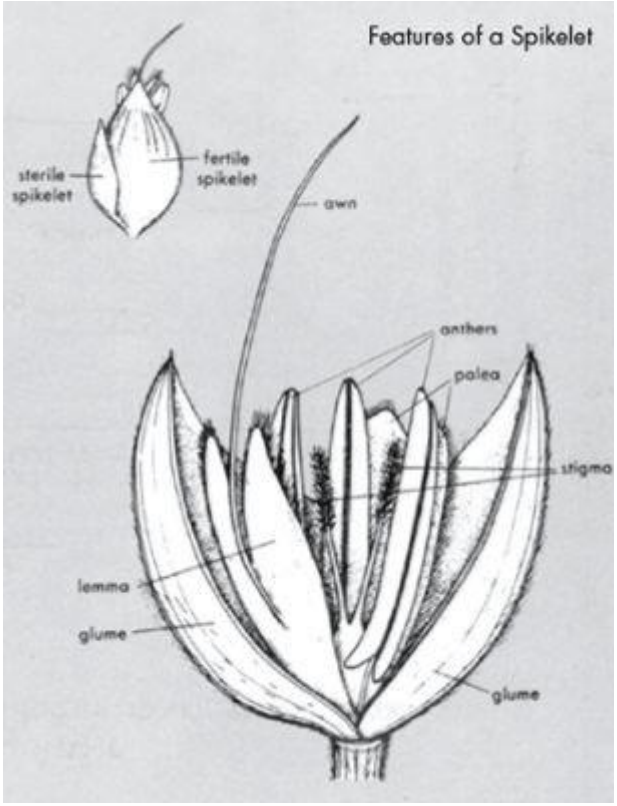


Diagram of the sorghum plant and its components. Illustration by Anthony B. Pearson

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- panicle grassy sorghum and lower in compact-panicle 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 (multi flower 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 2*n* embryo and the other nucleus fuses with the polar nuclei to form a 3*n* 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). Un-pollinated 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 900 and three anthers are lifted out. The emasculate 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 480℃ 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.

Exercise:

1. **Attempt controlled selfing and crossing in sorghum. Make labelled diagrams showing the procedures.**

Practical 7. Emasculation and hybridization methods in Green gram / Mung bean, Black gram and Pigeonpea

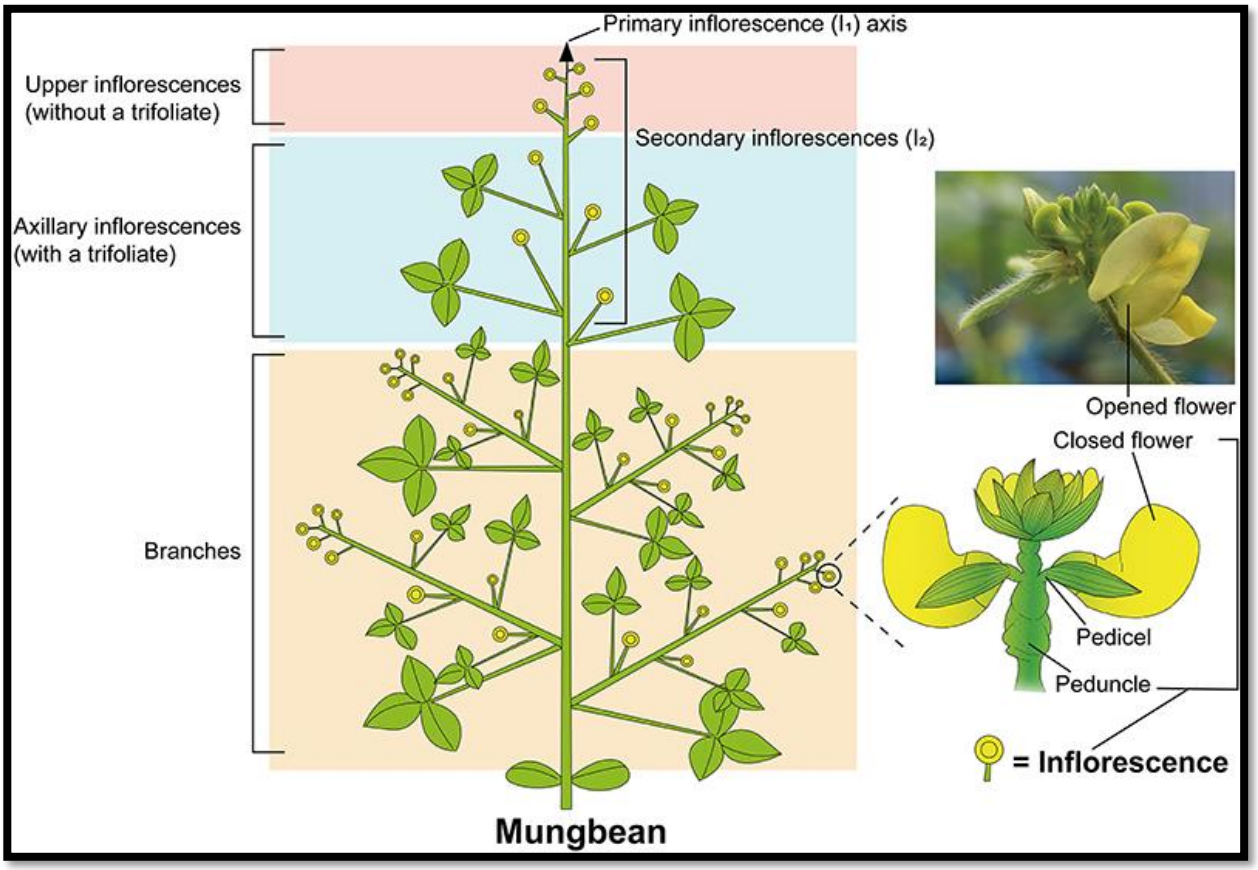
https://www.youtube.com/watch?v=fQ9pzl1ACh0

Green gram / Mung bean (*Vigna radiata* (L.) R. Wilczek) 2n =2x =22

- Mungbean or green gram 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, trifoliate, petiole long, stipules ovate, leaflets ovate upto 12x10 cm. Flowers are in axilary 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 slander 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:

- 4.00-6.00 pm- For emasculation the young bud is keep 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:

- 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%

Urd Bean / Black gram (*Vigna mungo*) (2n = 22 & 24)

Emasculation and pollination

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 Urd Bean

Selfing

As in green 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.

