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Emasculation and Pollination Techniques

Rice (*Oryza sativa*) (2 n = 24) (Family – Poaceae)

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

Receptivity remains for one day.

Emasculation and Crossing techniques

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

1. Clipping method

In the previous day evening, top 1/3rd and bottom 1/3rd portions in the panicle of the desired female parent are clipped off by using scissors leaving the middle spikelets. With the help of scissors again, top 1/3 portion in each spikelet is clipped-off in a slanting position. The six anthers present in each spikelet are removed wit the help of the needle (Emasculation). Care must be taken during emasculation for not to damage the gynoecium. Then to prevent contamination form the foreign pollen, the emasculated spikelets are covered with a butter paper big. In the next day morning (usually at 9.00AM), the bloomed panicle from the desired male parent is taken. The top portion of the butter paper bag which was originally inserted in the emasculated female parent is now cut to expose the panicle. The male parent panicle is inserted in an inverted position into the butter paper bag and sturned in both ways in order to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened butter paper bag is closed using a pin. Coloured thread may be tied at the base of the panicle to identify the crossed ones.

After ensuring pollination, the bag may be removed.

2. Hot water method

A method of hot water emasculation is used to about the same extent as the clipping method. Panicles in 3rd (or) 4th day of blooming are chosen as female parents. An hour or so before blooming (i.e. normally at 7. A.M.), the panicle is selected and under developed and opened spikelets are removed. Now, the tiller is bent over (carefully to avoid breaking) and the selected panicle is immersed in hot water contained in a thermos bottle at 40-440C for a period of 5 to 10 minutes. This treatment causes the florets to open in a normal manner and avoids injury. Then, emasculation is done by removing the six stamens by fine forceps or needles and then dusting should be done.

3. Dr. Ramiah method

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

4. Vacuum emasculation method

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

5. Cuttack Method

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

6. Brown paper method

The panicles are enclosed in a Brown paper cover before a couple of hours of blooming. Heat develops inside due to which the anthers extrude, but donot dehisce. This happens in 15-30 minute then the anthers are easily clipped off. Stigmatic surface is then dusted with pollen grains collected from the chosen male parent. The crossed panicle is then properly tagged and protected with paper cover which is retained in a position for 7 – 10 days.

7. Rhind's method

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

Wheat (*Triticum aestivum*) (2n = 42 Hexaploid) (Family – Poaceae)

Much of the pollen grains shed within the floret and the crop is largely self pollinated. The glumes normally open during the flowering process, the anthers protrude from the glumes and part of the pollen grains is shed outside the flowers. Entry of foreign pollen at flower opening may result in a small extent of cross pollination which is normally less than one per cent. **Selfing**

The inflorescence is covered with a butter paper cover prior to anthesis, and kept undisturbed till the flower opening completed.

Emasculation

On emergence of the ear upper 1/3rd of the spikelet is cut and lower spikelets are also removed. Of the remaining spikelets alternate ones on both sides of the axis are removed. The top spikele is held with forceps and pulled downwards and upwards to remove the upper florets of the spikelets. The glumes are separated and anthers left exposed are removed carefully and covered with butter paper cover.

Crossing

On the next day earhead selected from the pollen parent are used for crossing. The upper half of the glumes of the few medium spikelets are cut of and the ripened bright yellow anthers are rubbed on the styles of the emasculated florets and then covered.

Lecture 13

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

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

Selfing

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

Crossing technique

Female parent

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

Male parent

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

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

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

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

Sorghum is normally self-pollinated but some florets are ***protogyny*** resulting in cross pollination averaging about 6%. So, it is classified as often cross- pollinated. The amount of

natural cross pollination varies from 0.6 to 50 per cent in different varieties and places. The cross pollination is more in loose panicles than in compact ones. Anthesis starts from tip to downwards at the rate of 2-5 cm per day and completes within 7-10 days. Anthesis time 3-6 am.

The pollen grains are viable only for short period and stigma is receptive for 8-16 hours.

Selfing

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

Emasculation

1. Hand emasculation

Only a part of the panicle is emasculated and the remaining panicle is clipped away. During clipping, flowered tip and the lower panicle branches are removed. About 50 florets which would normally flower the following day are selected for emasculation. The needle is inserted at the middle of the floret and moved across the glumes. The needle is rotated at 900 and three anthers are lifted out. The emasculate panicle is covered by a suitable paper bag.

2. Hot water method

In this method, in the panicle flowered tip and lower panicle branches are removed. About 50 florets (in clusters of two or three) are immersed in hot water at 480C for 10 minutes.

