HYBRIDIZATION TECHNIQUE IN COCONUT

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CPCRI Technical bulletin No. 057

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INTRODUCTION

Coconut, *Cocos nucifera* L., is an important oil yielding palm mainly cultivated in the humid coastal tropics. India is at present the largest producer of coconut in the world with an annual production of 1584 million nuts (2006-07). Coconut oil accounts for about 7.8% of the total edible oil production in the country with a production of 4.4 lakh tonnes. In addition, India imports around 2000-7000 tonnes of crude coconut oil. To enable self sufficiency in edible oil production, the Government of India is encouraging establishment of coconut plantations in the country. Traditionally tall coconut cultivars have been commercially grown for copra/oil production in the coconut growing countries. However, since the reports on manifestation of hybrid vigour in coconut palms, first from India in 1937, hybrids have gained popularity because of their precocity and high yield. Hybrids usually express hybrid vigour in the nursery for vegetative characters such as height, girth at collar and number of leaves in the seedlings. These seedlings on planting have a rapid growth rate with a higher rate of leaf production, shorter pre-bearing period and significantly higher yield. These findings emphasized the importance of hybridization in coconut improvement. In India, 14 hybrids have been released for commercial cultivation. Production of seed nut of hybrids requires controlled pollination and this technique has been standardized. Details are provided in the subsequent sections.

FLORAL BIOLOGY

Inflorescence

For any hybridization programme, it is essential to know the floral biology for undertaking controlled pollination. The inflorescence of a coconut palm is the 'spadix'. As the coconut palm is monoecious, it produces both male and female flowers in the same palm.

The inflorescence develops within a strong, tough, pointed double sheath called spathe. When the spathe is fully grown, the entire structure collectively called the 'spadix' would be about 1 to 1.2 m in length and 14 to 16 cm in diameter at the broadest point. At this stage, it splits along its underside from top to bottom and releases the inflorescence. This usually occurs 75 to 90 days after the first appearance of its tip in the leaf axil.

Fig. 1. Coconut inflorescence

Male flowers

The inflorescence consists of many flower bearing spikelets situated on the spike - the central axis. The number of spikelets varies from 30 to 35. On the spikelets, the male flowers are located at the distal end and the female flowers towards the base. The number of male flowers may vary from 250-300 per

Fig. 2. Single male flower

spikelet and there may be about 8000 to 10000 male flowers per inflorescence.

Male flowers have three outer perianth measuring about 3 to 5 mm, three inner perianth measuring about 15 mm, six stamens and a rudimentary pistil.

Male phase

Generally, male flowers are the first to open immediately after splitting of the spathe. Flowering commences from the apex of the spike and extends downwards towards the base. Flowers bloom throughout the day but maximum blooming occurs during 8 to 10 AM. After shedding their pollen, the male flowers wither and fall off, usually within two days after their opening. In tall palms, the duration of male phase, that is the time duration between the opening of the first male flower and shedding of the last male flower is about 18 to 22 days, depending on the palm's characteristics, the growing conditions and the season.

The Pollen

When the anthers are fully mature, the pollen sacs burst along the two longitudinal slits corresponding to the partitions of the pollen sacs and shed their pollen before the opening of the male flower. The pollen grains are spherical and smooth when fresh but on exposure for a few seconds, turn ellipsoidal with a longitudinal groove in the middle. They measure about 0.063 mm in length and 0.020 mm in breadth. It is estimated that an inflorescence contains about 180 to 360 million pollen grains. The pollen output per anther in a flower of a healthy palm is estimated to be 111,000 to 221,000 pollen grains.