Pigeonpea (*Cajanus cajan*) (2n = 22)

Emasculation and pollination

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

Adaptations for self-pollination:

a. Bisexual
b. Close proximity of anthers and stigma
c. Simultaneous maturity of anthers and stigma.

Selfing, emasculation and pollination techniques in pigeon pea

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:

1. **Study the flower structure of different pulses and draw diagrams.**

2. **Attempt artificial selfing and crossing in black gram, green gram and pigeon pea.**

Practical 8. Emasculation and hybridization methods in Soybean and Sesame

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

https://www.youtube.com/watch?v=0Uux87T2riE

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.

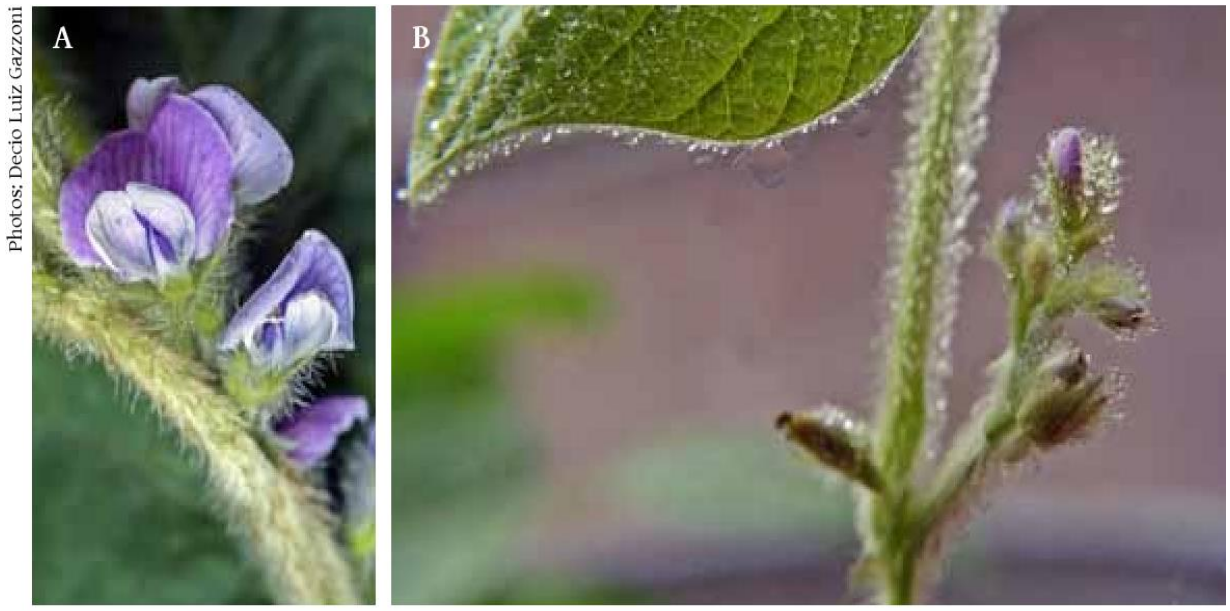


Figure 11. Soybean flowering: axillary and terminal inflorescence. A) Inflorescence in the axil of soybean; B)

Anthesis:

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

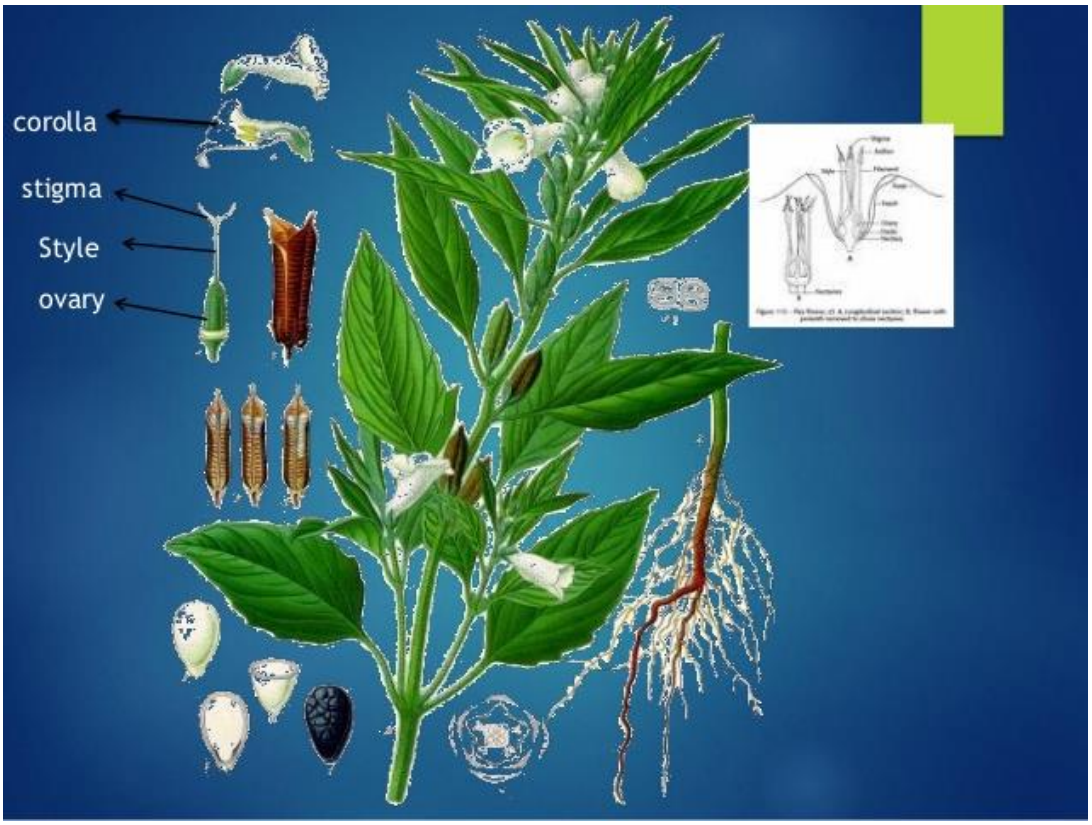
Hand emasculation is the method followed for crop breeding which is tedious since the floral parts are so small and seed set is also less. Emasculation is done in the evening and pollination is done in the morning hours.