3. Plastic bag/ mass emasculation technique

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

On a dry morning when pollen shedding is occurring between 6 and 7 A.M., the hand pollination may begin around 9.30 A.M. In rainy days, the operation may be started at 11.30 – 12.30 A.M. The pollen is collected in paper bags. Sorghum pollen kept in bags is viable for 1020 minutes. For collection, appropriate heads may be selected and bagged in the previous night itself.

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

Cumbu / Pearl Millet (*Pennisetum glacum*) (2n = 14) Family – Poaceae

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

Selfing

To ensure selfing, spikes may be bagged before emergence of the stigmas. As the spike elongates it may be necessary to adjust the bag to cover the lower most spikelets. Another procedure is to enclose within a bag two full spikes from the same plant, one day (or) 2 days older than the other and ready to shed pollen as the stigmas are emerging form the younger spike.

Crossing

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

Controlled cross pollination

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

morning. Care should be taken to cover pollen parent previous day with butter paper bag. The crossed heads are labeled.

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

Small Millets

Ragi/ Finger millet (Eleusine coracana) (2n:36) Family: Poaceae

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

Selfing, emasculation and pollination techniques

Selfing

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

Crossing

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

Approach Method or contact method

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

60-90% seed set is recorded in both methods.

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Lecture 14

Red gram (*Cajanus cajan*) (2n = 22) Family – Fabaceae

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

Adaptations for self pollination

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

Selfing, emasculation and pollination techniques in Red gram

Selfing

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

Crossing

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

Black Gram (*Vigna mungo*) (Diploid, 2n = 22 & 24) Family – Fabaceae

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

Selfing

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

Crossing

Young unopened bud is kept between thumb and fore fingers of the left hand. The point of dissecting needle is inserted just under the standard petal in an oblique position along the top of the bud. The left side of the standard and wing petal are pushed outward and held with thumb and left hand. The left side of the keel petal is removed with the forceps. The pistil and stigma are then exposed and the anthers are removed with the forceps. Evening

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

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

Pollination

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

Selfing

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

Crossing

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

Selfing and crossing are the essential procedures in crop improvement process. The exact procedures used to ensure self or cross-pollination of specific plants will depend on the floral structure and normal manner of pollination. Generally effecting cross-pollination in a strictly self-pollinating species is more difficult than vice-versa because for instance preventing selfpollination occurring inside the unopened flowers is cumbersome.

Bengal Gram – *Cicer arietinum* (2n = 14, 16), (Channa, Chick Pea), Family – Fabaceae

Chickpea is a self pollinated species with normal out crossing limited to 1.58%. self pollination takes place one or two days before opening up of the flower. The flower open in the morning and close in the afternoon and each flower opens on tow or three successive days.

Time of anthesis is 3 AM to 9 AM. For hybridization crossing work should be started when the first pod on the selected plant is already formed. In Northern India, emasculation is done a day prior to pollination. The pollination is done in the morning hours give better setting. In south India, pollination immediately after emasculation give higher seed setting.

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

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

Emasculation and crossing

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

Groundnut (*Arachis hypogaea*) (2n = 40), **Family –** Fabaceae

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

Selfing

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

Crossing

Mature flower buds which are ready to open in the next day are selected and emasculated in the evening. They can be easily identified by the size and length of calyx tube. The flower bud of groundnut is of crescent shape, being bulged on one side and slightly depressed on the other. The keel petal is located on the bulged side and the standard is present on the depressed side. For emasculation, hold the bud between the thumb and the index finger of the left hand and with the help of a razor blade in the right hand; make a cut on the depressed side at two-thirds the length below the tip so as to cut the standard and a portion of the wing

petals. Then gently pull the calyx and corolla by holding at the tip of the flower bud. By doing this, the sepals and the petals except the keel would be removed, with the help of the fine forceps gently liberate the bundle of stamens and the pistil from the keel and nip off the anthers.

With a hand lens, examine the tips of filaments so as to ensure complete removal of the anthers. Take a piece of straw tube (used for sipping cool drinks), 4 to 5 cm long and close one side opening by bending the tip. Cover the emasculated flower bud with the straw tube by slowly inserting calyx tube into it. This would ensure perfect protection to the stigma from any natural cross pollination. The next morning take out the straw tube, dust the stigma with the desired pollen and reinsert the tube. Pollination between 7 and 8 am was found to give more success. If the stigma is found dry, pollinate after smearing it with 2 per cent sucrose solution. Next day early morning between 7 am and 11 am pollen is collected from mature yellow anthers of the selected male parent and dusted on the receptive stigma. For cross pollination, the selected male flower is held between thumb and the middle finger after the standard and wing petals are removed. The flower with keel protruding is taken to the stigma of the emasculated flower. A gentle push on gently keel by the finger forces lumps of pollen grains of the cover the entire stigmatic surface. Five to seven days after pollination successful crosses will produce gynophores (pegs) with the dried flowers at their tips. These are then introduced into small wire rings of 4 mm diameter which are marked for respective crosses.

