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A practical manual on

Fundamentals of Plant Breeding

Credits: 3(2+1)

Subject code: 13A.209

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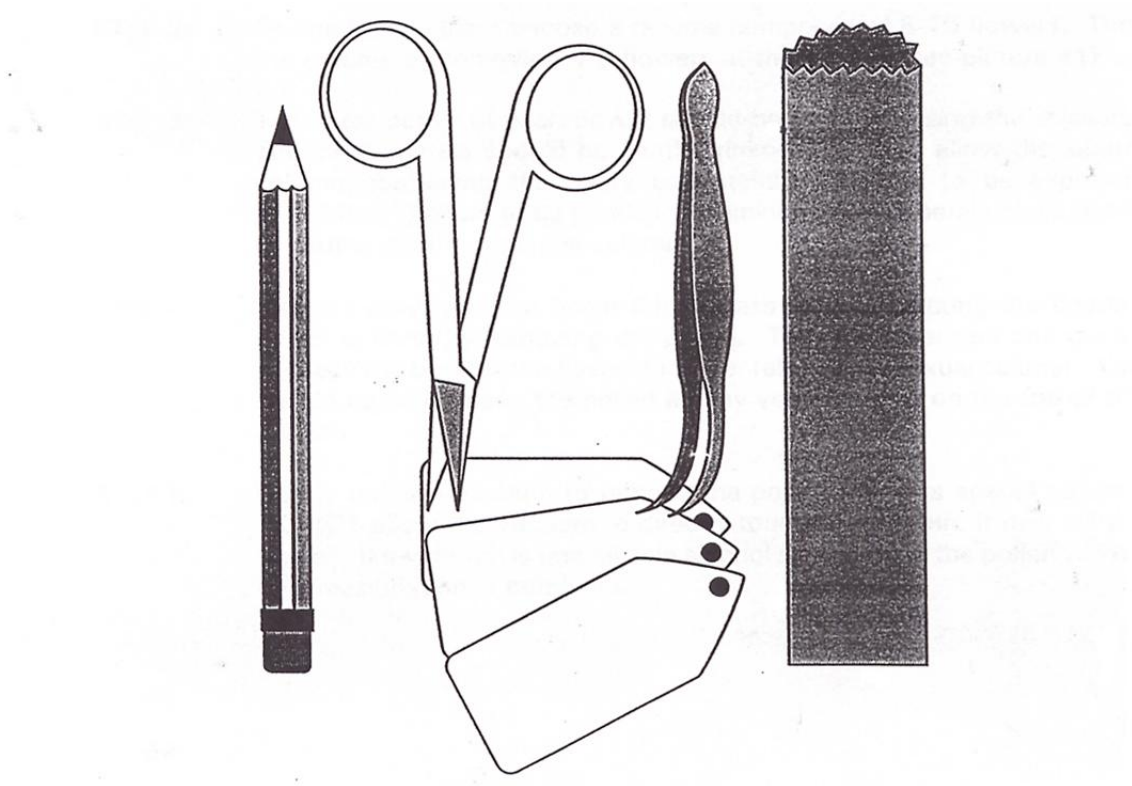
Laboratory Manual

Practical 1. Plant Breeder's kit, Study of germplasm of various crops

Materials required: Forceps, scissors, alcohol, tags, pencil and butter paper bags

Principle: Germplasm is living tissue from which new plants can be grown. It can be a seed or another plant part – a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant. Various institutes with different objectives are engaged in plant and/or germplasm collecting activities. The collecting of plant genetic resources primarily aims at tapping germplasm variability in different agri-horticultural (crop) plants, their wild relatives and related species. The germplasm so collected reveals the nature and extent of variability in different species, within species, cultigens, etc. as also their agro-ecological/phyto-geographical distribution. Knowledge of agro-ecology, crops and their distribution and harvesting time in areas of survey, local contacts, equipment required, transport arrangements and routes to be followed, distances involved, places of halt/camping sites available, transport of material, besides team-composition etc. is to be acquired before setting out on a collecting expedition. Of equal importance is to acquire knowledge on diversity in crop plants *vis-a-vis* its distribution to tap target areas and/or target species and the variability contained thereof.

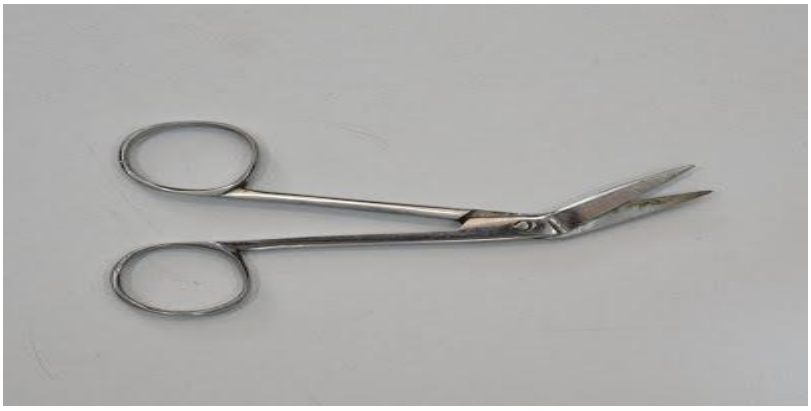
Plant breeder's kit:



Fine pointed forceps

Use: it is used for incising the floral buds and for removing the anthers from it. E.g. Tobacco, sesamum etc.

Small/ curved scissor



Use: for cutting the small florets in cereals and small flowers in the crops like lucerne, guar etc.

Long straight scissor

Use: it is used for clipping, cutting the vegetable parts and large size floral parts in cereals like wheat, sorghum, bajra, and tobacco.