Sesame (*Sesamum indicum*) (2n = 26), Family – Pedaliaceae

https://www.youtube.com/watch?v=y8QB37u5f-c

Emasculation and pollination

Sesame is a self-pollinated (Autogamous) crop. In some varieties cross pollination also takes place to a limited extend of 5-6 per cent. Very high cross pollination between 14 and 65 per cent has been recorded in a few varieties in India. Hence, the crop can be classified as often cross pollinated. Cross pollination may occur due to wind and bee activities. On a bright clear day, the flowers open between 5 and 7 am. In the mature flower bud, just before the flower opens, the four unripe anthers are much below the stigma which at this stage is not receptive. The anthers begin to burst longitudinally after 4am in the next day and commence to liberate their pollen. At this time, the stigma becomes receptive. The plant comes to flowering 4 weeks after sowing.



Selfing

1. **Tying with thread:** Selfing can be affected by tying the corolla of the unopened flower which is selected in the previous day evening itself.

2. **Smearing of semi-solid clay:** Selfing can be done by smearing a speck of semi-solid clay, on the upper portion of tubular petals of unopened flowers. The clay while on drying does not allow the tubular petals to open and hence self-pollination is the rule. This method is cheap and less time consuming one. This method is most effective during rainy days. During rainy days, fevicol may be applied on young flower bud to ensure selfing.

Crossing

Soda- straw method

The emasculation technique in sesame is easy for crossing due to epipetalous nature of the stamens. The flower bud which is expected to open in the next day morning is selected in the previous day evening between 3 P.M. and 6 P.M. and emasculated by just removing the corolla tube in which the stamens are attached. Then, the emasculated flower buds are covered with a piece of soda-straw tube, bent at the top in order to avoid contamination from foreign pollens. During the next day morning, between 7 A.M. and 9.A.M., pollen from the desired male parents were dusted gently on the surface of the stigmas of the emasculated flower buds after removing the soda-straw and again covered. The un-emasculated flowers are removed in the female parent.

Individual crossed flowers are tagged with coloured thread for the identification of crossed capsules. Different coloured threads are used for different type of crosses.

Exercise:

1. Self-fertilized the soybean. Make a diagram sowing the procedure and label them.

2. Cross the two varieties of sesame. Make a diagram sowing the procedure and label them.

Practical 9. Emasculation and hybridization methods in Groundnut

GROUNDNUT (*Arachis hypogaea*) 2n=4x=40 (Allotetraploid), Fabaceae

https://www.youtube.com/watch?v=zNQgf6T13A8

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, 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

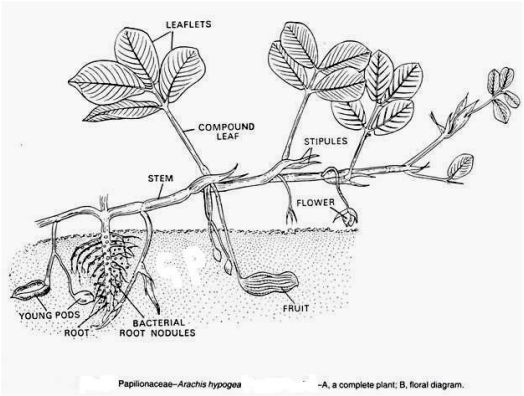
becomes diageotropic.

- 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.

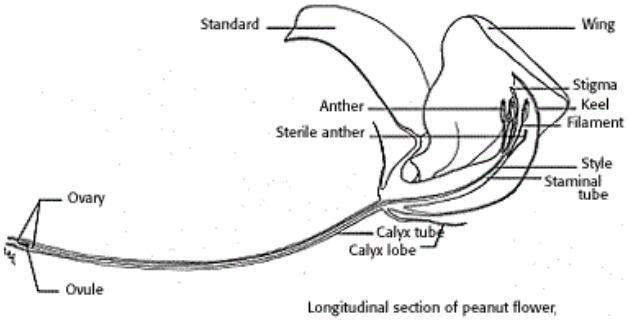
Flowering:

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.



Papilionaceae–*Arachis hypogea* –A, a complete plant; B, floral diagram.



Longitudinal section of peanut flower

Emasculation and pollination

Self-pollination is the rule in groundnut. Anthesis commences at 6 am and continues up to 8 am. Anther dehisces two hours prior to opening of the flower. Twenty-four hours before anthesis, the buds are very small. During the day, elongation of calyx, proceeds slowly but process gets accelerated during night.

Selfing

Since cleistogamous condition prevails in groundnut, selfing is most easy in this crop. Usually covering is unnecessary and difficult. Keeping the plants in insect proof cages will ensure self-pollination.