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

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

Selfing

1. **Tieing with thread:** Selfing can be effected by tieing the corolla of the unopened flower which is selected in the previous day evening itself.
2. **Smearing of semi-solid clay:** Selfing can be done by smearing a speck of semi-solid clay, on the upper portion of tubular petals of unopened flowers. The clay while on drying does not allow the tubular petals to open and hence self pollination is the rule. This method is cheap and less time consuming one. This method is noat effective during rainy days. During rainy days, fevicol may be applied on young flower bud to ensure selfing.

Crossing

Soda- straw method

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

Sunflower (*Helianthus annuus*) (2n = 20), **Family - Astraceae**

Sunflower in highly ***cross pollinated*** crop mainly through insects (***Entomophily***) and to a limited extent by wind (Anemophily). The flower opening starts from outerside of the head and proceeds towards centre. The head takes 5-10 days for complete blooming depend on size of head and season. Anthesis occurs between 5 to 8 A.M. Pollen viable for 12 hours. Stigma is receptive for 2-3 days. The staminal filaments elongate rapidly and the anthers appear above the top of the corolla. Anthers dehisce early than maturity of stigma. (***Protoandry***). In this crop, the cross pollination occurs due to protandry, limited area of

stigmatic surfaces for receptivity, ray floret colour attracts insects and abundance of sweet secretions in the disc florets.

Selfing

The flower head is protected with a suitable cover before the commencement of anthesis in any of the florets and the cover is retained till fertilization is over in all the florets. Artificial self pollination with pollen collected from the same flower or another flower of the same plant using a soft brush will enhance seed settings.

Crossing

i. Hand Emasculation

Emasculation is done in the early morning by removing the anthers of the disc florets in 2 to 3 whorls with forceps and the other florets in the head are removed. About 9-10 am the pollens from desired male parent are collected and dusted on the emasculated head. This process is continued for 2 to 3 days.

ii. Without emasculation

In sunflower, head emasculation is difficult. Considering this difficulty, the heads are pollinated without emasculation. On the basis of hybrid vigour, plants are distinguished from the selfed plants. The presence of marker genes for identifying hybrids is also utilised effectively. **iii. Chemical induction of male sterility.**

This is achieved by spraying of 100ppm GA (Gametocide) during bud-initiation stage consecutively for three days in the morning.

Pollination is carried out by collecting pollen from heads which are already bagged prior to flowering. Pollen may be collected from flowering heads into paper bags. Pollination is done in the morning by applying the freshly collected pollen by a small piece of cotton, a hair brush or through fine cloth bag. After each cross, care must be taken to avoid contamination by wiping the hands with alcohol.

Castor (*Ricinus communis*) (2n = 20), **Family – Euphorbiaceae**

Cross pollination is the rule in this crop. It is mainly wind pollinated. But insect activity is also seen to some extend since the young leaves just below the inflorescence exude copious nectar at the time of flower opening cause insect pollination. Unisexual flowers, protogynous, elevated position of female flower in the inflorescence, mechanisms to promote

wind pollination and nectar glands to attract insect promotes cross pollination. Here male flowers open first (*protoandry*). After one or two days of male flowers opening, female flowers open. However, *protogyny* is also reported. The opening is between 4.30 and 5.00 A.M. Pollen grains are viable for a 2 days and stigma is receptive for 3 days. Each candle takes 10-12 days to complete flowering.

Selfing

The whole inflorescence is protected with not yet opened are selected. From the selected inflorescence all the male flowers are removed and the female flowers protected with a suitable cover. Artificial cross pollination is done when the stigmas of the retained female flowers become receptive by rubbing the anthers of male flowers collected from the selected male parent. During the rain day old bags are to be replaced with new bags to avoid fungal attack, and free air movement.

Crossing

1. **Emasculation:** It can be achieved by removing or rubbing off the staminate flowers by finger and thumb.
2. **Crossing:** Pollen grains are collected from the desired male parent and are dusted on the stigma of the female parent. Again the inflorescence is covered.