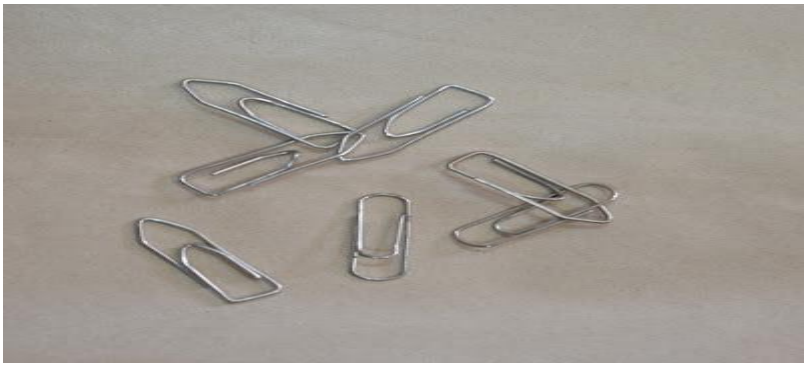
Sharp pointer

Use: it is used for incising the floral parts and for removing the anthers from the crops like bajra.

Eye lens or magnifying lens

Use: For observing the reproductive parts to confirm that there should not be any part of the anther left on the stigma or stigma is free from any foreign pollens.

U-pins(u- clips)



Use: It is used for fasting the bags on earheads or flowers to keep the bag in proper position.

Paint brush

Use: It is used for transferring the pollen grains in crops like Castor, Sorghum etc.
Advantage: Without injuring to stigmas or pollens, pollination is accomplished very smoothly.

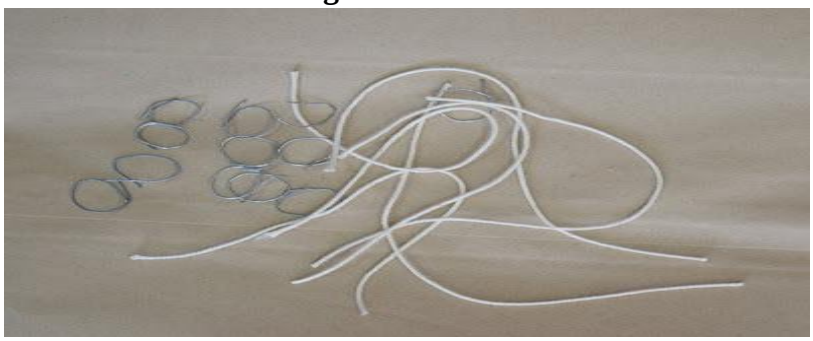
Pencil

Use: It is used for writing field labels or field bags. Sometimes it is also used for emasculating the sorghum flowers. Compared to ink or ball pen writing, pencil writing should be preferred as it will not erase or spread during rains, dew or under intense light.

Washing bottle

Use: It is used for filling sterilizing agent like alcohol or spirit to sterilize the scissors, pointers, forceps and brush during crossing work.

Wire ring and smooth thread



Use: It is used for selfing in crops like Cotton, Okra, Sesamum etc. Thread is used for fastening(tying) the bud, while ring is inserted in axis of flowers to identify it. Compared to bags, this method is more convenient and cheaper.

Small white tag

Use: It is used for identifying the internal flower or a small twig during crossing programme. The detailed information about crossing is written on it with pencil and then it is inserted on pedicel or peduncle e.g. Cotton, Bajra, Wheat, Sorghum, Sesamum etc.

Soda straw tubes

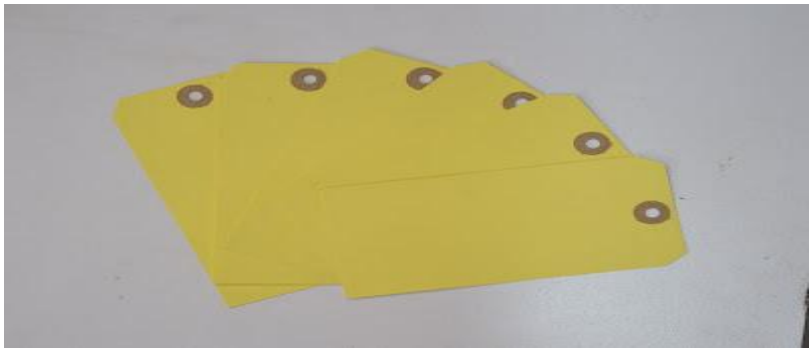
Use: it is used for protecting the emasculated or pollinated flower buds of cotton, tobacco etc.

Advantage: compared to paper bags it is very convenient, easy and cheaper method of selfing and crossing.

Waxy threads

Use: It is used for fastening(tying) the luggage labels on the plants.

Luggage labels (white or yellow)



Use: It is used for tagging the large sized plants like Tur or Castor while rouging or during selection.

Aluminium label with wire

Use: It is used for tagging the flowers in fruit crops or tree species after crossing. It is also used for identification of selected trees.

Muslin cloth bag (large size)

Use: To cover the whole plant while selfing or crossing in the crops like Chillies, Brinjal etc. In large sized plants like Tur it can be used for protecting individual branch also.

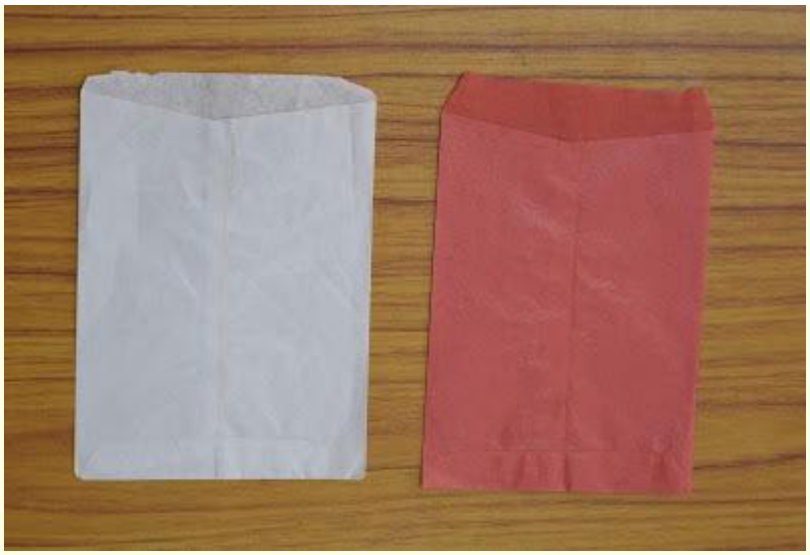
Yellow sample bag

Use: For storing the crossed seeds in small quantity.

