ANNAMALAI UNIVERSITY

FACULTY OF AGRICULTURE

DEPARTMENT OF GENETICS AND PLANT BREEDING

Record Material

GPB 225 Principles and Methods of Plant Breeding (2+1)

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Ex.No.1
Date :

LIFE CYCLE OF FLOWERING PLANTS

The life cycle of plants includes alternation of generations, where two generations complete a life cycle. A haploid gametophyte form alternates with a diploid sporophyte form.

Alternation of generations is a term used to describe the life cycle of organisms which have two different multicellular stages namely a diploid stage (sporophyte/plant) and a haploid stage (gametophyte/spore). The gametophytes produce sporophytes, and sporophytes produce gametophytes.

Gametophyte refers to the haploid (n) stage of plants which comprises the gametes (pollen and eggs). The pollen is the male gametophyte and the ovule is the female gametophyte. Though they are small, they are still multicellular.

The haploid male and female gametes fuse (fertilization) to produce a zygote which develops into a diploid (2n) sporophyte. The flowering plants around us are sporophytes. It produces two kinds of haploid spores, *viz.,* megaspores and microspores by meiosis. The haploid spores become the gametophyte. The gametophyte then grows and develops and makes the gametes by mitosis which, when fused through fertilization, make a new sporophyte.

For bryophytes (mosses, liverworts, hornworts) and some species of green algae, the gametophyte is the main form, while among vascular plants (ferns, seed plants) the sporophyte dominates. In the case of the seed plants - gymnosperms (cycads, pines) and angiosperms (flowering plants) - the female gametophytes are found only within ovules (which develop into seeds when fertilized), and the male gametophytes are individual grains of pollen.

Life cycles of plants and algae with alternating haploid and diploid phases are referred to as **diplohaplontic** (the equivalent terms **haplodiplontic**, **diplobiontic** or **dibiontic** are also in use). Life cycles of animals in which there is only a diploid multicellular phase are referred to as **diplontic**.

All animals develop differently. A mature animal is diploid and isequivalent to a sporophyte. However, an animal *directly* produces haploid gametes by meiosis. No haploid spores capable of dividing are produced, so neither is a haploid gametophyte. There is no alternation between diploid and haploid forms.

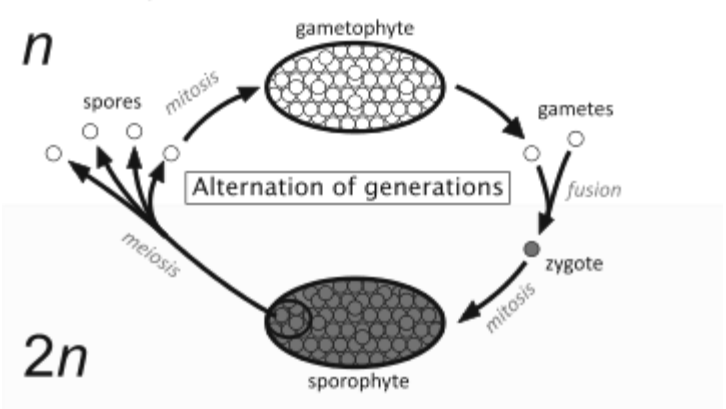


Fig 1. Alternation of generations

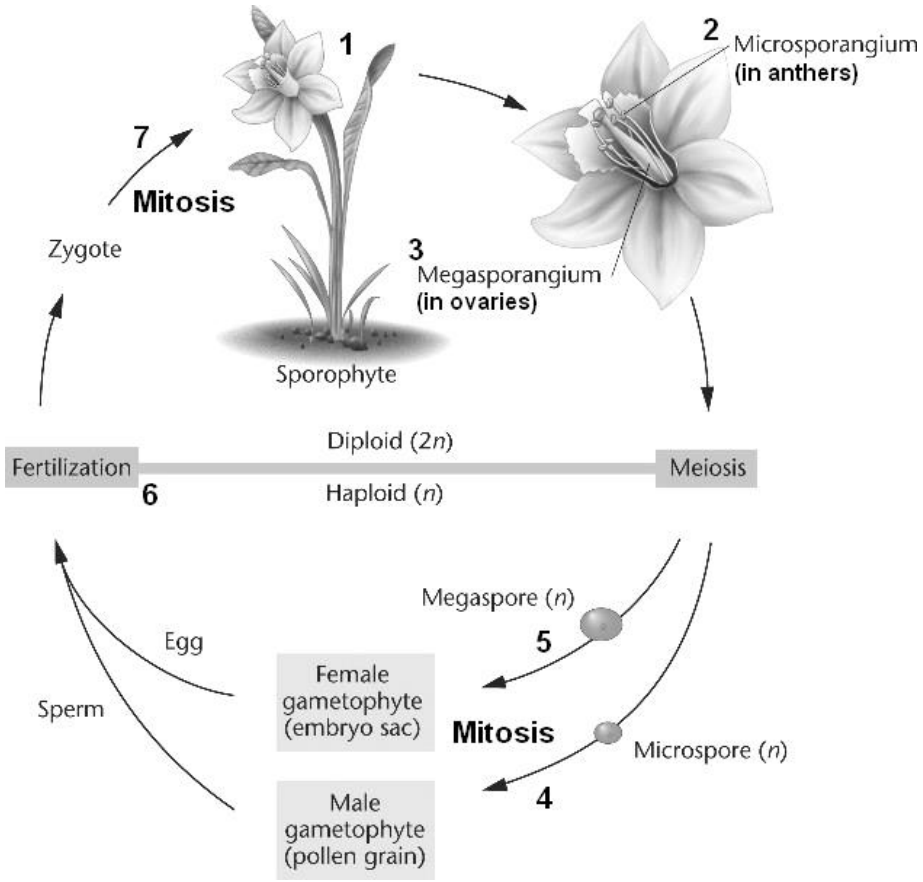


Fig.2. Life cycle in flowering plants

Ex.No.2
Date :

DETERMINATION OF MODE OF REPRODUCTION AND POLLINATION IN PLANTS

The mode of reproduction determines the genetic constitution of crop plants that is whether the plants are homozygous or heterozygous. The breeding methods are different for the self and cross pollinated crops. The knowledge of the mode of reproduction of crop plant is also important for making artificial hybrids. The production of hybrids between diverse and desirable parent is the basis for almost all the modern breeding programmes.

STEPS TO DETERMINE THE MODE OF REPRODUCTION

1. The first step is to find the mode of reproduction of a crop is to critically examine the flower.
2. Mechanisms like monoecy, dioecy, protogyny, protandry and cleistogamy are easily detected which reveal the mode of pollination.
3. The next step is to isolate single plants and record the seed set under isolation. Failure to set seed indicates that the crop is cross-pollinated. When seed set occurs under isolation then the crop is self- pollinated.
4. Finally the effect of selfing should be studied. Loss of vigour indicate that the crop is cross-pollinated.
5. To determine if a species is apomictic or not can be done by crossing a recessive strain (female) to a dominant strain (male). If sufficient number of recessive offsprings are recovered in the progeny, the species is likely to be apomictic.
6. To check if a crop species is having self-incompatability mechanism, observe if the flower is hermaphrodite, homogamous, hermaphrodite dichogamous and unisexual.

If flowers are hermaphrodite, observe the pollen viability using acetocarmine stain. If the pollens are viable, then the seed set is studied under isolation by bagging. Absence of seed set indicates self-incompatability. If the pollens are not viable and very low seed set when compared to normal is observed it is indicative of male sterility.

7. After identification of self-incompatability, study of flower morphology will show the type of self-incompatability. If the flower is morphologically different then it is heteromorphic self-incompatability.
8. In case of homorphic flowers, attempt all possible crosses including reciprocals and record the seed set. When normal seed set is observed and if reciprocal differences are absent then it is classified as gametophytic self-incompataility. Absence of seed set and presence of reciprocal differences indicate that it is sporophytic self-incompatability.

DETERMINATION OF EXTENT OF CROSS POLLINATION

The extent of cross pollination can be determined by growing two strains/varieties of a crop in a mixed stand. The strains should have marker characters say green and red pigmented of which one strain should be dominant and the other should be recessive. Planting should be done in such a

way that each recessive strain is surrounded by dominant strain. Later seeds from the recessive strains are harvested. The percentage of seeds carrying dominant allele represent the percentage of cross-pollination in that species. The proportion of dominant phenotypes in the progeny indicate the extent of cross-pollination.

MODES OF POLLINATION

Pollination refers to the transfer of pollen grains from anthers to stigma. Pollination is of two types namely, autogamy (self pollination) and allogamy (cross pollination).

I. **AUTOGAMY (SELF POLLINATION)**

When the pollen from an anther falls on stigma of the same flower it is called as self pollination or autogamy Self-pollinated species as a rule, must have hermaphrodite/bisexual flowers. Even in self pollinated crops cross-pollination may occur up to 5%. Example for autogamous crops are rice, wheat, barley, oats, greengram, blackgram, bengalgram, soybean, common bean, sesame etc.

II. OFTEN CROSS POLLINATION

When cross pollination occurs between 5 and 20 % then the crop is classified as often cross pollinated crop. Examples of often cross pollinated crops are cotton, bhendi, sorghum, pigeonpea, tobacco, triticale etc.

III. CROSS POLLINATION (ALLOGAMY)

Transfer of pollen grains from the anther of one plant to the stigma of another plant is called allogamy or cross pollination. This is the common form of out breeding. When cross pollination occurs more than 20 % the crop is classified as allogamous/ cross pollinated. Examples for allogamous crops include Maize, castor, coconut, sunflower, safflower, oilpalm, cabbage, cauliflower, onion, garlic, watermelon, pumpkin, cucumber, papaya, carrot etc.

A third situation *geitenogamy* results when pollen from a flower, of one plant falls on the stigmas of other flowers of the same plant. eg., Maize.

Ex no. 3
Date:

MECHANISMS FAVOURING SELF AND CROSS POLLINATION

I. MECHANISMS FAVOURING SELF-POLLINATION

When the pollen from an anther falls on stigma of the same flower it is called as self pollination or autogamy. Self-pollinated crops must have hermaphrodite flowers. The various mechanisms that promote self-pollination are as follows.

1. **Homogamy:** Maturation of anthers and stigma of a flower at the same time is called homogamy. Self pollinated species as a rule have homogamy.
2. **Cleistogamy:** In some species the flowers do not open at all. This condition is called as cleistogamy. This ensures complete self-pollination. Eg. Wheat, barley oats and several grass species.
3. **Chasmogamy:** In some species the flowers open, but only after the pollination has taken place. Eg. Wheat, Rice, oats.
4. **Position of anthers**: In some species, stigmas are surrounded by anthers in such a way that it ensures selfing. Eg. In crops like tomato and brinjal the stigma are closely surrounded by anthers ensuring self pollination. Also, in some species, flowers open but the stamens and the stigma are hidden by other floral organs. For example in legumes, the stamens and the stigma are enclosed by the two petals forming a keel.