Crossing

Mature flower buds which are ready to open in the next day are selected and emasculated in the evening. They can be easily identified by the size and length of calyx tube. The flower bud of groundnut is of crescent shape, being bulged on one side and slightly depressed on the other. The keel petal is located on the bulged side and the standard is present on the depressed side. For emasculation, hold the bud between the thumb and the index finger of the left hand and with the help of a razor blade in the right hand, make a cut on the depressed side at two-thirds the length below the tip so as to cut the standard and a portion of the wing petals. Then gently pull the calyx and corolla by holding at the tip of the flower bud. By doing this, the sepals and the petals except the keel would be removed, with the help of the fine forceps gently liberate the bundle of stamens and the pistil from the keel and nip off the anthers.

Exercise:

1. **Hybridized the groundnut. Make a diagram sowing the procedure and label them.**

Practical 10. Emasculation and hybridization methods in Okra and Cucumber

Okra Lady's finger (*Abelmoschus esculentus*) (2n=130)

https://www.youtube.com/watch?v=885lREX4Bzo

Okra is a common vegetable crop grown in warmer climate

Origin: India

Distribution: Asia, Europe, Africa and United States and Brazil. In India it is grown in Gujarat, Maharashtra, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Haryana and Punjab.

Species of *Abelmeschus*

Abelmoschus angulosus

A. crinitus

A. ficulneus

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 being 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.

Cucumber (*Cucumis sativus*) (2n=14)

https://www.youtube.com/watch?v=cOMsoS7fhYs

Cucumber is one of the Asiatic species and member of the cucurbitaceae which has 90 genera and 750 species.

Origin: India – It is considered as home of cucumber

Distribution: China, USA, Africa, Europe. In India it is grown in north and south and lower as well as higher hills

Pollination of Cucumber Plants

Identify the Male Flowers

- Both male and female flowers grow on the same plant, and pollination will only be successful if pollen is moved from the fresh male flowers to the female flowers.
- Examine the flowers to determine whether they are male.
- Male flowers have shorter stems than female flowers, and they grow in clusters of three to five.

Identify the Female Flowers

- Examine the flowers to identify those that are female. Female flowers bloom on their own, and there is one flower per stalk. Additionally, female flowers are identifiable by the small ovary they contain in their center, which male flowers do not have.

- You will be pollinating from fresh male flowers to the female flowers.

Wait 11 Days

- When hand-pollinating cucumbers, first allow the vines to grow and have well-developed flowers, which usually takes about 11 days after blooming begins. Pollinate flowers that open in the morning and pollinate on the same day that the flowers open.

Transfer the Pollen

- Locate the yellow pollen inside the male flowers. Use a clean, small paintbrush to dab the yellow pollen onto the paintbrush. Transfer the pollen from the paintbrush to the stigma in the center of the female flower. The pollen can stick to the paintbrush, so it takes some persistence.
- You can also remove an entire male flower and touch the center of the male flower to the center of the female flower or shake the male over the female to transfer the pollen.

Repeat the Process

Repeat this process for the most effective hand-pollination of cucumbers. Now that you know the technique, the same methods can be used to get optimal crops from squash and melons as well.

Exercise:

1. **Attempt crossing in okra. Make a diagram sowing the procedure and label them.**

2. Pollinate the cucumber. Make a diagram sowing the procedure and label them.

Practical 11. Emasculation and hybridization methods in Cotton

Floral Morphology of Cotton:

https://www.youtube.com/watch?v=vQz63hh2_D4

Cotton (*Gossypium spp*.) 2n = 2x = 26, 2n = 4x = 52

- 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 being 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:

1. **Attempt crossing in cotton. Make a diagram sowing the procedure and label them.**

Practical 12. Study of field techniques for seed production and hybrid seeds production in *Kharif* crops

https://www.youtube.com/watch?v=If8OspxeqjU

I. Rice

Hybrid seed production: Hybrid vigour in rice has been first reported by Jones (1926). This has led to speculation regarding the production of hybrid rice by utilizing cytoplasmic male sterility. Most japonica rice has normal cytoplasm, but indica varieties with sterile cytoplasm and fertility restoration system have been identified. But difficulties have been encountered in obtaining sufficient seed set cross pollination to make hybrid rice seed production economically feasible. After the implementation of UNDP / FAO project entitled "Development and use of hybrid rice technology in India" – the hybrid rice production in India has become a success story. Hybrid rice seeds were produced using (cytoplasmic male sterility) three-line system. The two genes Rf_1 and Rf_2 are the genes for fertility restoration.

The process of hybrid rice production involves continuous supply of agronomically improved cytoplasmic male sterile line (A), maintainer line (B) and fertility restorer (R) line in system. Maintainer and restorer lines are maintained by selfing, while CMS line and F1 seeds are produced with efforts to enhance cross pollination in field. F and S refer to fertile and sterile cytoplasm. Rf and rf are fertility restoration and non-restoring gene respectively.

Row ratio and spacing of A and R lines in the main field.

R	R	A	A	A	A	A	A	A	A	R	R
0	0	*	*	*	*	*	*	*	*	0	0
0	0	*	*	*	*	*	*	*	*	0	0
0	0	*	*	*	*	*	*	*	*	0	0

(male : female ratio = 2 : 8)

Techniques of hybrid rice seed production

The following points are to be taken into account for a successful hybrid rice production.

1. **Choice of field:** Fertile soil, protected irrigation and drainage system, sufficient sunshine. No serious disease and insect problem.