Paper Bag

Use: For selfing Bajra, Wheat, Sorghum, Castor etc.

Kite paper bag (white/red)



Use: It is used for protecting small size flowers of Pulses, Oilseeds and Other food crops during selfing and crossing.

Germplasm collecting stratagies

A. For seed collections (*cultivated and wild species*)

1. Collect from (30- 100) individuals per site (50 seeds of each as one sample or less, if necessary, at random. One inflorescence per plant is generally suitable.
2. Sample as many sites as possible according to availability of time.
3. Choose sampling sites over as broad an environmental range as possible. This should capture all alleles with frequency of 5 percent or more in the population.
4. Change tactics, where necessary, for wild species, that is, where individuals are scattered, you may need to consider that a population for sampling spreads over several square kilometres.
5. If considerable morphological variation is present in a population, make separate samples of each type.
6. Add biased sampling if some morphotypes are not included in random sampling.
7. Take whole inflorescences, as well as seeds, where necessary, as vouchers.
8. Make herbarium specimens, where possible.
9. Take photographs.
10. Write meticulous field notes.

B. i) For vegetatively propagated cultivated species

1. Sample each distinct morphotype in a village.
2. Repeat at intervals over an area.

3. Supplement with seed collections, where possible, and give same collection numbers if seeds come from the same plants as the vegetative samples. If they do not or are bulked samples, give separate collection numbers.

ii) *For collecting wild vegetatively propagated species*
Collect just a single propagule from each of 10-15 individuals as a bulk sample (less if organs are very large, more if smaller, from area of about 100 x 100 m).

Germplasm cataloguing, Data storage and Retrieval

Each germplasm accession is given an accession number. This number is pre fixed in India, with either IC (Indigenous collection), EC (exotic collection) or IW (Indigenous wild). Information on the species and variety names, place of origin, adaptation and on its various feature or descriptors is also recorded in the germplasm maintenance records. Catalogues of the germplasm collection for various crops are published by the gene banks. The amount of data recorded during evaluation is huge. Its compilation, storage and retrieval is now done using special computer programmes.

National Bureau of Plant Genetic Resources (NBPGR)

NBPGR establishment in 1976 is the nodal organisation in India for planning, conducting, promoting, coordinating and lending all activities concerning plant.

Practical 2 Study of floral structure of self-pollinated and cross pollinated crops

1. **Floral structure of Rice (*Oryza sativa*): A self pollinated crop**

Panicle

- The inflorescence of rice plant, borne on terminal shoot and thus called as panicle.
- It is determinate type and at maturity, it is droopy in nature.

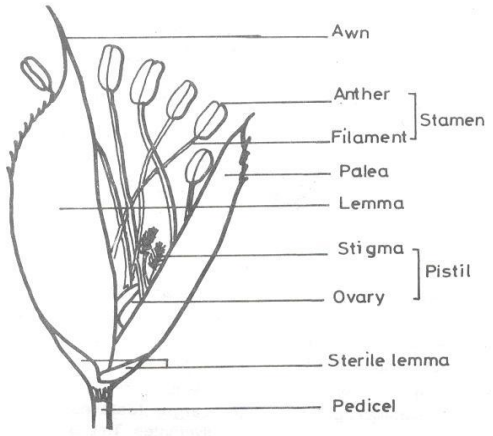


Fig: A flowered rice spikelet

Spikelet

- A spikelet is the floral unit and consists of two sterile lemmas, a lemma, a palea and the flower.
- Its parts are:
    1. Lemma: It is a 5- nerved hardened bract with a filiform extension (of the middle nerve) known as awn.
    2. Palea: IT is a 3- nerved bract slightly narrower than lemma.
    3. Flower: It consists of 6 stamens with two-celled anthers and a pistil with one ovary and two stigmas. The pistil contains one ovule.

2. Floral Structure of wheat (*Triticum aestivum*): A self pollinated crop

Floral Biology

The inflorescence of wheat is a spike bearing two opposite rows of lateral spikelets and a single terminal spikelet on the primary axis. The unit of spike is called spikelet. Two to five florets are born in each spikelet, subtended by a pair of glumes. Each floret contains three anthers and a pistil bearing two styles each with feathery stigma and two ovate lodicules which are modified perianth structure. Florets at anthesis are forced open by swelling of the lodicules. Flowering starts several days after the wheat spike emerges from the boot. Florets on the main culm flower first and those on the tillers flowering later. Flowering begins in the early morning and continues throughout the day. Two to three days are required for a spike to finish blooming. A wheat grain is caryopsis, a small dry, indehiscent, one seeded fruit with a thin pericarp consisting of a germ or embryo and an endosperm.

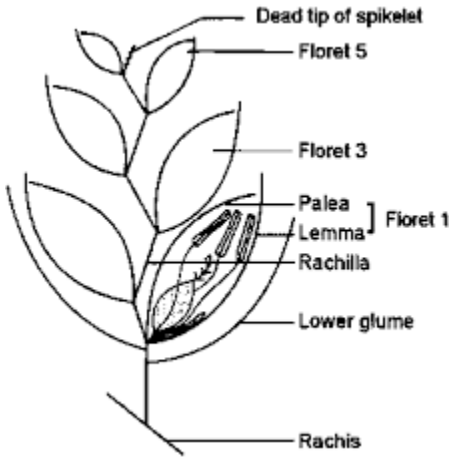


Figure: A wheat Spike

3. Floral structure of Maize (*Zea mays*) A cross pollinated crop

Floral biology

It is monoecious plant bearing male flower are the growing tip as tassel and female flower at the axial of the leaf on the shoot. The tassel is terminal with staminate flowers in several roots. Each pairs of flower consist of sessile and pedicillate spiklet. Each spiklet contains two similar

glumes. The flower contains membranous palea with three stamens and two lodicules. The pollens remain viable for 18 to 24 hours.