GENETIC CONSEQUENCES OF SELF-POLLINATION

Self pollination leads to increase in homozygosity and the populations of self pollinated species are highly homozygous i.e they do not segregate upon selfing and produce true to type pure lines.

Self pollinated crops do not show inbreeding depression, but may exhibit considerable heterosis. Therefore, the aim of breeding methods generally is to develop **homozygous varieties**.

II. MECHANISMS FAVOURING CROSS POLLINATION

Transfer of pollen grains from the anther of one plant to the stigma of another plant is called allogamy or cross pollination. The various mechanisms hich promote allogamy are as follows.

1. **Dicliny:** In refers to the presence of unisexual flowers. This is of two types: viz. *(i) Monoecy and (ii) Dioecy*. When the male and female flowers are separate but present in the same plant, it is known as monoecy Eg. Maize. In some, crops, the male and female flowers are present in the same inflorescence Eg. Castor, coconut. When staminate and pistillate flowers are present on different plants, it is called dioecy. Eg. Papaya, date palm and spinach.
2. **Dichogamy:** It refers to difference in maturation time of anthers and stigma of the same flower. Dichogamy is of two types viz. *(i) Protogyny and (ii) Protandry.* When pistil matures before anthers, it is called Protogyny Eg. Pearlmillet. When anthers mature before pistil, it is known as protandry Eg. Maize and sugar beet.
3. **Heterostyly:** When styles and filaments in a flower are two different lengh, it is called heterostyly Eg. Linseed.
4. **Herkogamy:** Hindrance to self-pollination due to some physical barriers such as presence of hyline membrane around the anther is known as harkogamy. Such membrane does not allow the anther dehiscence of pollen and prevents self-pollination such as in alfalfa.
5. **Self incompatibility:** The inability of fertile pollen to fertilize the same flower is referred to as self incompatibility. It prevents self-pollination and promotes cross pollination. Self incompatibility is found in Brassica, Radish, Nicotiana and many grasses.
6. **Male Sterility:** The presence of non-functional pollen grains is known as male sterility. It is of three types namely, genetic, cytoplasmic and cytoplasmic genetic male sterility.

GENETIC CONSEQUENCES OF SELF-POLLINATION

Cross pollination preserves and promotes heterozygosity in a population i.e., upon selfing the offsprings will segregate. These species have developed heterozygous balance and exhibit severe inbreeding depression upon selfing. Also, they exhibit high amount of heterosis. The breeding methods in such species aim at improving the crop species without reducing heterozygosity to an appreciable degree. Usually development of a **hybrid or synthetic variety** is the aim of breeder wherever the seed production is economically feasible.

SIGINIFICANCE OF POLLINATION

The mode of pollination influence the following five important factors *viz.*, gene action, genetic constitution, adaptability, genetic purity and transfer of genes.

Ex. No.4
Date:

STUDY OF POLLEN MORPHOLOGY, POLLEN FERTILITY AND STERILITY

The study of external morphological features of mature pollen grain is referred to as ***palynology***. Hyde & Williams(1843) first used the form of palynology. A schematic illustration of a pollen grain is called '***palynogram'***. The major research centres in India are National Botenical Research Institute(Lucknow), Birbal Institute of palenobotany (Lucknow), Osmania University(Hyderabad) and Bose Institute(Calcutta). The study of pollen is called palynology and is highly useful in paleoecology, paleontology, archeology, and forensics.

Pollen grains are initially formed in groups of four called as tetrad. Each pollen grain has two poles viz, the proximal pole (PP) at the centre, the distal pole facing away from the centre of the tedrad. The pollen grains are said to be heteropolar if their two faces are different and isopolar if the two faces are similar. In heteropolar grains one face has an aperture while the other face has none.

POLLEN APERTURES

Pollen apertures are various modifications of the wall of the pollen grain that may involve thinning, ridges and pores. An aperture is a weak area on the pollen surface which aids in germination of the pollen grain. They serve as an exit for the pollen contents and allow shrinking and swelling of the grain caused by changes in moisture content.

Long apertures are called colpi and short apertures are called as pores. Apertures may be simple or compound. Pollen with simple colpi (long apertures) are called colpate and pollen with simple pores (short apertures) are called as porate. The pollen with compound colpi is known as colporate pollen and there with compound pores are called as porporate pollen. The colpi and the pores form a major criterion for the identification of classes of pollen

POLLEN WALL

The pollen wall protects the sperm while the pollen grain is moving from the anther to the stigma. It protects the vital genetic material from drying out and solar radiation. The wall of the pollen grain always consists of two layers: the inner ***intine wall*** and the outer ***exine wall***. Both inner and outer cell wall of pollen grains often have a typical structure that depends on the species.

The deposition of these walls begins already during meiosis and continues until final maturation of the pollen grain. The inner layer is laid by the cells themselves, the outer wall is deposited by the tapetum (t in figures on pollen development). The inner wall consists of cellulose and hemicellulose, but callose is always present. Callose is nearly absent in "normal" cell walls.

The outer wall consists mainly of sporopollenin, a mixture of stable biopolymers containing fatty acids. Sporopollenin protects the living vegetative

and generative cell in the pollen grain against mechanical damage, chemical break-down and too rapid desiccation and it provides a shield against the aggressive ultraviolet radiation of the sun. On the surface of the outer wall and in cavities remnants of the tapetum can be found. Sometimes the grain is covered by a liquid, fatty substance, so-called "pollenkit". Intine, exine and cytoplasm can all three contain allergens that may cause hay fever.

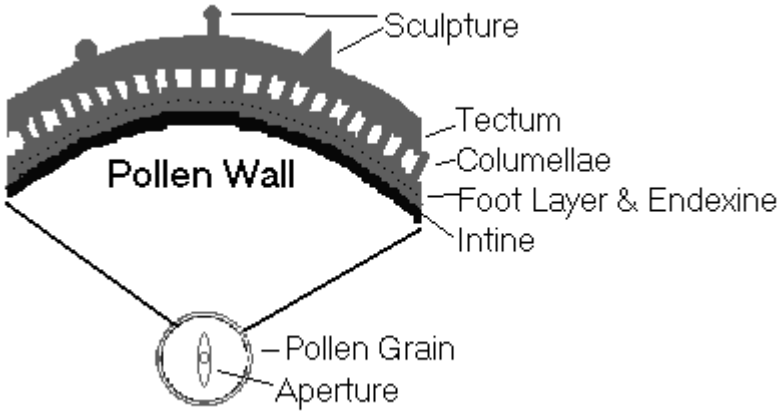


Fig. 1. Cross section of pollen wall

POLLEN WALL FEATURES

The two main layers of pollen wall are intine (inner wall) and extine (outer wall). The extine is further divided into an outer sculptured layer called sexine and inner non-sculptured layer called nexine. The sculpturing may be smooth, pitted, grooved, warty, spiny, punctuate, reticulate etc.

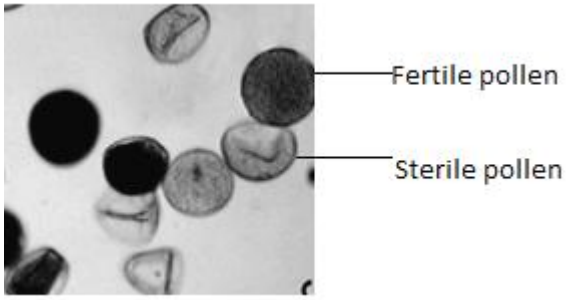
In seed plants, pollen grains embody the male partners in sexual reproduction. Pollen grains are generally shed in a desiccated condition and the moisture level is less than 20%. At the time of shedding pollen grains are two celled (a large vegetative cell enclosing a generative cell) or three celled (vegetative cell / and two sperm cells formed by the division of generative). Pollen biology thus encompasses pollen production, its transfer to the stigma and details of pollen-pistil interaction leading to fertilization and seed set.

POLLEN STERILITY AND FERTILITY

Treating the pollen grains with non vital stains such as acetocarmine, Iodine in Potassium iodide and aniline blue in lactophenol essentially imparts color to the contents of the pollen in fresh as well as fixed / dead pollen. Staining with non-vital stains is highly useful to determine the degree of pollen sterility in plants. Usually acetocarmine or 1% I-KI stain is used for assessing the pollen fertility stains. In rice, following classes were used to categories the plants.

0-0.99% fertility	:	sterile
1-20.99% fertility	:	partially sterile
30-59.99% fertility	:	partially fertile

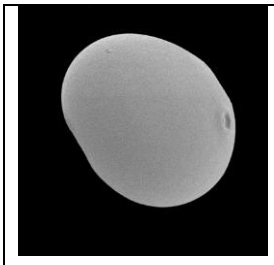
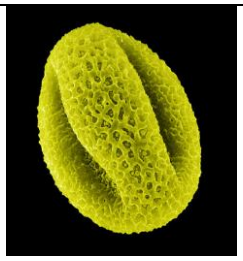
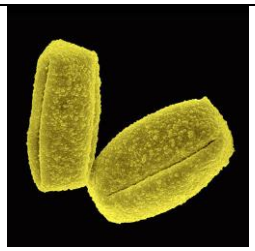
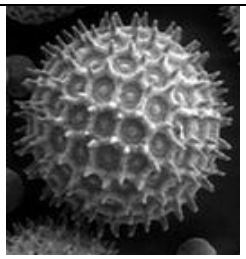
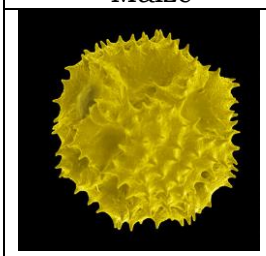
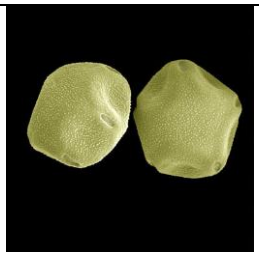
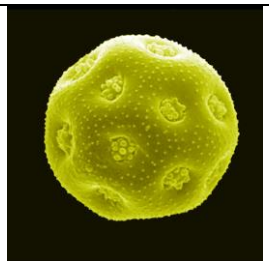
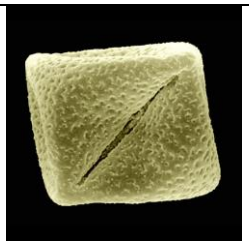
> 60% fertility : fertile

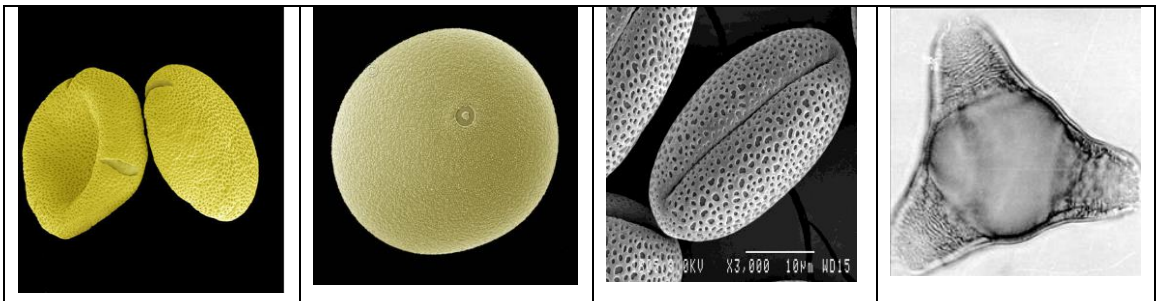


POLLEN IDENTIFICATION

Pollen identification can reveal the season in which a particular object picked up the pollen. Pollen has been used to trace activity at mass graves in Bosnia and to catch a burglar who brushed against a Hypericum bush during a crime. The palynologicacl research can be classified as Geopalynology (fossil pollin grains), Aeropalynology (pollen found in atmosphere), Iatropalynology (use in criminology) and Melittopalynology (study of pollen in honey).