2. **Isolation:** To ensure purity of hybrid seed avoid pollination by unwanted pollen isolation is a must.
    a. **Space isolation:** No other rice varieties should be grown expect pollen parent with a range of 100 m distance.
    b. **Time isolation:** A time of over 20 days is practiced. The heading stage of other variety over a 100 m range should be 20 days earlier or later over the MS line.
    c. **Barrier isolation:** Topographic features like wood lot, tall crops to a distance of 30 m / artificial obstacles of (plastic sheet) above 20m height.
3. **Optimum time for heading and flowering:** Favourable climatic condition for normal flowering are:
    i. Mean temperature 24 - 28℃
    ii. Relative humidity 70 – 80%
    iii. Day and night temperature difference 8 - 10℃
    iv. Sufficient sunshine
    v. Sufficient breeze

4. Synchronization of flowering: As the seed set on MS line depends on cross pollination it is most important to synchronize the heading date of the male and female parents. In addition, in order to extend the pollen supply time, the male parent is usually seeded twice or thrice at an interval 5 -7 days.

5. Row ratio, row direction and planting pattern: Row ratio refers to the ratio of number of rows of the male parent to that of the female parent in the hybrid seed production field. The layout of row ratio depends on

i. The growth duration of the R line
ii. Growth vigor of the R line
iii. Amount of pollen shed and
iv. Plant height of the R line

The Principles include

- R line should have enough pollen to provide
- The row direction should be nearly perpendicular to the direction of winds prevailing at heading stage to facilitate cross pollination.

Practically, a row ratio of 2:8 is currently widely used in indica hybrid seed production. Generally, the R line is transplanted with two to three seedlings per hill and separated by a spacing of 15 cm from plant to plant, 30 cm from one row of restorer to another and 20 cm from CMS line. The MS line is transplanted with one to two seedlings per hill with a spacing of 15 x 15 cm.

A good population structure to get more seed yield is given below.

(a) Seedling/hill (b) Hills/sq.m (c) effective tillers/sq.m

A line = 1- 2	A line = 30	A line = 300
R line = 2 – 3	R line = 5	R line = 120

6. Production and adjustment of heading date:

Even if the seedling interval between both parent is accurately determined, the synchronization of their flowering might not still be attained because of variation in temperature and difference in field management. Hence it is necessary to predict their heading date in order to take measures as early as possible to make necessary adjustments by examining the primordial initiation of panicle.

7. Leaf clipping, gibberellin application and supplementary pollination: These techniques aee very effective for increasing the outcrossing rate.

a) **Leaf clipping:** The leaves taller than the panicles are the main obstacles to cross pollination and therefore, should be cut back. Generally, leaf clipping is undertaken 1-2 days before the initial heading stage, and more than $2/3^{rd}$ of the blades of flag leaves are cut back from the top.

b) **Application of gibberellin (GA_3):** GA_3 can adjust physiological and biochemical metabolism of rice plants and helps in hybrid seed production by stimulating the elongation of young cells. In most of the CMS lines about 20 to 30% of spikelets of a panicle are inside the flag leaf sheath (exertion is only 70%). GA3 affect excretion of panicle completely out of flag leaf sheath. In India, recommended dose of GA3 is 50 g/hec using knapsack sprayer and 25 g/hec with ultra-low volume sprayer.

Advantage of GA_3 application

- Enhance panicle and stigma excretion
- Speed up growth of late tillers and increase effective tillers
- Flag leaf angle is increased
- Reduces unfilled grains

- Enhances seed setting and seed yield
  Spraying stage: 5% of panicle emergence
  Spraying time: 8 -10 am is the best time

c) Supplementary pollination: Shaking the R lines panicles by rope-pulling or rod driving during anthesis can enhance the crossing rate. This is carried out during peak anthesis (10 -12 am)

8. Rogueing: To get 90% purity of CMS lines and R lines, in addition to strict isolation, a thorough rogueing is also necessary.

9. Harvesting and Processing

- The male parent harvested first
- Care should be taken to avoid admixture of male and female lines
- Female line should be threshed separately in a well cleaned threshing floor.
- Seed field dried in shade to 12% moisture content
- Packed in suitable cleaned gunny bags after grading

Hybrid rice Indira Sona

- Year of released: 2007
- Parentage: IR 58025 x R 710-437-1-1
- Maturity: 125 days (medium)
- Grain type: Long slender
- Biotic stress: Resistant to gall midge and tolerance to blast
- Abiotic stress: Non-lodging and non-shattering
- Recommended regions: Irrigated ecosystem
- Area of adaptation: Chhattisgarh
- Special attributes: High milling and head rice recovery (55%) and translucent kernel type
- Average yield: 6.0 -6.5 t/ha

II. Pigeon pea

Hybrid seed production of (CoRH1)

In the exploitation of hybrid vigour for commercial cultivation, efficient production of hybrid seed is essential for which a full knowledge of various steps involved in hybrid seed production

is necessary to achieve the twin objectives of maximizing the hybrid seed production and improvement in quality of hybrid seed.

For hybrid seed production, a ratio of 4 : 1 of male sterile pollen parent is adopted. Sufficient isolation distance i.e., more than 200 meters for the hybrid seed production plot is needed. There should not be any Pigeonpea crop within a radius of 200 meters from the seed production plot. Since the male sterility is maintained in heterozygous state following the test cross principle, there would be fertile and sterile plant in the ratio 1 : 1 in the male sterile population. It is therefore imperative to remove male fertile plants in the male sterile population before flower opening. The rogueing should be done thoroughly to avoid contamination by the pollen from any left out fertile plants.

Steps involved in hybrid seed production

1. **Selection of site**
    i. Fertile field with an irrigation source
    ii. Previous crop should not be pigeon pea
    iii. Isolation distance of 200 mt from any other variety of Pigeonpea
2. **Fertilizer**
    i. Farm yard manure @20 cart loads per hectare
    ii. 25 kg N + 50 kg of P as basal application
3. **Sowing**
    i. The female and male parents are sown in the ratio of 4:1 with two border rows of pollinator parent.
    ii. The pollen parent (ICPL 87109) should be sown one week after sowing the female parent (MST. 21).
    iii. Row spacing 45 cm.
    iv. Plant to plant spacing should be 15 cm.
    v. Dibble 2-3 seed per hill for the female parent.
    vi. Seed rate (per hectare) for 4 : 1 ratio 40 kg of female parent, 5 kg of male parent.
    vii. Sowing should be done during first fortnight of June or first fortnight of December.
    viii. The whole plot should be bordered with sunflower to increase the bee activity to effect cross pollination.
4. **Irrigation**
    i. First irrigation after sowing and life irrigation 2 -3 days after sowing.
    ii. Irrigate the plot at 7 – 10 days' interval depending upon the moisture in the field.