The female inflorescence is a spadex known as cob or ear. It is modified lateral branch developed from lateral bud. The shoot is composed of compressed internodes from which husk rise and terminates in an ear on which the sessile are borne. Spiklets are in pair. Each spiklet having two flowers, the lower one is reduced to lemma and palea is non-functional, while upper one contained knob shaped ovary surrounded by broad lemma and thin palea. One carpel is provided with long silky hair, which behaves as style and style stigma throughout the length.

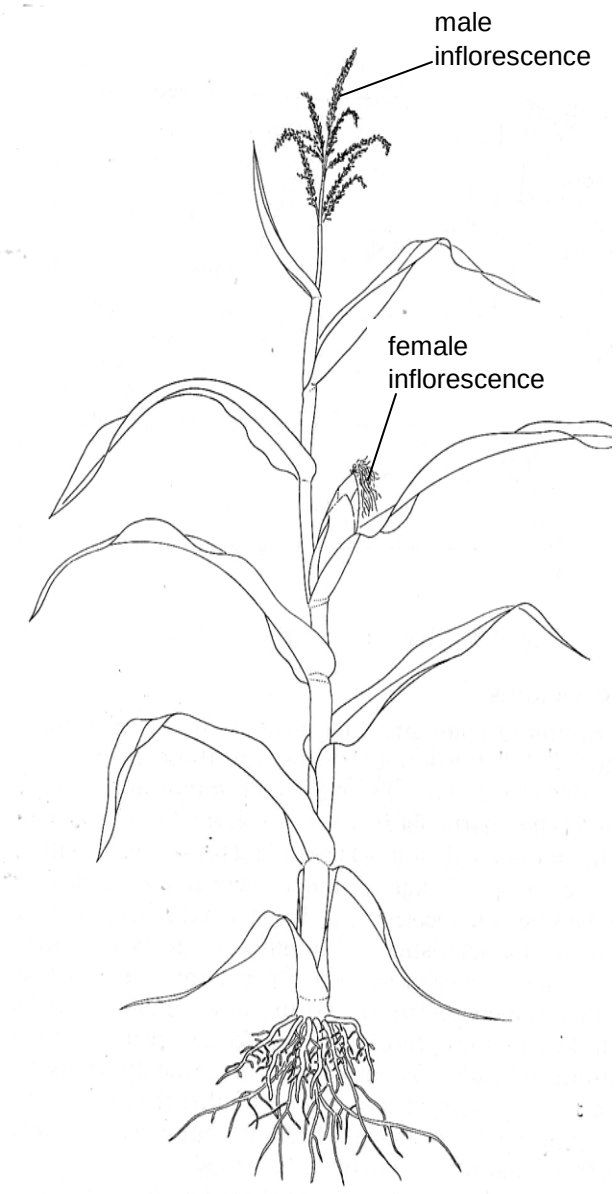


Figure 1: Maize plant showing position of male and female inflorescences

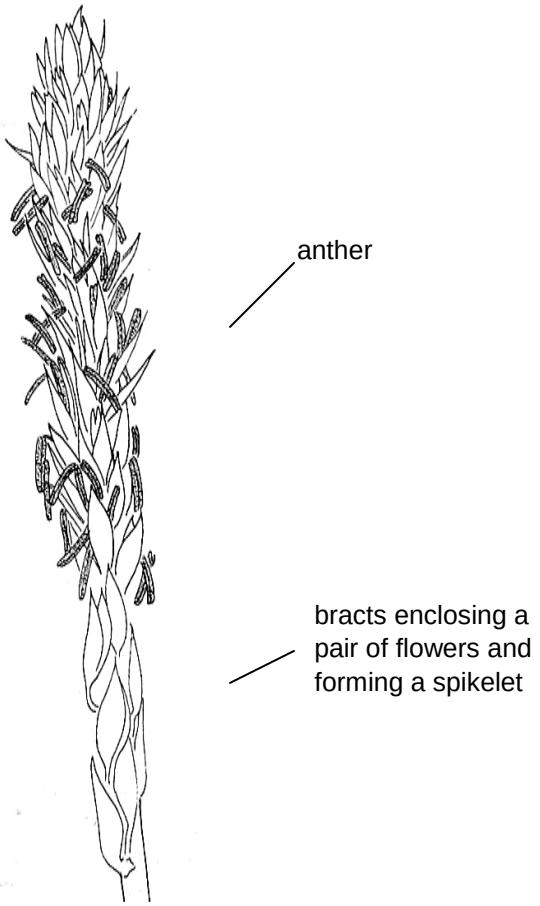


Figure 2: Part of male inflorescence

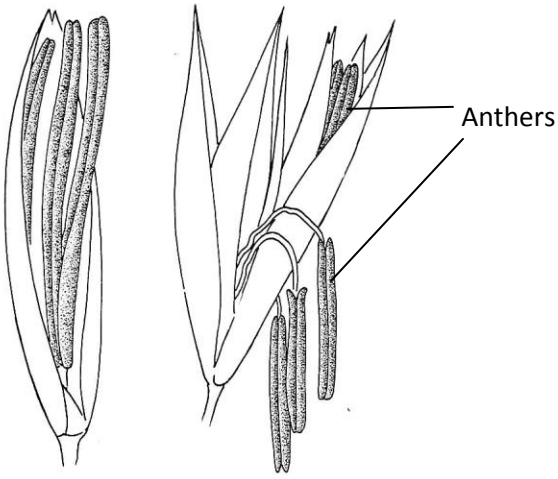


Figure 3: Male flowers

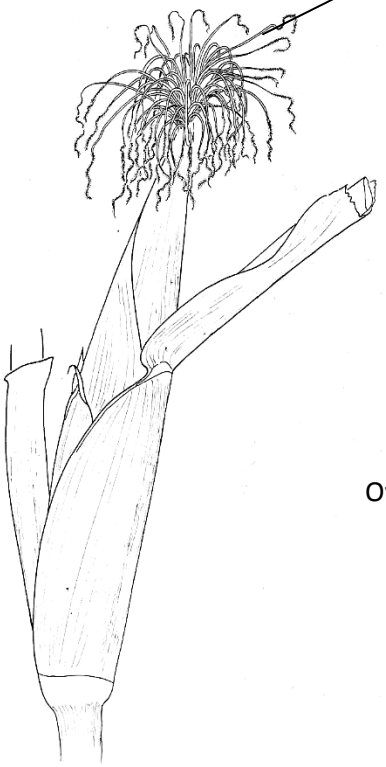


Figure 4: Female inflorescence

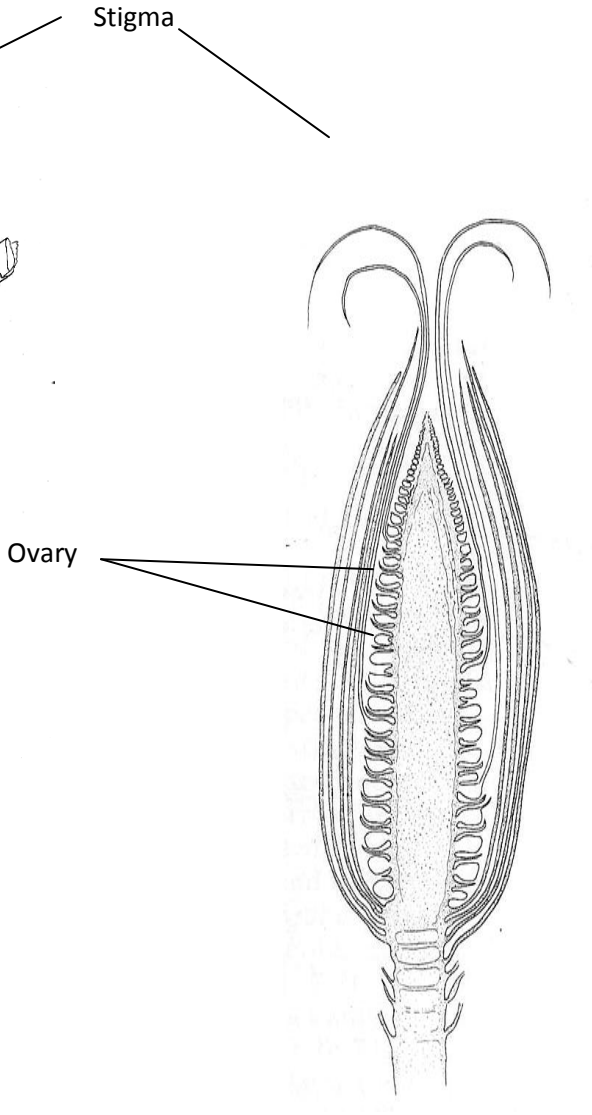


Figure 5: Longitudinal section through female inflorescence

Practical 3: Emasculation and hybridization techniques in self-pollinated crops

Methods of Emasculation

1. Hand Emasculation

In species with large flowers, removal of anthers is possible with the help of forceps. It is done before anther dehiscence. It is generally done between 4 and 6 PM one day before anthers dehisce. It is always desirable to remove other young flowers located close to the emasculated flower to avoid confusion. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with **epipetalous stamens** e.g. gingelly.

In cereals, one third of the empty glumes will be clipped off with scissors to expose anthers. In wheat and oats, the florets are retained after removing the anthers without damaging the spikelets. In all cases, 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 the anthers from the flowers. The amount of suction used is very important which should be sufficient to suck the pollen and anthers but not gynoecium. In this method considerable self-pollination, upto 10% is like to occur. Washing the stigma with a jet of water may help in reducing self-pollination, However self pollination can not 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 sorghum 42-48^{O}C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-44^{O}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. In Lucerne the inflorescence immersed in 57% alcohol for10 second was highly effective. It is better method of emasculation than suction method.