Identification of a pollen grains can be done by taking into account of the (i) Size and shape (ii) the number of colpi and/or germination pores and their position and (iii) the structure of the cell wall.

 Maize	 Willow	 Oak	 Hibiscus
 Dandelion	 Alder	 Chick-weed	 Sorrel



Lime	Grass	Redbud (*Cercis*)	Gaura

Fig. 2. Variation in pollen shape, size and external features

Ex.No.5
Date :

BREEDER'S KIT

To accomplish the selfing and controlled pollination a breeder needs the following instruments.

1. Needle: Required to open the small flower buds.
2. Forceps: For emasculating small anthers and holding stamens during transfer of pollen to the stigma.
3. Brushes: Camel hair brushes of size 3 or 4 are required when pollen production very low.
4. Alcohol/Methylated spirit : To sterilize the forceps, scissors, needles, brushes as well as hands.
5. Magnifying lens : It is required to observe the small flowers.
6. Paper bags: These are required to cover the inflorescence prior and after pollination. Parchment paper, butter paper and craft paper are used for making bags.
7. Tags: These are required to cover the inflorescence prior to and after pollination. Parameters like number of cross, name of the parents, date of emasculation and pollination etc., have to be written.
8. Meter Scale / Tape: It is used to measure biometrical observation during selection.
9. Field note book: It is used to record of the crosses made, date of sowing, germination etc.

Ex.No.7
Date :

SELFING AND CROSSING TECHNIQUES IN DIFFERENT CROPS

I.SELFING

The objective of selfing is to develop homozygous plants that can be used as parents in a crossing programme. The technique of selfing varies from one crop to the other depending upon the mode of reproduction. In self pollinated crops, selfing is the natural mode of reproduction and to ensure selfing no operation is needed. But in legumes like alfalfa, hand tripping of flower is essential for self-fertilization since the stigma has a waxy covering which must be removed to make it receptive.

In the case of often-cross pollinated species, the flowers are generally bagged to prevent cross-pollination. In species with bisexual flowers or with both male and female flowers in a single inflorescence, bagging the entire inflorescence, or sometimes the whole plant is adequate. In maize, the male and female inflorescences are bagged; the pollen is collected in the tassel bag and dusted on the silk of the female inflorescence. Alternatively, the tassel may be cut and enclosed in the bag-covering cob. The cut end of tassel may be kept in water contained in a small bottle to keep the tassel alike for a longer period.

II.CROSSING

Synchronization of flowering is a prerequisite so that the pollen collected from one variety can be immediately dusted on the stigma of the other variety. If the two parents have different durations to reach flowering, sowing should be staggered suitably so that they reach flowering at the same time. By raising the parents in glass houses under controlled conditions of light, temperature and humidity, synchrony in flowering can be achieved.

The technique of crossing consists of removing the anthers before pollen shedding and is called emasculation. Collecting viable pollen from the male parent and transferring it to the receptive stigma of the emasculated flower. The removal of the anthers is usually done one day before the pollen is ripe. Hand emasculation can be adopted in crops with large flowers such as cotton. Mass emasculation can be done by the 'hot water method' in rice. [40-44°C for 10 minutes] and Sorghum (42 -48°C for 10 minutes). Cold treatment for emasculation in rice (0-6°C) and wheat (0-2°C) requires a duration of 15-24 hours. Treatment with alcohol (57 per cent) for 10 seconds helps in emasculation in sweet clover.

Only 10 to 20 of the best developed spikelets on the central part of a wheat or sorghum head are emasculated, the other spikelets being clipped off. Sufficient panicles or heads, say 25 to 50 are emasculated to obtain the

required number of hybrid seeds. The emasculated head is enclosed in a butter paper bag or a muslin bag or otherwise protected from outside pollen, and labeled. When the stigma becomes receptive pollen collected from the male parent is dusted on the stigma of the emasculated head and the butter- paper bag is replaced immediately. Ten days after pollination, the bag is replaced by a perforated butter paper bag to provide for ventilation and to prevent moulds. The bag is left as such to protect the head from bird damage.

The number of flowers crossed and F_1 seeds to be produced depend on

(1) The number of seeds produced by each flower, which may be just one per spikelet as in cereals, a few per flower as in cotton, bhendi etc. or many per flower as in tobacco, tomato etc.
(2) The population size required in the F_2 and subsequent segregating generations,
3) The genetic differences between the parents
(4) The number of characters to be recombined and
(5) The degree of linkage between the genes involved.

Ex.No.8
Date :

FLORAL BIOLOGY OF DIFFERENT CROPS

1. RICE : The inflorescence of rice is called panicle. The spikelet is the unit of inflorescence. The spikelets are borne on the branched panicle.
Spikelet Morphology: The spikelet consists of two short sterile lemma, called glume 1 and glume 2, the grain a normal fertile lemma and palea. The fertile lemma and palea enclose the spikelet. The sexual organs *viz.,* six stamens arranged in two whorls and a pistil at the centre. The stamen consists of bilobed anthers borne on slender filaments, while the pistil consists of ovary, style and feathery bifid stigma.

2. WHEAT: Inflorescence is a terminal compound, distichous spike, with rachis, awned or awnless, glabrous or hairy. Self pollinated.
Spikelets: Sessile, and borne singly at the nodes on alternate sides of the zigzag rachis. Each spikelet consists of two to five florets attached alternately on opposite sides of a short central axis called rachilla, and is covered by two sterile or empty glumes. Lemma broad with an acute tip or awn, palea thin. The lower lemma are fertile while the top one or two sterile. Lodicules are two in number, stamens three with thin filaments and large anthers, superior ovary, styles two, single ovule, bifid feathery stigma

3. PEARLMILLET: The inflorescence of pearl millet is a speciform terminal cylindrical panicle. Panicle bears two types of florets, one being bisexual, and the other staminate. A single pistil with 2 feathery and 3 anthers enclosed between the lemma and palea. The unisexual staminate florets are sessile and borne below the bisexual flowers. They have 3 anthers and lack the female organs. Cross pollinated crop.

4. REDGRAM :
Inflorescence: Flowers are borne in axillary corymbose racemose, often forming a terminal panicles. Self pollinated crop.
Calyx: Five tubular campanulate.
Corolla: Papilionaceus, five petals, one standard, two wing and two keel, petals, polypetalous.
Androecium: Stamens 10, diadelphous. The stamens show dimorphism of the 10 stamens, four have short filaments and six including the old posterior one, have long filaments.
Gynoecium: The ovary superior, sub sessile, densely pubescent and glandular punctate with 2-9 ovules. The style is long filiform, and glabrous. The stigma is capitate.

5. SOYBEAN:

Inflorescence: Axillary racemes. Self pollinated crop.
Flowers: Bracteate, bracteolate, bracteoles two, shortly pedicellate, bisexual, hypogynous, zygomorphic, complete, pentamerous, cyclic.

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Calyx: Five gamosepalous, tubular and five unequal lobes.
Corolla: Papilionaceous corolla, posterior standard petal two lateral wing petals and two anterior keel petals.
Androecium: The ten stamens diadelphous Pattern (9+1).
Gynoecium: The single pistil is unicarpellate and has one to four campylotropous ovules. The style is about half the length of ovary and curves backward towards the free posterior stamen. The stigma is capitate. Hairs are present on the pistil, and the outer surface of the calyx tube.

6. SESAME:

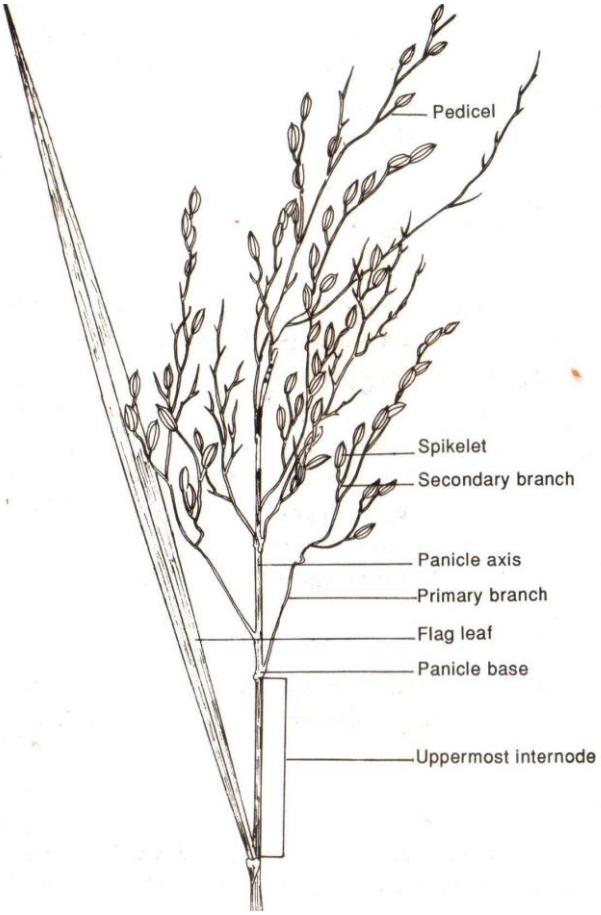
Inflorescence: Axillary, solitary, shortly pedicellate.
Calyx: Five, gamosepalous.
Corolla: Tubular, two lipped, the upper lip of two lobes usually smaller, the lower bigger and of three lobes.
Androecium: Stamens 5, Four are fertile, epipetalous.
Gynoecium: Superior ovary, bicarpellary, tetralocular, looks like a tetra carpel, by the formation of many ovules on axile placentation. Self pollinated crop.