5. **Rogueing**

(a) Male sterile line or female parent

i. Remove the off type plants.

ii. Remove the male fertile plants by examining the colour of anthers (yellow) at the time of first flower formation, one-day before flower opening.

iii. Rogueing should be completed in 7 – 10days times.

iv. Remove the late flowering plants also.

(b) Male fertile line or pollen parent:

i. Rouge out off types

ii. Remove the immature pods sets in the plants from time to time to induce continuous flowering and to ensure pollen availability for longer period.

6. **Harvesting:** Collect the pods from the female parent i. e., male sterile parent. This will give the hybrid seeds.

Production and Maintenance of male sterile line

Genetic male sterility is utilized in hybrid seed production. In case of pigeon pea, the male sterile line will segregate in 1 : 1 ratio of fertile to sterile. For the maintenances of male sterile population (to be raised under isolation), the male sterile plants have to be identified and tagged and the fertile plants have to be retained without tagging. The male sterile plants will be pollinated naturally by the pollen from the male fertile plants in the population through insect pollinators. After maturity, the seeds from tagged male sterile plants are collected and will be used for producing male sterile lines again or far producing hybrid seeds.

The main difference between the hybrid seed production and the male sterile line maintenance is, during hybrid seed production the male fertile plants from the male sterile population are to be rogued off, while they are retained during male sterile line maintenance.

Exercise:

1. What are the different steps involved in hybrid seed production of rice and pigeon pea?

Practical 13. Estimation of heterosis, inbreeding depression and heritability

https://www.youtube.com/watch?v=8SUqOblE17E

Heterosis: The superiority of F1 (hybrid) in one or more characters over its parents

Types:

A. Average heterosis
B. Standard or useful heterosis
C. Heterobelthiosis
D. Luxuriance heterosis

HOW TO ESTIMATE:

A. **Average Heterosis (A. H.) -** When the F_1 is superior than over mid parent.

A.H. = F_1 – MP / MP x 100

Example 1. What is the average heterosis if yield of F1 is 48 qtls/ hec and yield of P1 and P2 respectively 40 and 50 qtls/ hec

Answer:

F1 = 48 qtls/hec

P1 = 40 qtls/hec

P2 = 50 qtls/ hec

MP = P1 + P2 / 2

= 40 + 50 / 2 = 45

A.H. = F1 – MP / MP x 100

= 48 – 45 / 45 x 100 = 6.66%

B. **Standard or useful Heterosis (S. H.):** when the F_1 is superior than standard check variety (SCV)

S.H. = F1 – SCV/ SCV x 100

Example 2: What is the standard heterosis if yield of F_1 is 50 qtls/hec and yield of standard check variety 48 qtls/ hec

Answer:

F1 = 50 qtls/hec

SCV = 48 qtls/hec

S. H. = F1- SCV / SCV x 100

= 50 – 48 / 48 x 100 = 4.16%

C. **Heterobelthiosis H(BP):** when the F_1 is superior over than better parent.

$$\mathbf{H\ (BP) = F1 - BP / BP \times 100}$$

Example 3. What is the heterobelthiosis if yield of F_1 is 50qtls/ hec and yield of P1 and P2 respectively 40 and 48 qtls/hec

Answer:

F_1 = 50qtls /hec

P1 = 40qtls/hec

P2 = 48 qtls/hec

H (BP) = F_1 – BP / BP x 100

= 50-48 / 48 x 100 = 4.16%

Inbreeding depression: when loss or decrease in vigour of F_1 (hybrid) due to inbreeding.

$$\mathbf{I.\ D. = F_1 - F_2 / F_1 \times 100}$$

Inbreeding depression is reduced fitness in a given population as a result of breeding of related individuals. Breeding between closely related individuals, called inbreeding, results in more recessive deleterious traits manifesting themselves. The more closely related the breeding pair is, the more homozygous deleterious genes the offspring may have, resulting the very unfit individuals. Another mechanism responsible is overdominance of heterozygous alleles leading to a reduction of fitness of a population with many homozygous genotype, even if they are not deleterious. Currently it is not known which of the two mechanism is more important. In general, populations with more genetic variation do not suffer from inbreeding depression. Inbreeding depression is often the result of a population bottleneck. Inbreeding depression seems to be present in most groups of organisms, but is perhaps most important in hermaphroditic species, most prominently in plants. The majority of plants are hermaphroditic and thus capable of the most severe degree of inbreeding.

Calculation: The inbreeding is computed as a percentage of chances for two alleles to be identical by descent. This percentage is called "inbreeding coefficient". There are several methods to compute this percentage, the two main ways are the path method and the tabular method.

Typical inbreeding percentage are as follows:

- Father / daughter – mother / son –brother / sister – 25%

- Half –brother / half- sister – 12.5%
- Uncle / niece – aunt / nephew – 12.5%
- Cousin – 6.25%

An inbreeding calculation may be used to determine the general genetic distance among relatives by multiplying by 2, because any progeny would have a 1 in 2 risk of actually inheriting the identical alleles from both parents. For instance, the parent / child or sibling / sibling have 50% identical genetics.

NOTE: For siblings, the degree of genetic relationship is not an automatic 50% (as it is with parent and their children), but a range from 100% at one extreme – as in the case of identical twins (who obviously could not mate as they are the same sex) – to an exceedingly unlikely 0%. Siblings share an average of 50% of their genes, but unlike the 50% ratio between parents and children, the actual ratio between siblings in any given case can vary widely.