5. Cold Treatment

Cold treatment like hot water treatment kills the pollen grains without damaging gynoecium. In the case of rice, treatment with cold water $0.6^{O}C$ kills the pollen grains without affecting the gynoecium. This is less effective than hot water treatment.

6. 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 pearlmillet, 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 (e.g.) Cumbu.

7. Use of Gametocide

Also known as chemical hybridizing agents (CHA) 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.

Bagging

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

Crossing

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

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 may be recorded on the tag with pencil.

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

1. In Paddy

Emasculation:

It is done in the afternoon on preveious day or early in the morning on the day of pollination. The ear just emerged is selected and all spiklets already opened are clipped the spiklets which

are likely to be opened are selected and six anthers from each spiklet is removed with needle and fine pointed forceps. The emasculated ear after examination with lens covered with perforated butter paper bag and labelled.

In mass emasculation method hot water having temperature 42 to 45 0C is carried in thermos flask in the field. The panicle of the proper stage is selected and inserted in the water for 2 to 3 minutes. The flask is unopened spiklets are clipped off.

Pollination:

It is done on next day morning. Matured anthers are collected from protected male parent in petri dish and dusted on the stigma of emasculated flower with brush and forceps and covered with butter paper bag to protect natural cross pollination.

Practical 4: Emasculation and hybridization techniques in cross pollinated crops

In Maize

Emasculation:

The tassels of the female plants are removed immediately as soon as appeared. The process is called as detasseling. It is always done in the morning. Ear shoot which emerging from the leaf sheath is bagged 1 to 2 days below the tip of the preveious day of pollination.