7. SUNFLOWER:

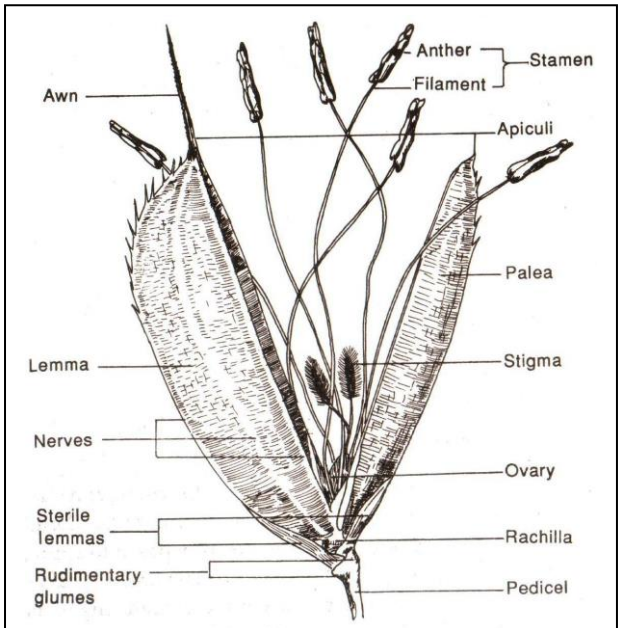
Inflorescence: Head or capitulum which contains two type of florets a) Disc floret b) Ray floret.
Disc floret: Bracteate, sessile, bisexual, complete, actinomorphic and epigynous.
Calyx: calyx is reduced to pappus hairs, gamosepalous.
Corolla: United to form a tube.
Androecium: Stamens five, syngenesious anthers, epipetalous stamens, ovary inferior, monocarpellary with single ovule and the style passes through syngenesious anthers with bifid stigma.
Ray floret: With ligulate corolla, yellow in colour zygomorphic and calyx is reduced to pappus hairs. Normally florets are sterile or sometimes pistillate. Highly cross pollinated crop.
Fruit: Achene (or) cypsela.

8. COTTON:

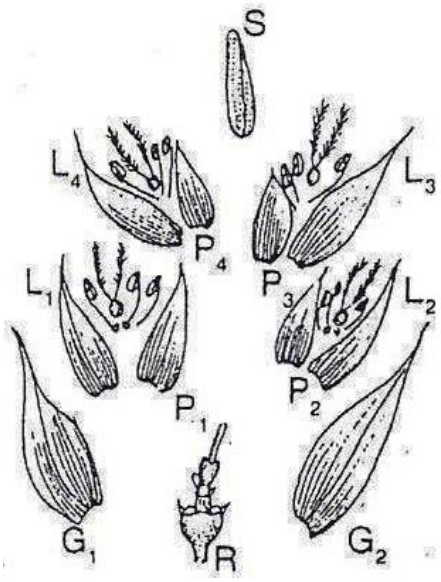
Flowers: Extra axillary, terminal or solitary opening of flower is spiral in acropetal and centripetal succession. Petal colour vary with colours white, yellow, cream yellow purple.
Bracteoles: Three in number, usually foliar and persistent some times small or minute and cordate, toothed or entire, rarely caducous.
Calyx: Cup shaped, truncate, undulate with five toothed and persistent.
Corolla: Five in number, colour vary, White, creamy, yellow or purple.
Androecium: Anthers unilocular, reniform and monodelphous.
Gynoecium: Ovary vary with 3 – 5 locules, superior, ovules one to several in locules. Style glandular, club shaped and clavate shortly into, as many as lobes as locules in the ovary. Often cross pollinated crop.



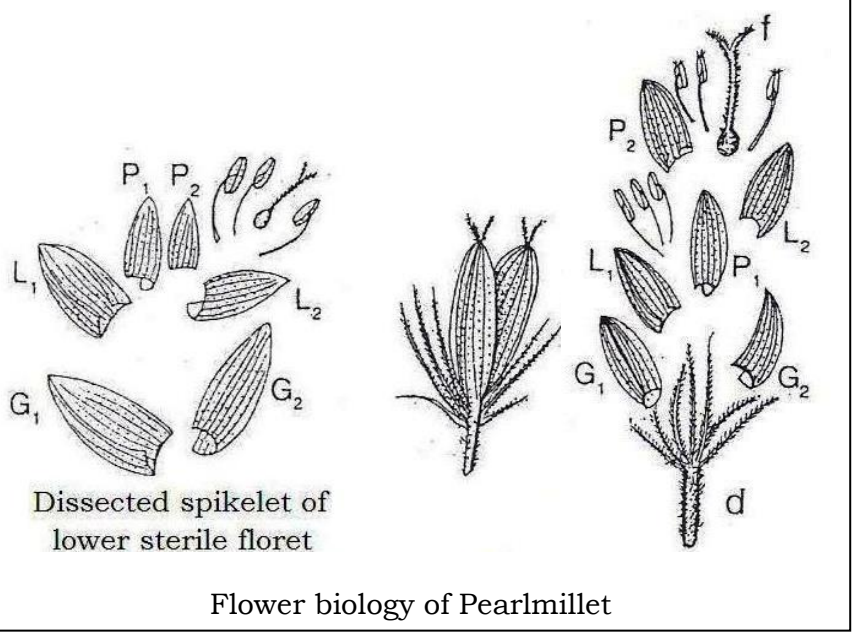
Floral biology of Rice



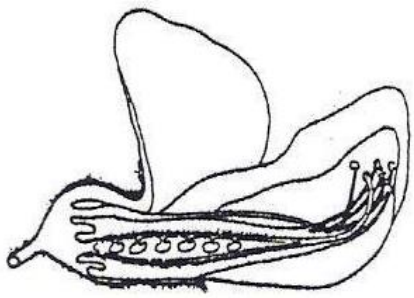
Parts of a rice spikelet



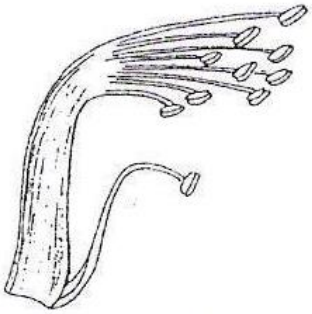
Floral biology of wheat



Flower biology of Pearlmillet

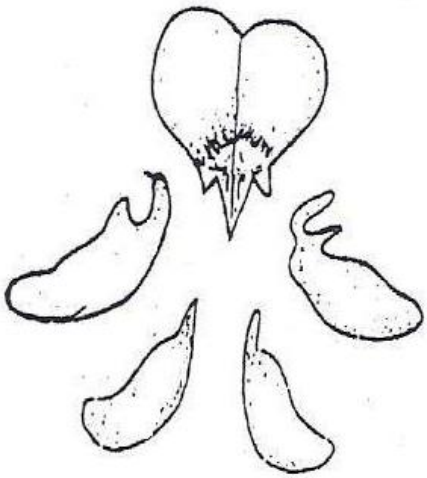


L. S. of flower



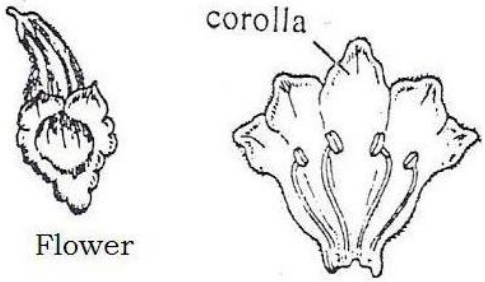
Diadelphous stamens
(9+1)