Example 4. What is the inbreeding depression if yield of F_1 is 50 qtls / hec and yield of F_2 40 qtls/hec

F_1 = 50 qtls/hec

F_2 = 40 qtls/hec

I.D. = F_1 - F_2 / F_1 x 100

= 50-40 / 50 x 100 = 20%

Exercise:

In maize, 15 hybrids along with 8 parents were evaluated for heterosis on seed yield. The genotypes were raised in RBD with three replications. Calculate the different types of Heterosis and interpret the result.

Sl no.	Genotypes	RI	RII	RIII
1.	P1	17.0	17.2	17.1
2.	P2	15.0	15.6	15.6
3.	P3	15.3	15.0	15.0
4.	P4	14.7	14.7	14.4
5.	P5	19.8	19.4	19.3
6.	P6	22.2	22.3	22.7
7.	P7	20.1	19.9	20.3
8.	P8	26.8	27.2	26.7
9.	P1 x P6	23.2	23.5	23.5
10.	P2 x P6	22.1	22.1	22.9
11.	P3 x P6	21.3	21.1	19.8

12.	**P4 x P6**	**19.1**	**19.0**	**18.6**
13.	**P5 x P6**	**24.0**	**24.6**	**24.0**
14.	**P1 x P7**	**22.6**	**23.2**	**22.9**
15.	**P2 x P7**	**22.8**	**23.2**	**22.7**
16.	**P3 x P7**	**19.6**	**19.7**	**20.1**
17.	**P4 x P7**	**19.9**	**20.0**	**19.5**
18.	**P5 x P7**	**15.4**	**16.0**	**16.3**
19.	**P1 x P8**	**20.1**	**20.6**	**20.5**
20.	**P2 xP8**	**16.0**	**16.5**	**16.4**
21.	**P3 x P8**	**20.6**	**20.9**	**20.6**
22.	**P4 x P8**	**17.3**	**17.9**	**17.9**
23.	**P5 x P8**	**20.7**	**28.0**	**27.9**
24.	**SC**	**17.6**	**14.8**	**15.0**

Practical 14. Study of quality characters, donor parents for different characters

https://www.youtube.com/watch?v=F-UN3Ny4ql0

COMMON NAME	SCIENTIFIC NAME	CHARACTER NO.	QUALITY CHARACTERS
Rice	*Oriyza sativa*	2n=24	• Grain size and shape • Texture of endosperm • Quality of starch in endosperm • Aroma and cooling quality • Milling out form
Wheat	*Triticum aestivum*	2n=14 2n=28 2n=24	• Glutamine content • Dough • Flour quality • Water holding capacity of humus
Maize	*Zea mays*	2n=20	• Protein content • Protein in grain is 20% balanced one
Pegion pea	*Cajanus caja*	2n=22	• Protein content(23%)
Soyabean	*Glysine max*	2n=20	• Protein content(42-45%)
Green gram	*Vigna radiata*	2n=22	• Methionine (high)
Black gram	*Vigna mungo*	2n=22	• Protein content (24-27%) • Methionine
Ground nut	*Arachis hypogea*	2n=20	• Protrin content (45-55%) • High oil content • Kernel • High selfing
Sesamum	*Sesamum indicum*	2n=42	• High oil content
Sunflower	*Heliantha s annus*	2n=34	• High oil content (46-54%)
Mustard	*Brassica compestris*	2n=20	• High oil content (40-45%) • Protein content (30-41%)
Chili	*Capsicum annum*	2n=24	• Fruit length • Ascorbic acid content • Capsacino content

Okra	*Ablmoscnus esculentus*	2n=130	• Pod length, seed / pod test weight
Cucumber	*Cucumis sativa*	2n=14	• Shape of fruit firmness of fruits
Chrysanthemum	*Chrysanthemum monifolium*	2n=13	• Flowering quality, average flower weight, flower diameter
Gerbera	*Gerbera*	2n=50	• Colour of flower leaf and leaf area no. of flowers/plant shelf life of flowers buckers production capacity
Mango	*Mangifera indica*	2n=40	• Self-life of fruit high ascorbic acid %
Guava	*Psidium guajava*	2n=22	• Colour, flower, texture, taste of fruit
Rose	*Rosa sp*	2n=14	• Large flower bud maximum bud diameter, no. of petals/flowers
Papaya	*Carica papaya*	2n=18	• TSS, pH, ascorbic acid
Banana	*Musa sp*	2n=22	• Fresh appearance of fruit self like, shape of fruit at pedicel
Tomato	*Solanum lycopersicum Lycopersicum esculantum*	2n=24	• Fruit uniformity

Exercise:

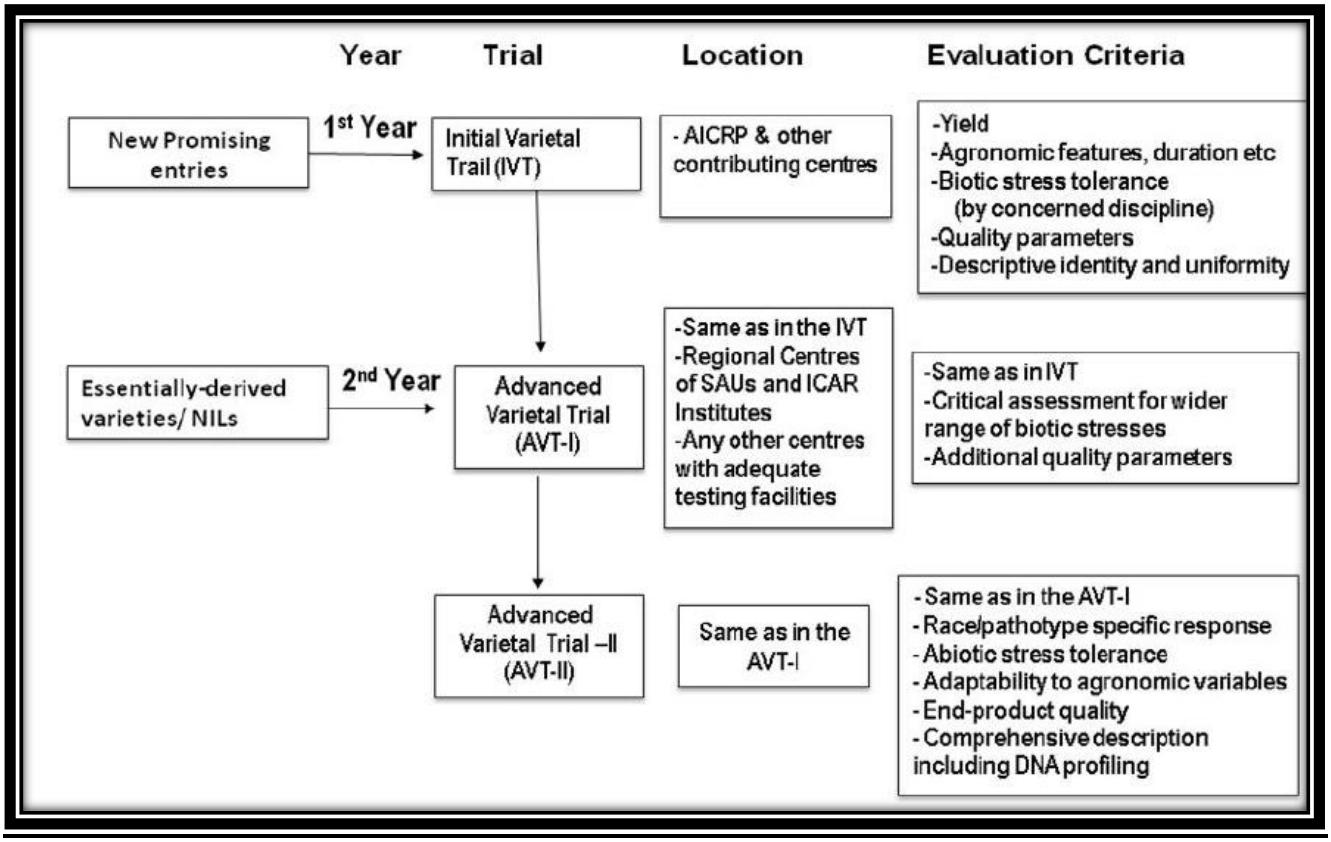
1. Mention the different quality characters of *kharif* crops under your study.

Practical 15. Visit to any research station to know seed production and certification plots, AICRP trails and programmes

Mandate of AICRP

- Development of agro-ecological specific high yielding, nutritionally superior varieties with in-built resistance/ tolerance to major biotic and abiotic stresses.
- Generation of location specific agro-production and protection technologies.
- Conduct of multi-location testing
- Technological assessment through conduct of front line demonstration.
- Production of breeder seed.

Testing Procedure



All India Coordinated Research Project on Sesame, Tikamgarh

1. **Name of AICRP**: Sesame, College of Agriculture, Tikamgarh
2. **Year of start:** 1981
3. **No. of Scientists**: 03 [01-F, 02- V]

Most important achievement and their quantifiable impact (Importance wise):

- Six varieties of white seeded sesame having export demand *i.e.* TKG-21 (1993), TKG-22 (1995), TKG-55 (1999), JTS-8 (2000), TKG-306 (2004) and TKG-308 (2009) have been developed and released.

- TKG-308 white seeded early maturing variety (82 DAS) notified in 2008 having 662kg/ha seed yield with 2.80g test weight and 49.6% oil content with moderately resistant and phytophthora blight. Most dominating variety of Bundelkhand as evidenced by National indent of breeder seed.
- TKG 306 white seeded early maturing variety (80 DAS) notified in 2004 having 570 kg/ha seed yield with 2.80g test weight and 46.6% oil content with multiple resistant to phytophthora blight, phyllody and moderately resistant to macrophomina, cercospora, powdery mildew and alternaria leaf spot and also resistant to Antigastra at capsule stage. It is one of the most dominating varieties of Bundelkhand as evidenced by National indent of breeder seed.
- JTS-8 notified in 2000 white seeded medium maturing duration (86 DAS) JTS-8 having 629 kg/ha seed yield with 2.94g test weight and 52.3% oil content with resistant to Phylloldy moderately resistant to macrophomina stem and root rot, Alter aria Leaf spot and resistant to lepidopteron antigastra designated as **Zonal check** for Zone II.
- TKG 55 white seeded extra early maturing (78 DAS) notified in 1998 having 630kg/ha seed yield with 2.94g test weight and 52.3% Oil content with resistant to macrophomina stem and root rot and tolerant to phytophthora blight and antigastra.
- TKG 22 white seeded early maturing variety notified in 1994 being used as **National Check** for AICRP trials having 602-716 kg/ha seed yield with 2.94g test weight and 53.3 % oil content. It has resistance to macrophomina stem and root rot and tolerance to phytophthora blight and antigastra.
- TKG 21 white seeded extra early maturing notified in 1992 having 952Kg/ha seed yield with 3.13 g test weight and 55.6% oil content with tolerance to Phytophthora, Alternaria, Bacterial leaf spot, phyllody, leaf curl and stem blight. It is highly responsive to higher dose of fertilizer application and tolerant to lodging.

Exercise:

1. Fill in the following information during the visit

Place of Visit:

Date of the visit:

Objectives:

Crop Name	Name of the AICRP	Location	Year of starts	No. of scientists	Achievements	Remarks