The tassels of selected male parents is also covered with bag on following day in the morning between 9.00 to 10.00 a.m. pollens from tassel bag is dusted over the silk of the female cob/eat. The bag covered ear shoot is torn and bag from the male parent may be placed over the cob. Care should be taken to avoid contamination of silk with foreign pollens.

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.

Practical 5: Consequences of inbreeding on genetic structure of resulting populations

Inbreeding is a form of mating system in sexual organism. It implies mating together of individual that are close to each other by ancestral or pedigree relationship.

When the individuals are closely related E. g Full sib mating, half sib mating. The highest degree of inbreeding is achieved by selfing. The chief effect of inbreeding is to increase homozygosity in the progeny, which is proportionate to the degree of inbreeding. Cross – pollinated and asexually reproducing species are highly heterozygous in nature. These species show a severe reduction in fertility and vigour due to inbreeding (inbreeding depression). It contrast to this hybridization between unrelated strains leads to an increased vigour and fertility (hybrid vigour or heterosis). These two aspects are of great significance in breeding of these species. In fact heterosis and inbreeding depression may be considered as the two opposite sides of the same coin.

Inbreeding Depression:

It refers to decrease in fitness and vigour due to inbreeding or it may be defined as the reduction or loss in vigour and fertility as a result of inbreeding.

The most revealing impact of inbreeding is the loss of vigour and the physiological efficiency of an organism characterised by reduction in size and fecundity. For example selfing reduces heterozygosity, by a factor ½ in each generation. In fact the degree of inbreeding in any generation is equal to the degree of homozygosity in that generation. Inbreeding depression results due to fixation of unfavourable recessive genes in F2, while in heterosis the unfavourable recessive genes of one line (parent) are covered by favourable dominant genes of other parent.

The primary genetic consequence of inbreeding is increased homozygosity (Falconer and MacKay 1996). Two hypotheses for the genetic basis of inbreeding depression are put forth, both of which depend on the idea that homozygosity wll increase with inbreeding. Either the overdominance or partial dominance hypotheses are invoked to model the negative consequences of inbreeding (Charlesworth and Charlesworth 1987; Lynch 1991; Karkkainen et al. 1999). In the overdominance hypothesis, inbreeding depression is attributable to higher fitness of heterozygotes versus homozygotes for the loci in question (Frankham et al. 2003). For the partial dominance hypothesis, negative fitness consequences for inbred lines are due to the fixation of recessive or partially recessive deleterious alleles (Frankham et al. 2003). Current thought favors the latter hypothesis, where inbreeding depression is attributable to many genes of small effect (Keller and Waller 2002, Frankham et al. 2003). However, distinguishing between the two genetic mechanisms is complicated by linked sets of deleterious recessives that imitate overdominance effects (Keller and Waller 2002).

Practical 6: Study of male sterility system

1. Palynology

This is the science involving the study of pollens. The pollen has a very minute structure. It is unicellular and usually round although it may be oval, pyramidal, polyhedral etc. It is provided with two coats-an inner, delicate, cellulose layer called **intine** and an outer tough, cutinised layer called exine or **extine**. The exine is often sculptured or provided with spines, warts etc., occasionally, it is smooth. The exine may have a waxy coating to render the pollen more or less waterproof. Very often, there are some definitely thinner circular spots or slits in the exine called **germ pores** or **slits**. These weak spots are utilized during the germination of the pollen.

2. Preparation of Acetocarmine Stain ($C_{22}H_2O_{13}$)

It is one of the most widely used stain for pollen study. A mixture of 4 ml glacial acetic acid and 55 ml of distilled water is boiled. A quantity of 1 g of carmine (according to the strength required) is added to 100 ml of the above mixture at about boiling point and then boiled for few minutes. After boiling, the contents are removed from the flame and allowed to cool and filtered in a clean bottle. The filtrate is reddish in colour and known as 1% acetocarmine. Ferric chloride or ferric acetate may be added if necessary for deep staining and preservation.

Fertility and sterility in A, B, R and TGMS lines

Male sterility is characterized by nonfunctional pollen grains, while female gametes function normally. It occurs in nature sporadically.

Types of male sterility, maintenance and uses:

Male sterility may be conditioned due to cytoplasmic or genetic factors or due to interaction of both. Environment also induces male sterility. Depending on these factors male sterility can be classified in to

a) Cytoplasmic male sterility (CMS)

b) Genetic male sterility (GMS)

c) Cytoplasmic-genetic male sterility (CGMS)

d) Environmental induced male sterility which is again sub divided in to

i) TGMS (Theromosensitive)

ii) PGMS (Photo sensitive)

A line or ms line: It represents a male sterile line belonging to any one of the above categories. The A line is always used as a female parent in hybrid seed production.

B line or maintainer line: This line is used to maintain the sterility of A line. The B line is isogenic line which is identical for all traits except for fertility status.

R line or restorer line: It is other wise known as Restorer line which restores fertility in the A line. The crossing between A x R lines results in F_1 fertile hybrid seeds which is of commercial value.

Pollen fertility count

a. Different crop species

Crop species	**Number of pollen grains**		**Total**	**Percentage of pollen fertility**
	Unstained	**Stained**		

b) A, B & R Lines of rice, cumbu.

Lines	Number of pollen grains		Total	Percentage of pollen fertility
	Unstained	Stained		
A				
B				
R				

Practical 7: Handling of segregation populations

Maintenance of Records

1. Accession Register
2. germplasm Bank
3. Descriptive blank register
4. Cropping programme
5. Single plant selection register
6. Row test
7. Replicated row test
8. Preliminary/Initial evaluation trial
9. Comparative yield/ yield evaluation trial
10. Multilocation I, II trials.
11. Quality observations Note book
12. Record of crosses
13. F1 generation
14. F2 segregation generation note book.

Accession Register

This will contain the details of the seeds/ planting material with regard to receipt date, source, their number, number assigned at the receiving unit, short description of the planting material, to whom sent for evaluation, date, feed back information about the genotype, now how maintained etc., Accession number given by the serial number followed by the year of entry i.e. serial 145 in 1991. Then accession number will be 145191 or 91145. It will be mentioned as EC = Exotic collection IC = Indigenous collection.