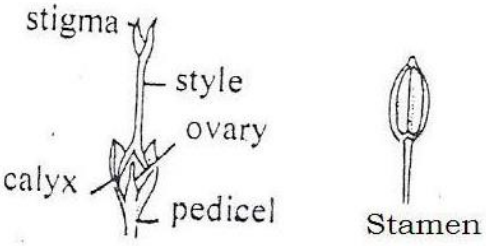
Floral biology of Redgram and soybean



Corolla dissected

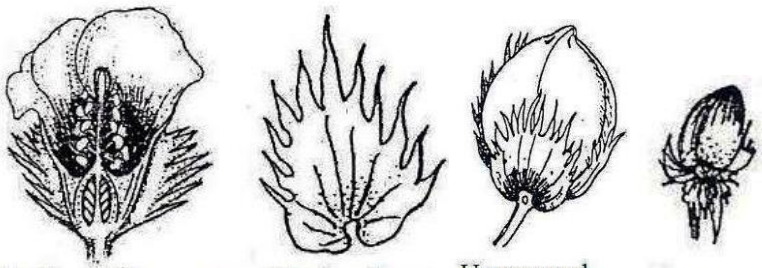
Floral biology of Redgram
and soybean



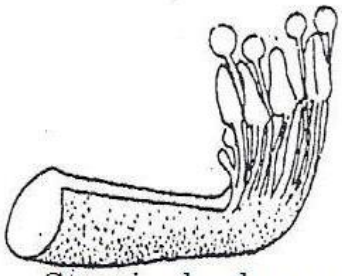


Gynoecium with
calyx

Floral biology of Sesame

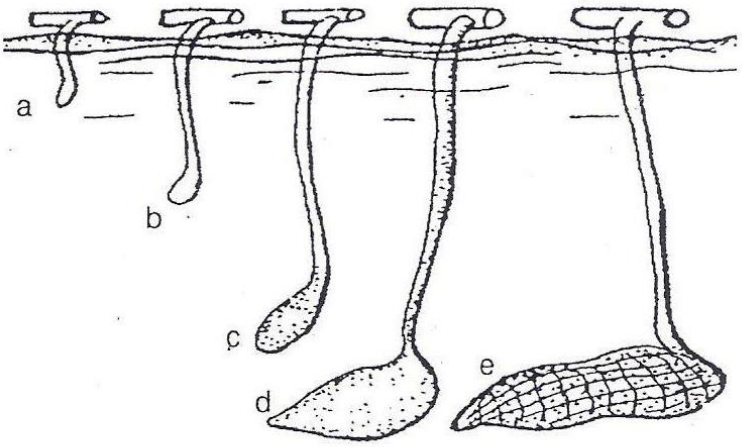


Floral biology of Cotton



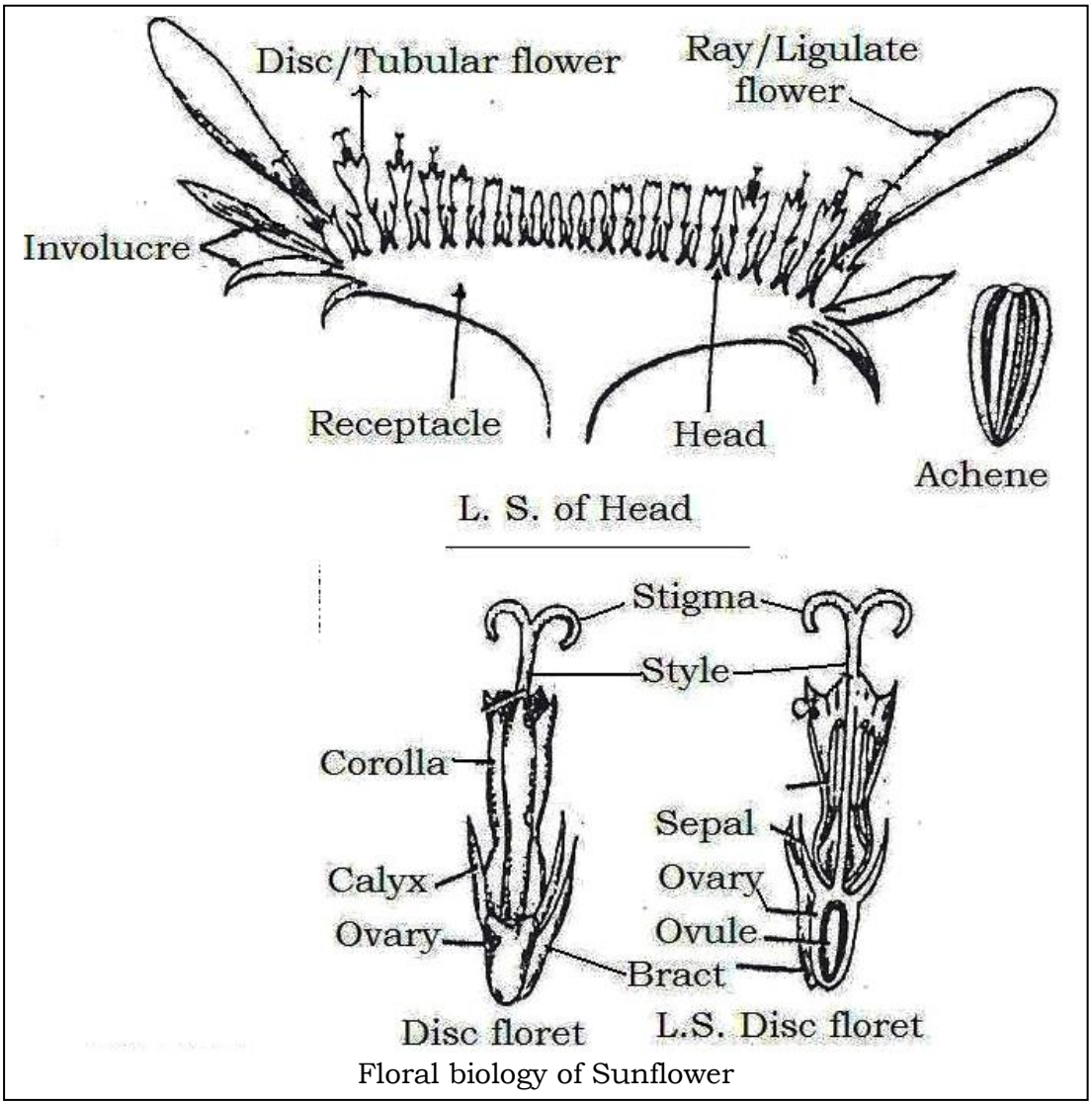
Staminal column
(4 Long globose, 4 short elongated and 2 staminodes)

Floral biology of Groundnut



a. Peg or gynophore , b & c. Positively geotropic growth
d. Horizontal growth, e. Pod development

Development of pod in groundnut



Floral biology of Sunflower

Ex.No.9
Date :

EMASCULATION, KINDS OF EMASCULATION AND POLLINATION TECHNIQUES

EMASCULATION

The removal of stamens or anthers or killing of pollen grains in a bisexual flower before dehiscence and without affecting the gynoecium is known as emasculation. In monoecious crops like maize, the male inflorescence is removed to prevent self pollination. In dioecious crops eg. Papaya, the male plants are removed

TYPES OF EMASCULATION

1. **Hand Emasculation**

Hand emasculation is generally carried out between 4 and 6 pm, one day before anthesis. The corolla of the selected flower bud is opened with the help of forceps and needles and the anthers are carefully removed. When the stamens are epipetalous (stamen attached to corolla) corolla may be completely removed eg. Sesamum. The gynoecioum must not be injured during emasculation.

2. **Hot water emasculation**

As pollen grains are very sensitive to both genetic and environmental factors when compared to female reproductive organs, the treatments with alcohol, cold water, hot water are used for emasculation. The temperature of hot water and the duration of treatment differs from crop to crop. For example, Sorghum requires a temperature of 42-48^{o}C for ten minutes to effect emasculation while in rice, the temperature should be between 40-44^{0}C for 10 minutes. Hot water is generally carried in a thermos flask.

3. Alcohol treatment

It is not a commonly used method as female reproductive organ is also drastically affected under certain conditions. In this method, the flower bud or the inflorescence is immersed in alcohol for a particular period of time followed by rinsing with water. Eg. In sweet clover and Lucerne, treatment with 57 % alcohol for 10 seconds was highly effective.

4. **Cold treatment**

Cold treatment is less effective when compared with hot water treatment. Eg. In rice, cold water treatment at 0.6 C kills the pollen grains without affecting the gynoecium. In wheat, the temperature required is 0.2 C for 15-24 hours.

5. Genetic emasculation

When emasculation is effected by using genetic or cytoplasmic male sterile mechanism, it is referred to as genetic emasculation. In crops like onion, sorghum, maize, pearlmillet, wheat genetic emasculation is widely used. In genetic emasculation the pollen formation is hampered or even when formed it

will be sterile and cannot fertilize. In case of self-incompatible crops eg. Brassica, emasculation is not necessary, because the self-fertilization is ineffective. In some crops with protogyny mechanism (stigma matures earlier than anthers) emasculation is not needed to produce hybrid seeds.

BAGGING

Immediately after emasculation, the flowers or the inflorescence should be covered with butter paper cover to prevent random cross-pollination. In crops like maize, the male inflorescence (tassel) is also bagged to maintain the purity of the pollen used for pollination.

TAGGING

After bagging, the emasculated flowers are tagged. The plant tags are available in different sizes (3 x 2 or 6x3 cm). The information regarding, date of emasculation, date of pollination and names of the female and male parents should be written on the tag with carbon pencil. The name of the female parent is written first followed by the name of the male parent eg. ADT 43 x ASD 16. ADT 43 is female parent and ASD 16 is male parent.

POLLINATION

Pollination procedure consists of collecting pollen grains from freshly dehisced anthers and dusting this pollen on the stigma of emasculated flowers. In rice, the inflorescence of male parent is shaken over the emasculated inflorescence. In case of bajra and jowar, the male and female inflorescences are enclosed in the same paper bag to effect crossing. Also, the pollen grains can be collected in a bag or petridish and dusted on the stigma with the help of camel hair brush or forceps or toothpicks.

(Draw diagrams of clipping method and hot water method)

Ex.No. 10
Date :

Handling of segregating material

F_2 Generation

Seed source

All the seeds of the selected F_1 should be bulked crosswise and sown. If the corresponding original hybrid seeds are also available, it is desirable to raise the F_1S and parents with the respective F_2'S. The local proven variety is grown as the standard check. The F_2'S are grouped first into sets according to the parentage and objective wise preferably separate sets for each of the objective of crossing should be raised.

Lay out

A randomized design replicated 4 to 6 times is followed. A population of 40 to 50 plants per row for each of the cross in each replication is maintained to have a maximum of 1000 and a minimum of 200 plants, for each cross. If seeds are inadequate non replicated layout may be adopted with local checks at frequent intervals.

Spacing

Only single seed is sown per hole. Thinning of F_2 population should not be done. If necessary gap filling may be done to maintain maximum stand required.

Selfing

Generally selfing is recommended for F_2. This step may be skipped in practical plant breeding.

Observation

The spectrum of variability for the objective with which it was crossed should be recorded. For example for earliness, observe the variation and mark the early segregants by special tags. Similarly, in resistance breeding, the plants with different disease reactions are marked, with different labels. The data on yield and yield components are recorded on the single plant basis and the quality attributes are estimated for all the single plant harvested.

Harvest

Individual plant selection and harvest of selected F_2s can be made in the pedigree breeding. Main criterion is the elimination of poor hybrids. The breeders should develop an attitude for rejection of undesirable types.

After discarding poor F_2 plants, all the available plants of selected crosses should be harvested on single plant basis.

Statistical Analysis

1) Draw the frequency distribution for each of cross and estimate the variance, S.E. and C.V for each cross.

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2) The parameters like genotypic variance, heritability and genetic advance G.C.V and effective factors may be determined.
3) If combination breeding is involved the correlation coefficient between the characters should be assessed.

Disposal

Selecting at F_2 stage is done in two steps.

1) Selecting the crosses on their high values for mean, variability, heritability and genetic advance and
2) Selection of individual plants or genotypes from among selected crosses based on the deviated of the mean for the cross to capitalize the transgressive variability of the cross. With these principle in mind first reject the crossed which have a) low mean and low variability and b) low heritability and low genetic advance.

Ex.No.11
Date :

MUTATION BREEDING

Hugo de Vries introduced the term mutation in 1900. Mutation is a sudden heritable change in characteristics of an organism other than due to recombination or segregation. Mutations produced by changes in the base sequence of a gene is known as *gene or point mutation*. The mutations produced by changes in chromosome structure and number are termed as *chromosomal mutations*. The changes in cytoplasm is known as cytoplasmic or plasmagene mutation. Generally mutation refers to gene mutation. Muller discovered the mutagenic action of x-ray in 1927 on Drosophila and by Stadler in 1928 on barley.

Induced mutation

Mutation produced by treatment with either a chemical or physical agent is called induced mutation. The agents which induce mutation is called as a *mutagen*. The individual which undergoes mutation is termed as *mutant*. The utilization of induced mutations for crop improvement is known as *mutation breeding.*

Spontaneous mutation

Mutations that occur in natural population at a low rate without any treatment by humans is known as spontaneous mutation. The frequency of spontaneous mutation is 1 in 10 lakh i.e 10^{-6}. Spontaneous mutation occurs due to error in DNA replication and mutagenic effects of the environment.

CHARACTERISTICS OF MUTATION

1. Mutations are generally recessive
2. Most mutations have harmful effects
3. Mutations are random
4. Mutations are recurrent

CLASSIFICATION OF MUTATION

1. Effect on Survival

Lethal - lethal mutations kills all the =]individuals
Sub lethal - It kills more than 90% but not all the individuals
Sub vital - It kills less than 90% of the individuals.
Vital - Do not affect the viability. Wild alleles are referred as vital genes
Super vital - It enhance the survival of the individual (resistant genes)

2. Direct of mutation

Forward - Mutation from wild type allele to mutant allele
Reverse - Mutant allele to normal / wild allele

3. Cell type

Somatic mutation - Mutation occur in somatic cell
Gametic mutation - Occurs in sex cells producing a heritable change

4. Quality

Substitution - Substitution of a single base for another

Deletion - Loss of some portion of gene
Addition - Addition of one/more base to the gene

5. Frame shift mutation

Insertion or deletion of a base can alter the amino acid sequence due to a shift of reading frame. It has a serious effect on the mutant phenotypes.