Lecture 15

Sugarcane (*Saccharum officinarum*) (2n = 80)

Cross pollination is the rule in sugarcane. Self male and female sterility, protogyny and hanging down of anthers away from the stigma at the time of anthesis promote self pollination. Usually anthesis will be in early morning between 5 am and 6 am. Maximum anthesis between 6 am and 8 am. Stigma protrudes out first and anthers dehisce afterwards. Flower opening will be from top to downwards. It will take about 10 days for complete opening of spikelets. Flowering in sugarcane is location specific and influenced by environment. Natural pollination is by wind (*Anemophily*)

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Selfing

Selfing is done by covering the arrow with a bamboo frame work or cage which is covered with muslin cloth or polythene paper. Such a cover is commonly called lantern. It prevents accidental cross pollination. The lantern has to be supported by bamboo poles. The lantern has to be opened once in a day to reduce the temperature that my build up inside during the day time. This is done preferably during the afternoon hours between 12am and 4pm. Usually the cover has to be retained in position till the seeds are set. Within one week or 10 days we can get selfed seeds. This selfing method is followed in Sugarcane Breeding Institute, Coimbatore.

Crossing

Hybridization is very difficult.

1. It is mostly vegetatively propagated. Some varieties seldom flowers outside tropics. Some varieties flowers once in 6 to 8 months. It is highly controlled by photoperiods.
2. Spikelets are minutes. So, hand emasculation is not possible.
3. Self sterility of both pollen and ovule predominates in almost in all the varieties.
4. Hot water treatment can not possible.

Hybridization methods

Coimbatore method

During flowering period, the sugarcane stem will be cut leaving one or two bud. The cut stem can be transferred to a mud pot having moist mud. Within 10 days the buds will develop into roots and there will be good root system. This can be transferred to the breeding block. In the crossing block, the male and female plants are covered with common **lantern**. Free shedding pollen over female plant will occur. We can harvest both selfed and crossed seeds from the female parent. The selfed seeds can be identified by chromosome number by raising it in the nursery. Selfed seeds thus removed retaining crossed seeds.

Marcotting method

During flowering, cut around the stem and tie a polythene bag with nutrients (growth medium). The bud near cut end give rise to roots. This can be cut and used for hybridization purposes. This method is called marcotting. Practiced in Sugarcane Breeding Institute, Coimbatore.

Lantern method

Providing Lantern for a female plant before anthesis starts. From the desired male parent cut the arrow. That arrow can be introduced into the Lantern and shaken up and thereby crossing can be effected. This will be repeated for 2-3 days in order to have more seed set.

Hawaii method (Sulfurous acid Technique)

A sulfurous acid solution keeps the inflorescence alive for several weeks. Here, we cut both mal and female flowering arrows along with small portion of stem. These cut end will be immersed in a vessel containing 0.01% sulphuric acid and 0.01% phosphoric acid. The cut end at the lab is brought nearer and effect cross pollination. They absorb the weak acids. We have to add weak solution daily to replace the acid taken by stem. Once in a week we have to completely change the solution. This is done for 20-30 days. During this time, the seed will mature.

In modified method of this, the female plant alone is cut and kept in weak acid at the time of flowering the male parent can be brought nearer and the pollen can be shed by shaking as done in Lantern method.

Emasculation and Pollination Techniques in Cotton

Selfing

In the selfing of cross-pollinated species, it is essential that the flower are bagged or otherwise protected to prevent natural cross-pollination. Selfing and crossing are essential in crop breeding. It is important that the breeder, master these techniques in order to manipulate the pollination according to his needs. The exact procedure that he may use to ensure self or cross pollination of specific plants will depend on the particular species with which he is working. The structure of the flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself with the **flowering habit** of the crop.

In the case of wheat, rice, barely, groundnut etc., the plant is permitted to have self pollination and the seeds are harvested. It is necessary to know the mode of pollination. If the extent of natural cross pollination is more, then the flowers should be protected by bagging. This will prevent the foreign pollen to reach the stigma. Seed set is frequently reduced in ear heads

enclosed in bags because of excessive temperature and humidity inside the bags. In crops like cotton which have larger flowers the petals may fold down the sexual organs and fasten, there by pollen and pollen carrying insects may be excluded.

In certain legumes which are almost insect pollinated, the plants may be caged to prevent the insect pollination. In maize, a paper bag is placed over the tassel to collect pollen and the cob is bagged to protect from foreign pollen. The pollen collected from the **tassel** is transferred to the cob.

Emasculation

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

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˚C for 10 minutes is found to be suitable. In the case of rice, 10 minutes treatments with 40-44˚C is adequate. Treatment is given before the anthers dehiscence and prior to the opening of the flower. Hot water is generally carried in thermos flask and whole inflorescence is immersed in hot water.

4. Alcohol Treatment

It is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. 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 0.6OC 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

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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.

Pollination

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 emasculate