Proforma for Accession Register

Accession No. 1	Name of variety 2	Date of Receipt 3	Source of seed 4	Source No. 5	Pedigree record 6	Description of the material 7	How disposed to whom sent 8	Feed back information 9	Re-marks 10

Standard form of a Field Note Book

Each field note book should contain the following information.

A. Yield Trial

i) First Page

a) Number and title of the project
b) Season of raising the crop

c) Unit under which the trial is being conducted

ii) Second page

a) A full plant of the field showing the location of the trial with the approach path.
b) North East directions should be specified.

iii) Third Page

a) Plan of the experiment
b) Experiment details
1. Name of the experiment
2. Season
3. Number of variants
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/planting
9. Date of harvest
10. Name of the Principal Investigator

iv) Fourth page

Details of cultural practices followed for the plot/ field

a. Date of ploughing
b. Date of layout of the trial
c. Manurial schedule adopted
   Basal :
   Topdressing :
d. Irrigation schedules with date from life irrigation onwards
e. Plant protection schedules followed
f. Details of intercultural operations A (hoeing, weeding, and earthing up etc.,)
g. Date of harvest
h. Duration of processing till storage
i. Rainfall received during the crop growth
j. General remarks on the seasonal condition prevailed and its effects on crop growth including the occurrence of pests and disease.

v) Fifth page

One page for each variant per replications 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.

e.g.	Rice	: Date of earhead emergence in the main shoot number of tillers, : Date of earhead emergence in tillers and : Number of tillers.
	Cotton	: Number of sympodial branches : Number of monopodial branches
	Cumbu	: Date of emergence of female flowers : Date of emergence of male flowers : Number of tillers
	Sunflower	: Duration of flower opening etc.,

Generation study

This field note book will contain the following information.

a. Plan for the segregation generation
b. Details of the generation

1. Name of the generation study
2. Number of crosses
3. Details of the cross
   Cross No. Female parent, Male parent, Number of families, number of seed sown.
4. Length of row
5. Spacing (cm)
6. Date of sowing
7. Dates of harvest
8. Name of the Principal Investigator

c. Plan for the segregation generation

d. Details of the generation

1. Name of the generation study
2. Number of crosses
3. Details of the cross
   Cross No. Female parent, Male parent, Number of families, number of seed sown.
4. Length of row
5. Spacing (cm)
6. Date of sowing
7. Dates of harvest
8. Name of the Principal Investigator

Practical No 8: Methods of calculating mean, range, variance, standard deviation, heritability

The Mean

The sample mean is the average and is computed as the sum of all the observed outcomes from the sample divided by the total number of events. We use x bar as the symbol for the sample mean. In math terms,

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x$$

where n is the sample size and the x correspond to the observed valued.

Example

Suppose you randomly sampled six acres in the Desolation Wilderness for a non-indigenous weed and came up with the following counts of this weed in this region:

34, 43, 81, 106, 106 and 115

We compute the sample mean by adding and dividing by the number of samples, 6.

$$\frac{34 + 43 + 81 + 106 + 106 + 115}{6} = 80.83$$

We can say that the sample mean of non-indigenous weed is 80.83.

2.Variance, Standard Deviation and Coefficient of Variation

The mean, mode, median, and trimmed mean do a nice job in telling where the center of the data set is, but often we are interested in more. For example, a pharmaceutical engineer develops a new drug that regulates iron in the blood. Suppose she finds out that the average sugar content after taking the medication is the optimal level. This does not mean that the drug is effective. There is a possibility that half of the patients have dangerously low sugar content while the other half have dangerously high content. Instead of the drug being an effective regulator, it is a deadly poison. What the pharmacist needs is a measure of how far the data is spread apart. This is what the variance and standard deviation do. First we show the formulas for these measurements. Then we will go through the steps on how to use the formulas.

We define the variance to be

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2$$

and the standard deviation to be

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2}$$

Variance and Standard Deviation: Step by Step

Calculate the mean, x.

Write a table that subtracts the mean from each observed value.

Square each of the differences.

Add this column.

Divide by n -1 where n is the number of items in the sample This is the variance.

To get the standard deviation we take the square root of the variance.

Example

The owner of the Ches Tahoe restaurant is interested in how much people spend at the restaurant. He examines 10 randomly selected receipts for parties of four and writes down the following data.

44, 50, 38, 96, 42, 47, 40, 39, 46, 50

He calculated the mean by adding and dividing by 10 to get

x = 49.2

Below is the table for getting the standard deviation:

x	x - 49.2	(x - 49.2)2
44	-5.2	27.04
50	0.8	0.64
38	11.2	125.44
96	46.8	2190.24

42	-7.2	51.84
47	-2.2	4.84
40	-9.2	84.64
39	-10.2	104.04
46	-3.2	10.24
50	0.8	0.64
Total		2600.4

Now

$$\frac{2600.4}{10 - 1} = 288.7$$

Hence the variance is 289 and the standard deviation is the square root of 289 = 17.

Since the standard deviation can be thought of measuring how far the data values lie from the mean, we take the mean and move one standard deviation in either direction. The mean for this example was about 49.2 and the standard deviation was 17. We have:

49.2 - 17 = 32.2

and

49.2 + 17 = 66.2

What this means is that most of the patrons probably spend between $32.20 and $66.20.

Practical 9: Designs used in plant breeding experiments, analysis of Randomized Block Design

Laying out of Field Experiments

The basic objective of plant breeding is the ultimate crop improvement. It results in development of high yielding varieties hybrids etc., over the existing cultivars and so on. The performance of the new varieties are confirmed from the results obtained from the field experiments. To be explained scientifically the field experiments are laid out following certain rules and the data thus collected are analyzed statistically. The steps involved in this process are explained here under.

Any designing of experiments involves three major steps.