MUTAGENS AND TYPES OF MUTAGENS

The agent which induces mutation, is known as mutagen. It is of two types.

1. PHYSICAL MUTAGENS

The radiations having mutagenic property are known as physical mutagens. Radiations are grouped into two classes viz., Ionizing and non ionizing radiations. The radiation is measured in Roentgen (R) units.

a. Ionizing Radiation

The process of ion production (either positive or negative ion) is known as ionization. Ions are produced when an atom either gain or loses one electron. There are two types of ionizing radiation, particulate and non-particulate radiations. The particulate radiations consists of high energy atomic particle generated due to radioactive decay (eg. Alpha, Beta, Fast neutrons). The non particulate ionizing radiations are represented by high energy radiations composed of photons.

b. Non ionizing Radiation

UV rays present in solar radiation, also produced by mercury vapour lamp is the only non ionizing radiation. UV is relatively low energy radiation. The maximum absorption of UV rays is by thymine. In plants, pollen grains may be irradiated and used for pollination.

2. CHEMICAL MUTAGENS

1. Alkylating agents	-	Ethyl Methane sulphonate (EMS) Methyl Methane sulphonate (MMS) Ethylene Imine (EI)
2. Acridine dyes	-	Acridine orange, Ethiliumbromide
3. Base analogous	-	5-Bromo Uracil, 5-chloro Uracil
4. Deamination agents	-	Nitrous acid (NHO_2)
5. Others	-	Sodium azide, Hydroxylamine (HA)

DOSAGE AND HALF-LIFE PERIOD

The radiation dose is determined by the intensity of the radiations and the length of the exposure and is expressed in Roentgen(R) units. But in mutation breeding experiments it is expressed as kR or Gray. 1 kR = 10 Gy. 1 Gy = 100 rad. Half life period is the time taken for degradation of half of the initial amount of the mutagen.

GAMMA GARDEN

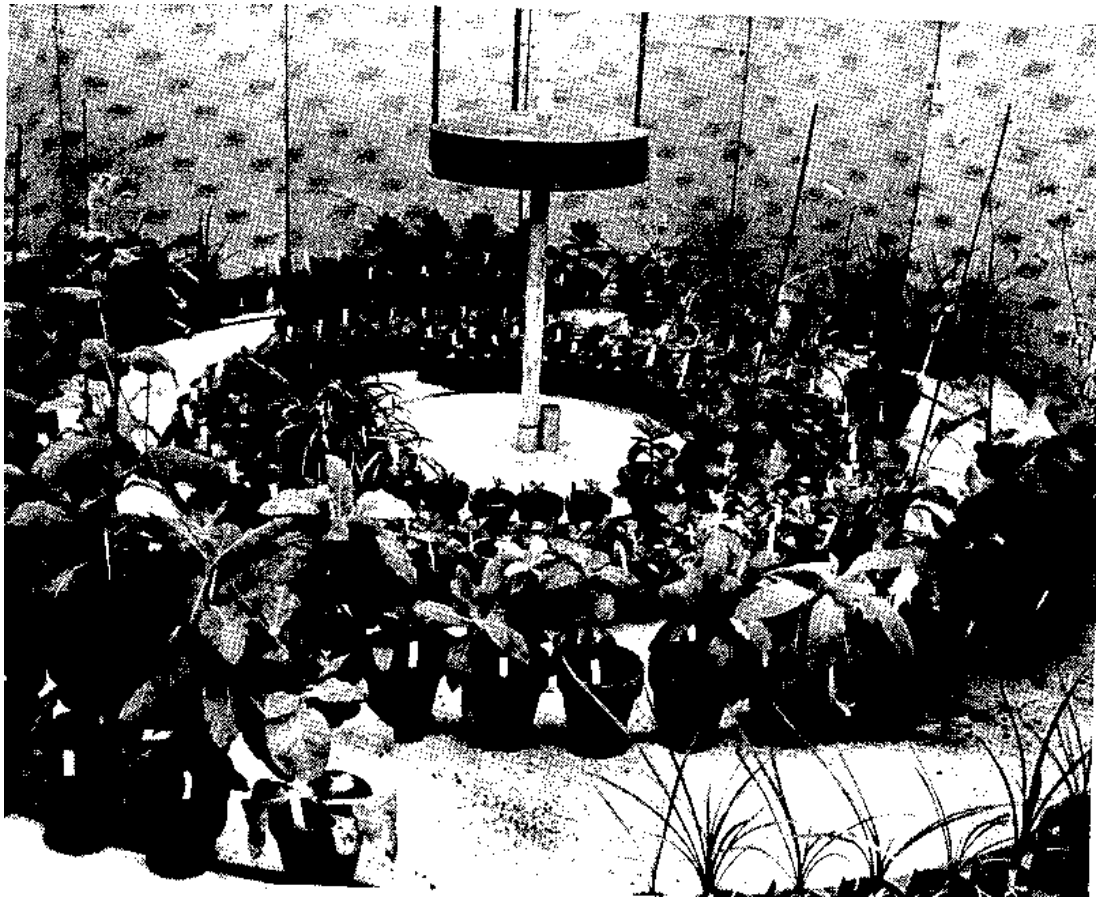
Gamma garden is an area enclosed by thick and high walls to protect the plants and animals from radiation damage. The purpose of gamma garden is to irradiate whole plants, seeds and other propagules. The first gamma garden was built in long island near New York, USA. The first gamma garden in India was

built in Calcutta at Bose Research Institute in 1959, at IARI in 1960 and at BARC, Trombay.

The IARI gamma garden has an area of 3 acres surrounded by a wall of 12 feet height and 3 feet thick. The gamma ray source consists of 6 g ^{60}Co sealed in an aluminum capsule. The strength of ^{60}Co is 200 curies. The sealed ^{60}Co is kept in a lead container, since gamma and x-rays do not penetrate leaS. For irradiation, the lid of the lead container is lifted along with aluminum capsule. After irradiation the ^{60}Co aluminum capsule is lowered into the lead container and the lid is closed. The lead container is opened and closed by a remote control device.

ACHIEVEMENTS

Wheat	-	Sharbati Sonara (Gamma ray mutant of Sonara-64)
Rice	-	Jagannath (γ ray mutant of T 141)
Urd	-	Co 4 (MMS mutant of Co 1)
Ground nut	-	TG 17
Cotton	-	MCU 7
		MCU 10 (MUC4 treated with γ-ray)
Ginger	-	Suravi
Turmeric	-	BSR 1
Tobacco	-	Jayasri



(Take print out of gamma garden and paste it in your record note)

Ex.No.12
Date :

CALCULATION OF PCV, GCV, HERITABILITY AND GENETIC ADVANCE

In pure lines or homozygous parental population, theoretically there should not be any variation between individuals for a character. But in practice, variations occur due to environmental factors. Also, the F_1 population will possess physical uniformity (homogenous), though genotypically the F_1s are heterozygous. Any variation in the F_1 population is due to environment or impurity of parents. In F_2 and other segregating populations, the individuals are genotypically different from one another. Under such conditions, finding out the proportion of variation due to heredity and that due to environment becomes necessary. In quantitatively inherited characters, the phenotype is the result of genotype and environment.

Phenotype =Genotype + Environment

The analysis of variance (ANOVA) divides the total variation into two main parts Viz., Variation between varieties and variation within varieties. i.e. environmental variation. It helps in partitioning of phenotypic variation into genotypic and environmental components as given below. In plant breeding, normally randomized block design (RBD) is used for field experiments.

CO-EFFICIENT OF VARIATIONS

For comparing the variability of different populations or between characters of the same population, the estimation of co-efficient of variation is required. The formulae for estimating the phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) as suggested by Burton (1952) are as follows.

1. $$PCV = \sqrt{\frac{Phenotypic\ Variance}{General\ Mean}} \times 100$$

2. $$GCV = \sqrt{\frac{Genotypic\ Variance}{General\ Mean}} \times 100$$

3. Heritability: The ratio of heritable portion of variance to the total variance or phenotypic variance is known as heritability. Thus heritability indicates the proportion of phenotypic variance that is due to heritable factors.

$$\text{Heritability } (H^2) = \frac{Genotypic\ Variance}{Total\ Phenotypic\ Variance}$$

4. Genetic Advance

Genetic advance (GA) also called genetic gain, gives a measure of the expected progress under a selection scheme.

$$\text{Genetic Advance (GA)} = H^2 \times \sqrt{Vp} \times K$$

K = Selection Intensity 2.06

(Write the solved problem)

Ex.No.13
Date :

MAINTENANCE OF A, B AND R LINES AND HYBRID SEED PRODUCTION IN RICE

Hybrid seed production in rice involves continuous supply of agronomically adapated male sterile (A) line, maintainer (B) line and fertility restorer (R) line. The maintainer and restorer are maintained by selfing, while the CMS line is maintained by crossing A line with B line. F_1 seeds are produced by crossing A line and R line.

TECHNIQUES IN HYBRID SEED PRODUCTION

1. Field selection: Fertile field with good irrigation and drainage system should be selected.
2. Isolation: To ensure purity of seed, avoidance of unwanted pollination is a must. This can be done by
   a. Space isolation: Though the isolation distance is 3 mts for rice, for seed production no other variety should be grown within 100 m distance.
   b. Time isolation: 20 days difference is practiced. The heading stage of other variety over 100 m should be 20 days earlier or later over the male sterile line.
   c. Physical barrier: Tall crops or wood lot to a distance of 30m or plastic sheet upto 2 m height should be maintained.

3.Planting should be done perpendicular to the direction of wind so that it favours pollen transfer from R line to A line.

4. Synchronization of flowering: Staggered sowing of R line twice or thrice at an interval of 5 to 7 days is followed. However, transplanting should be done on the same day.

5. Row ratio, direction of planting: The row ratio of R line depends upon the growth duration, vigour, pollen shedding and plant height of R line. Normally 8:2 (female : male) ratio is followed. Generally, the R line is transplanted with two or three seedlings per hill with a spacing of 15 cm between plants in a row and 30 cm between rows. The male sterile line is transplanted with one or two seedlings per hill with a spacing of 15 x 15 cm.

6. Adjusting date of heading: Adjustment of flowering date can be made by applying quick releasing nitrogenous fertilizer on early developing parent and late developing parent should be sprayed with 2% DAP. A difference of 4 to 5 days shall be adjusted by these methods.
7. Leaf clipping, GA3 spray and supplementary pollination:
   a). Leaf clipping is generally take up 2 days before initial heading and more than 2/3 rd of the flag leaf is clipped from the top.

b). Application of GA_3: In CMS lines, about 20 to 30% of the spikelets of a panicle are inside the flag leaf sheath i.e. the panicle exertion is only 70%. Application of GA_3 @ 50g/ha results in complete exertion of panicle from the flag leaf sheath. It also enhances seed set and hence seed yield. It is sprayed when the 5% of panicles have emerged during 8 to 10 am.

c). Supplementary pollination: Shaking the panicles in R lines by rope pulling or rod-driving during anthesis helps in pollen dispersal. It is done at 10-12 am.