1. Selection of experimental units

The objects on which the treatments are applied is known as experimental units.

Eg. Plots in the field, plant, etc.,

2. Fixing of treatments

The objects of comparison are known as treatments

Eg. Varieties, spacing etc.,

3. Arrangement of treatments in the experimental Units.

It comprises of three basic principles of design

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.

The objective of replication, randomization and local control is to minimize the Experimental Error (EE). EE is nothing but differences in the responses from the experimental unit to experimental unit under similar environments. Apart from these, EE

can be reduced further by proper selection of the experimental units and choosing of most appropriate experimental design for a given number of treatment.

Types of basic experimental designs

1. Completely Randomized Design (CRD)
2. Randomized Block Design (RBD)
3. Latin Square Design (LSD)

Among these, RBD is the widely used design.

Laying Out of RBD

A. The experimental material (field) is divided first into blocks consisting of homogenous (uniform) experimental units. Each block is divided into number of treatments equal to the total number of treatments.

B. Randomization should be taken within each block and the treatments are applied following the random number table.

C. Collection and analysis of data: After the collection of data from the individual experimental unit (treatments) ANOVA (Analysis of Variance) table is formed.

The significance of the ANOVA table is that it indicates the sources of variation exhibited by the treatments, the magnitude of variation derived from different sources and their worthiness (significant/ non significant).

D. 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 treatments mean is less than CD it can be concluded both the treatments are on par.

RT: Row trial

Row trial is generally conducted in F_3 and F_4, when the seeds are not sufficient for replication with individual plant progeny rows. Each row consists of about 20 or more plants. Individual plants with desirable characteristics are selected from superior progeny rows. Pest, Disease and lodging susceptible progenies with undesirable characteristics are eliminated.

RRT – Replicated Row Trial

It is generally conducted from F_3 generation onwards. Depending on availability of seeds, 3-4 more rows are grown for each progeny to facilitate comparison among progenies

adopting suitable replications. Families, which have become reasonably homozygous may be harvested in bulk. From those families showing segregation, single plants are selected for characters under study. The breeder has to visually assess the yielding potential of progenies and reject the inferior ones in the field and the yield potential has to be assessed in the laboratory for confirmation.

PYT – Preliminary Yield Trial Or (IYET) Initial Yield Evaluation Trial

It is conducted from F_5 generation onwards. Preliminary yield trial with three or more replications are conduct to evaluated the comparative performance of the culture and to identity the superior cultures among them. The cultures are evaluated for plant height, lodging, pest and disease resistance, flowering time, duration and yield, etc., Quality tests may also be carried out. Standard commercial varieties must be included as checks for comparison. Ten to fifteen outstanding cultures, if superior to checks, would be advanced to the Advanced yield trails.

AYT – Advanced Yield Trial

Advanced Yield Trial is conducted from F8 generation on wards. The superior cultures identified from Preliminary Yield Trial are tested in Replicated Yield Trial. In this trial, the cultures are evaluated for yield, pest, disease and lodging resistance, duration, quality, etc.

Multi location trial is conducted from F_{13} onwards for 3 years by the Research Station Scientists. Multilocation Trial are useful for suitability studies i.e. whether a particular culture is able to perform well in all the locations or not. Stable performance of a culture over all the locations will be promoted to ART.

ART – Adaptive Research Trial

It is conducted after MLT for 3 years by the Department of Agriculture. Nearly 3-4 cultures are tested and based on the performance of 3 Years in the farmers field, the best culture over the check may be proposed to SVRC (State Variety Release Committee) for releasing.

If the SVRC finds that the cultivar is suitable for any particular area or through out the state, then the variety is released and is notified by the State Department of Agriculture.

Practical 10: To work out the mode of pollination in a given crop and extent of natural out-crossing

AIM: To work out the mode of pollination in a given crop and extent of out crossing.

1. To work out the mode of pollination in a given crop.

There are several approaches:

a) Morphological examination of flowers:

Mechanism like dioecy , monoecy, protogyny, protandry and cleistogamy are easily detected. They indicate the mode of pollination.

b) Space isolation:

Growing single plant of a crop in isolation and recording the seed sit, determines the extent of pollination. Failure o set seeds in isolation proves the crop to be cross pollinated and seed set is indicative of self-pollination.

c) Effects of selfing (inbreeding):

Vigour due to inbreeding is common in cross pollinated species while self-pollinated crops show no inbreeding depression.

2) To work out the extent of out crossing:

The amount of cross-pollination is determined by planting two strains of the concerned species in a mixed stand. One of these two strains is homozygous for a dominant character, preferably an easily recognizable seeding or other phenotypic character, while other strain is recessive for that character. The two strains are planted in such a manner that each plant of the recessive strain is surrounded by plants of dominant strain to provide abundant pollen. Seeds produced on the recessive strain are harvested and grown in the next generation. The percentage of plant carrying the dominant allele of the character represents the percentage of cross-pollination

Practical 11: Prediction of performance of double cross hybrids

The performance of double cross hybrids can be predicted by comparative evaluation of the predictions based on the performance of single cross.

The method was developed by Jenkins (1934). According to this method, the predicted performance of any double cross is the average performance of the four non-parental single crosses involving the four parental inbred.

For example:

If the 4 inbred are I1, I2, I3 and I4. The possible single cross among these inbred would be 6, viz I1 × I2, I2 × I3, I3 × I4, I1 × I3, I1 × I4 & I2 × I4.

These single crosses can combine to produce 3 double crosses, Viz,

(I1 × I2) × (I3 ×I4)

(I1 × I3) × (I2 ×I4)

(I1 × I4) × (I2 × I3)

The performance of any of these double crosses can be predicted from the performance of the four single crosses, not involved in producing that particular double cross.

For example:

The performance of double cross (I1 × I2) × (I3 ×I4) would be the average of the performance of the four single crosses (I1 × I3), (I1 × I4), (bv+I2 × I3) and (I2 ×I4)