9. Rogueing: Through rogueing, in addition to isolation, is necessary to get 98 % purity of CMS and R lines.
10. Harvesting and processing: The male parents (R line) are harvested first. Then, the A lines (hybrid seeds) are harvested and threshed separately, dried to 12 % moisture and packed in gunny bags.

SOURCE OF STERILE CYTOPLASM IN VARIOUS CROPS

Rice: Wild abortive cytoplasm (WA) is the most commonly used cytosterility source in case of rice.

Sorghum (*Sorghum bicolor*) : The male sterility in this system is caused by the interaction of *milo* cytoplasm and Kafir nuclear genes. The milo cytoplasm which induces male sterility in the female parent is passed into hybrid.

Sunflower ***(Helianthus annus)*** *:* The breeding of hybrid sunflowers has been greatly assisted by the discovery of cytoplasmic male sterility among progenies of the interspecific cross *H.petiolaris X H. annus.*

Maize (*Zea mays*) : Maize cytoplasm that exhibit male sterility are classified as T (Texas), C (Charrna) or S (USDA) on the basis of the ability of specific nuclear (Rf) genes to restore male fertility.

Difficulties in using CMS for hybrid seed production

1. Male sterility must be stable. Instability leads to partial self-fertilization, giving rise to heterogeneity and lower yield of the commercial seed.
2. Fertility restoration is generally under the control or more than one gene. So, more than one Rf gene must be introduced into the breeding material.
3. The genetic background affects the expression of Rf genes.
4. The restorer genes are under the environmental influence which vary with day length, temperature, relative humidity, light intensity and plant density
5. In some crops abnormalities in growth, development or plant morphology are observed in connection with the introduction of (S) cytoplasm or Rf genes.

Ex.No.14
Date :

WILD SPECIES IN WIDE HYBRIDIZATION

Crossing between two different species of the same genus or two different genera of the same family is called distant hybridization. Such crosses are called as wide crosses or distant crosses. Wide hybridization is an effective mechanism in transferring desirable genes into cultivated plants from related species and genera.

It mainly depends on chromosome number, crossability, fertility and homology between the two species. Distant hybridization is of two types (a). interspecific hybridization and (b). intergeneric hybridization.

A. INTERSPECIFIC HYBRIDIZATION

Crossing two different species of the same genus is called as interspecific hybridization. It gives rise to three types of crosses namely, fully fertile cross, partially fertile cross and fully sterile cross.

1. FULLY FERTILE CROSS

When the two parents in a cross, have complete chromosomal homology then normal pairing of chromosomes during meiosis results in fully fertile hybrids. Examples of fully fertile crosses in various crops are as follows:

Cotton: *G. hirsutum x G. barbadense*
G. arboretum x G. herbaceum
Wheat: *Triticum aestivum x T. compactum*
Oats: *Avena sativa x Avena byzantiana*
Soybean: *Glycine max x Glycine soja*
Sugarcane: Wakker made the first reported interspecific cross between *S.officinarum* and *S.spontaneum* for incorporating disease resistance into nobel canes.
Pearlmillet (Pennisetum sp) : In, *Pennisetum* the primary gene pool includes the wild, grassy, and weedy *P.americanum* subspecies (2n = 14) *monodii* and *stenostachyum* that readily cross with pearlmillet and produce fertile hybrids. The *subspecies monodii* is a rich source of germplasm that can be used to improve pearlmillet.

2. PARTIALLY FERTILE CROSSES

When the two species involved in a cross differs in chromosome number then the hybrids become partially fertile and partially sterile. Examples are

Wheat: *Triticum aestivum* x *Triticum durum* ⟶ Partially fertile F_1
2n = 42 (AA BB DD) 2n=28 (AA BB) 2n = 35 (AABBD)

3. FULLY STERILE CROSSES

When the two species involved in a cross have no chromosomal homology then the hybrids become sterility. The lack of homology does not permit pairing

of chromosomes during meiosis. Such hybrids are made fertile by doubling their chromosomes by colchicine treatment. Example

Nicotiana sylvestris x Nicotiana tomentosa

(2n=24) ↓ *(2n=24)*

F_1 (Fully sterile)

↓ *Chromosome doubling with colchicine*

Fully fertile tetraploid (2n=4x=48)

B. INTERGENERIC HYBRIDIZATION

It refers to cross a between plants from two different genera of the same family. Examples are as follows:

(i). Wheat x Rye cross: A cross between wheat (*Triticum aestivum*) (2n=42) and Rye (*Secale cereale*) (2n=14) resulted in *Secale cereal (2n=56).* The F1s were sterile and became fertile upon colchicine treatment resulting in an amphidiploid called triticale.

DRAWBACK IN WIDE HYBRIDAZATION

A key observation on utilizing wild species germplasm for crop improvement is the predominance of sterility in F_1 hybrids. Even when partially fertile interspecific hybrids are produced, linkages with undesirable genes many times limit the usefulness of these hybrids. The most common fertility restoration method is colchicine treatment of sterile F_1 plants, but naturally occurring unreduced gametes have also played a role in fertility restoration for a few groups. Many examples could be cited when the initial hybrids were made, fertility was restored, and the hybrids were abandoned after a few generations, due to addition of sterility barriers.

Ex.No.15
Date :

ESTIMATION OF HETEROSIS

The term heterosis coined by Shull in 1914. It refers to the superiority of F_1 hybrid over its parents. In other words, heterosis refers to increase of F_1 in fitness and vigour over the parental values. While heterosis refers to the phenomenon (cause), hybrid vigour is the phenotyphic expression (effect) of the genetical phenomenon. In current usage, heterosis and hybrid vigour are used as synonyms an interchangeable.

Estimation of Heterosis

The term heterosis can be classified into three types on the basis of estimation. They are relative heterosis over mid parent, heterobeltiosis over better parent and standard heterosis over commercial hybrid / variety.

i) Relative Heterosis : The superiority of F_1 hybrid over the mid parental value (i.e. mean values or average of two parents involved in the cross) is known as mid parent heterosis, which is estimated as follows.

Relative heterosis percent $\frac{F_1 - MP}{MP}$ x 100 where

F_1 is the mean value of F_1 hybrid

MP is the average of two parents involved in the cross.

ii) Heterobeltiosis : The superiority of F_1 hybrid over the better parent or superior parent out of two parents involved in the cross is referred to as heterobeltiosis, which is estimated as follows.

Heterobeltiosis $\frac{F_1 - BP}{BP}$ x 100 where

BP is the mean value of the particular cross.

iii) Standard Heterosis: The superiority of F_1 hybrid over the standard commercial variety/hybrid is known as standard heterosis.

Standard heterosis $\frac{F_1 - SV}{SV}$ x 100 where

SV is the Standard Variety/hybrid

A total of 15 hybrids and their parents was evaluated in a RBD with four replications. The following is the data on yield in gms. Work out the heterosis percent on all the three base and comment on the results. Assume P_3 is the standard check.

(Write the solved problem)

Result:

The hybrid P3 x P7, P4 x P7 and P5 X P7 and excelled other hybrids by recording significant superior heterosis all the three bases.

Ex.No. 16
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LAY OUT OF FIELD EXPERIMENTS - COORDINATED NATIONAL AND INTERNATIONAL TRIALS

Before release as a variety, the selected strain is evaluated in various trials to determine its superiority over the already existing best variety in yield and its suitability for consumption. In general there are different types of trials (1) Station trials, (2) Multi-location trials, (3) National trials (4) Adoptive trials (5) Minikit trials and (6) Quality tests (7) Disease and insect tolerance test.

1. Station Trial (PYT)

Station trial is conducted by the breeder who has developed the new strains. It is also called as preliminary yield trial (PYT) and may be conducted for one or more years. The objective of station trial is to make sure that the new strains developed by a breeder are superior in performance as these strains would have to complete with the best existing variety and other new strains in coordinated trials.

2. Multi-location Trials (MLT)

The objective of MLT is to evaluate the performance of newly developed strain at several locations distributed over a region. These trials are conducted under the respective "All India Coordinated Crop Improvement Project". The various trials conducted under the coordinated projects, may be grouped into (a) Initial evaluation trials (IET), (b) Uniform regional trials (URT), (c) Agronomical trial and (d) Model agronomic experiments.

a. Initial evaluation trials (IET)

A new strain is first included in an initial evaluation trial within the zone in which it was developed. The strain included in IET or URT is commonly called as an *entry*. The objective of IET is to eliminate relatively inferior entries so that their number is reduced to a manageable size for URT evaluation. The IET in conducted at 10-12 locations within a zone. The plot size in IET is 6 x 1.38 m and the number of replications is generally 6. The entries are tested in IET for one year only and are promoted to Uniform Regional Trials (URT) if their performance is outstanding, otherwise they are rejected.

b. Uniform Regional Trial (URT)

The outstanding entries, both in yield and disease resistance, in the IET are promoted to the uniform Regional Trial. The URT is conducted at 25 to 30 different locations, under the same condition under in which the concerned entry was evaluated in IET. The plot size in wheat URT is 6 x 2.76m and the number of replications is usually 6.

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c. Agronomic Trials

An entry showing superior performance in URT is included in Agronomic trials of the respective All India Coordinated Crop Improvement Project. The objective of agronomic trials is to determine the suitable dates of sowing and the optimum number of irrigations. The plot size in for Agronomic Trials is 10 x 1.61 m with three replications

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3. National Trials

The national trials are conducted throughout the country in all the zones. The entries in National Trials consist of one entry from each that ranked first in that zone in URT previous year. The National Trials serve as IET for an entry in zones other that in which it was developed and tested in URT. The purpose of National Trials is to evaluate the performance of outstanding entries of one zone in the other agro climatic zones.

4. Adoptive Research Trials (ART)

Adoptive Research Trials are conducted on research stations or farms of the State Government. The entries are identified by the workshop of the concerned coordinated project. The plot size is 0.5 acre and there is no replication. The agronomic practice used in Adoptive research trials are the same for which the concerned entry has been identified. The data from these trials are considered for release of the identified entry as a new variety. Thus, each identified entry has to undergo adoptive trials before it is released as a variety.

5. Minikit Trials

The Minikit Trials are conducted in the farmer's field. These trials are conducted along with the adoptive trials in the following crop season when the entry is identified for release. The Minikit Trials are conducted under the supervision of Director, Ministry of Agriculture. The Minikit Trials are conducted at 300 to 400 places within a zone.

11. Quality test

Quality tests are conducted to determine the suitability of an entry for various uses. For example, chapatti and bread making quality in wheat. Quality tests ate generally done for all entries in URT/CVT.

12. Disease and Insect Tolerance Test

The entries are evaluated for disease and insect resistance from IET as well as URT under both natural epidemic as well as artificial epiphytotic conditions. The disease resistance tests for different diseases are carried out at places where epidemics of that disease occur regularly.

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Ex.No. 17
Date :

SCREENING METHODS FOR BIOTIC AND ABIOTIC STRESSES

In breeding for resistance, exposure of the varieties to the disease to select resistant genotypes for involving in the hybridization programme and exposure of the segregating or mutation progenies to the disease to select resistant recombinants or mutant is necessary. Since natural disease epidemics may not occur in the field every season, it would be necessary to establish disease epiphytotics by artificial means in the field or in the green house. Inducing epiphytotics in the field may not be as easy as in the green house because several environmental factors like relative humidity, temperature, moisture etc. cannot be easily controlled under field conditions.

It is essential that the epiphytotics is uniform and of intense proportions through out the population. Otherwise, many plants may escape infection and may be mistaken as resistant ones.

SOIL-BORNE DISEASES

In case of soil-borne diseases such as root rot, damping off soil borne mosaic, and wilt the soil borne pathogens enter the host plant through the roots or other underground parts. Field tests to screen resistant, plants are conducted in 'hot spots' where the fungalinfection is endemic. The 'Sick plots' have the disease producing pathogens naturally pravalent in the soil. The disease intensity may be increased by transporting soil from highly infected fields, or by incorporating choped plants already infected severely or by inoculating the soil with cultures of the causal organism on a sterile nutrient medium. The same sick plot is used for screening year after year.

For testing in the green house under controlled conditions, the pots are filled with soil form sick plots and by mixing cultures of the pathogens.

I. SCREENING FOR DISEASES

a. AIR BORNE DISEASES

Foilage diseases such as spots, rusts, mildew, scab, smut blight and Anthrachose get the infection through stomata or lentice is or wounds caused by insect. Such as loose smut are also air-borne.

For creating artificial epiphytotics, the host plants are sprayed with a suspension of the pathogen during day time when the stomata are open spores of the pathogen collected from infected plants are dusted or a suspension of spores sprayed on the leaves or flowers. In case of bacterial diseases, the bacterial inoculum is sprayed or injected by hypodermic syringe. Dry spores are introduced with a pair of forceps or a hypodermic syringe or spore suspension injected into the inflorescence for floral infecting diseases.

A highly susceptible variety is also grown in between and all around the plot and it forms the infector row that spreads the inoculum by wind.

Spray inoculation and clip inoculation of bacterial inoculum in rapid and large volume evaluation of disease resistance. The clipping technique involves clipping the upper one-third of the leaves with scissors, after clipping the scissor blades in a bacterial inoculum. The cut ends of the leaves are inoculated and the lesions develop rapidly. Scoring is done 14 to 18 days after inoculation in the case of bacterial blight of rice.

The temperature around the host plant is maintained at the optimum for the growth of the pathogen during the infection period. Growing the host plants in a moist chamber controls the humidity during infection. A mild detergent may be added for even spread of the inoculum and in case of plants with waxy bloom. The bloom is wiped out before infecting.

b. Seed-Borne Diseases

Seed-Borne Diseases such as smut of sorghum, oat or barley, and common bunt of wheat get infection by spores clinging on to the seeds. Hence dry spores are dusted on the seeds before planting or the seeds are soaked in a spore suspension under vacuum.

c. Virus Diseases

Virus Diseases are either mechanically transmitted or transmitted by insect vectors. Artificial inoculation of virus diseases is done by macerating the diseased plant and rubbing the extracted juice over the leaves of healthy plants with force to cause slight mechanical injury.

A fine abrasive such as Carborundum powder may be dusted over the leaves before rubbing the extracted juice or may be mixed with juice to aid. in mechanical injury. In case of vector transmitted viral diseases, the insects are allowed to feed on virus infected plants and transferred to healthy plants grown in insect tight cages.

II. SCREENING TECHNIQUES FOR INSECT RESISTANCE

1. Field Screening : The varieties to be evaluated or the segregating population to be screened for resistance may be raised in fields under natural infestation, either in endemic areas or by adopting techniques for increasing field infestation. Cultural practices such as closer spacing to create the most desirable humid microclimate within the crop, application of additional dose of nitrogen and irrigation to induce vegetative growth may be adopted. The test material may be sown or planted early, before the adults emerge in an area already infested in the previous season.

If the insect population under natural condition is not to the required density, artificially reared insect population may be released in the crop at the appropriate growth stage.

In case of insects such as plant hoppers, flies etc that have a tendency to move at a rapid fibre glass mesh cages may kept on microplots and artificial reared insects released at a specified number per plant.

The insect population should be able to infest the plant population uniformly so that plants that escape infestation are not graded as resistant.

Highly susceptible plants can be interplanted as "Spreader rows" along with rows of the test material. Insect attractants such as fishmeal for sorghum shoot fly may also be used to attract and increase the insect density in the field.

2. Green House Screening:

Screening the varieties or segregants in green houses providing conditions conductive for infestation is more rapid and reliable than field screening. Special methods have been developed to increase the insect population to provide sufficient insect pressure for valid screening. Insects reared in the culture maintance cages are released on plants raised in seed boxes kept in green house. Fibre glass screen cages are used for each seed box.

Depending on the crop on one hand and the insect pest on the other, the stage of the crop at which the insect is released, the stage of the insect whether egg, larva or adult and the number of the insect population vary, as also the symptoms on the host to differentiable and grade the resistant plants from the susceptible.

3. Laboratory Screening

Laboratory screening for resistance can also be done using plant tips or leaf discs and allowing forced or free choice feeding by insects as the case of lucerne weevil.

4. Bioassay Technique

Bioassay techniques are also used to screen resistance to insects, such as *Heliothis sp*. and pink boll worm in cotton. Lyophilized square powder is incorporated in an artificial diet and dispensed into two-ounce plastic containers. Late first or early second instar larvae of *Heliothis* are wished and placed in the diet cup. By periodic observations, larval survival larval growth and percent population are recorded. Date on fecundity and longevity of emerging adults are obtained to screen resistant types.

III. SCREENING FOR SALINITY

Screening genotypes in natural saline soils is not a practice that can be recommended due to the variability in salinity in field soils and in these conditions tomato plants develop their root system in the less saline parts of the soil. Hence, screening should be done in soil-less culture watered with solutions of known Nacl concentration, with artificial sea water or with Nacl + $Cacl_2$ mixtures. The salt concentration at which genotypes are to be evaluated must be carefully chosen as the type of gene action active for characteristics like yield and other trait. The salt concentration to be used in the evaluations should be similar to that of water available for field cultivation. Selection for ECS above 9 ds m^{-1} is not advisable because at that salinity yield is reduced by more than 50% compared to fresh water cultivation. The evaluation of genotypes should be made at three saline concentrations: The lowest determined by the salinity of the best available water for the experiment, the highest and an intermediate level.

Heritabilities found in studies for most of the characteristics involved in salt tolerance suggest that those characteristics can be improved by selection.

Ex.No : 18
Date :

PROCEDURE FOR MARKER ASSISTED SELECTION

Introduction

Genome of each organism is the blue print that determines the ultimate life. Genes in each genome are identified based on the phenotypes produced by them. However, many of the genes are not identified because of complex phenotypic outputs.

Markers at DNA level

Markers at DNA level include short DNA sequences, whole genes or even longer sequences of DNA. These markers may be of different types.

1. Restriction Fragment Length Polymorphisms (RFLPs)

RFLP are genetic differences observable at the DNA level characterized by a number of variable length restriction fragment. The RFLP analysis involves DNA cleavage by restriction enzymes, electrophoresis of resulting fragments, southern transfer of separated fragments to a membrane support, radioactive labeling of suitable probes hybridization of probes to membrane supported fragments and detection as a banding pattern on X-ray film. RELP s are codominant markers, inherited in a simple mendelin fashion.

2. Polymerase Chain Reaction (PCR) Based Markers

The polymerase chain reaction is a recent revolution in the field of molecular biology PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequence which uses two oligonucleotide primes of about 10-20 nucleotides in length that specifically hybridise to opposite strands flanking the region to be annealing and extension of annealed primers by DNA polymerase, produce an exponential amplification of specific DNA sequence.

a. Sequence Tagged Sites (STS)

Informative primers are synthesized from known sequences, or end sequencing the anonymous clones to amplify the regions of interest. This approach is called sequenced Tagged Site (STS) approach. Recently, most of the genome mapping projects are underway to convert all the RFLP and other clones into information primer sequence to amplify specific sequences by PCR. STSs are also codominant markers as RFLPs.

Randomly Amplified Polymorphic DNAs (RAPDs)

The generation of RAPDs involves the use of single short random oligonucleotides. When these random primers are mixed with genomic DNA and thermo stable DNA polymerase and subject to PCR will prime the amplification of several DNA fragments. The DNA amplification with random primers exposes polymorphisms distributed throughout the genome. RAPDs do have their drawbacks. Among them repeatability and stability are potential snags. Further, RAPDs are usually dominant markers thus preventing the accurate detection of heterozygotes. Considering the difficulties dominant RAPD markers are converted into codominant RFLP markers for a stable performance.

C. Sequence Characterised Amplified Regions (SCARs)

Sequence Characterized Amplified Regions (SCARs) are PCR based markers that repreent a single genetically defined loci that are identified by PCR amplification of genomic DNA with pair of specific primers. Amplified RAPd products are cloned and sequenced. The sequences are used to design 20-24 mer oligonuclcotide primers. The polymorphism revealed by the RAPD markers as the present or absence of specific band amplified by PCR, which are dominant, can be converted into codominant SCAR markers.

d. Variable Number Tandem Repeats (VNTRs)

Variable Number Tandem Repeats (VNTRs). It includes microsatellites, minisatellites and hyper variable regions. Microsatellites arrays of tandemly repeated DNA sequences, which occur, dispersed throughout the genome. These are otherwise called as Microsatellites consist of around 10-50 copies of motifs from 1 to 5 Pb that can occur in perfect tandem repetition, as in perfect repeats or together with another repeat type. The minisatellites are highly polymorphic but are less common that microsatellites and have larger sequence motifs extending over more than 1 kb making them less amenable to PCR analysis.

e. Amplified Fragment Length Polymorphism (AFLP)

It combines both classical restriction-based and recent PCR based approaches. AFLPs are obtained by various means and selective Restriction Fragment Amplification (SRFA) is one among them. SRFA is a method by which a selective restriction fragments of a total genomic digest are detected by amplification using PCR. SRFA involves three major steps viz., i) cutting genomic DNA with restriction enzymes(s) ii) ligating double stranded adapters to the restriction fragments and iii) amplifying selective restriction fragments using universal primers. SRFA may be performed using universal primers. SRFA may be performed with a single enzyme, but the best results are achieved using two different enzymes, a rare cutter and a rapid assay with potential application in genome mapping, DNA fingerprinting and marker-assisted breeding.