Germination of pollen grain

The germination test of the collected pollen is desirable before using the same in

Fig. 3. Initiation of Male phase

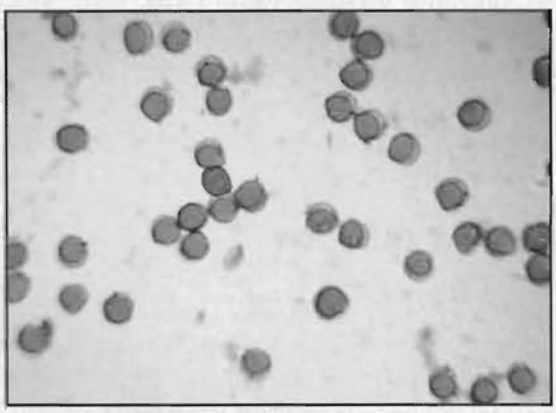

Fig. 4. Fertile pollen grains

pollination. The test is usually done by placing the pollen in a solution containing 800 mg of sucrose, 200 mg of gelatin and agar in 10 ml of distilled water. The pollen starts germinating within 1 to 2 hours of incubation in the germination media. The pollen lots exhibiting more than 50% germination alone should be selected for pollination.

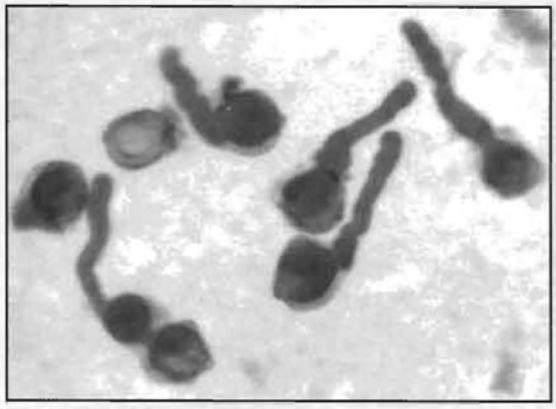

Fig. 5. Germinating pollen grains

Female flowers

Unlike the male flowers, the female flowers are comparatively few in number in an inflorescence. They may vary from 20 to 40 in tall palms. Dwarf palms, generally, carry a large number of female flowers in a spadix than that of tall palms. However, a few tall varieties also contain much more female flowers, even up to 100 female flowers per inflorescence.

At the time of opening of the spathe, the female flower is a small spherical body about 1.3 cm in diameter with great resemblance to a small nut and is popularly known as button. The female flowers, like male flowers, consist of six tepals that are thicker, imbricately arranged and tightly folded over the inner parts of the flower completely enveloping the spherical pistil. The ovary is tricarpellate and each carpel has a single

Fig. 6. Female flowers at the bottom of the spikelets

ovule. Normally, only one ovule develops while the other two either abort or degenerate. But in exceptional cases bicarpellate and even tricarpellate fruits are also produced.

The stigmas are sessile. When the stigmas are receptive (ready to receive pollen), nectar

Fig. 7. Receptive female flowers

is secreted at the base of the stigmas and at the three pores on the pericarp towards the top of the ovary. When the female flowers become receptive, it opens at the apex and the three stigmas protrude from it like a three-pointed star. At this stage, the stigmas are ivory in colour.

Female phase

Female phase is the time interval between the receptive stage of the first female flower and the last female flower. The duration of the female phase varies with the variety and the environment of the palm. It is much shorter than the male phase and lasts for about 5 to 7 days in tall palms and twice as long in some dwarfs.

Fig. 8. An inflorescence in female phase

Interval between male and female phases

The interval between the end of male phase and the beginning of the female phase has an important bearing on the nature of pollination. In coconut, especially in the tall variety, there is a distinct gap between the male and the female phases as the female flowers become receptive after all the male flowers in

Fig. 9. Inter-spadix overlapping

Fig. 10. Intra-spadix overlapping

the same spadix have shed their pollen. This makes cross pollination customary. However, some chances of self pollination exist from the succeeding inflorescences (inter spadix overlapping) especially during the summer season. In dwarf palms, the time interval between the two phases, is either nil or negligible thereby increasing the chances for self pollination. The duration of female and male phase along with the overlapping of phases in the common parental lines at CPCRI, Kasaragod is provided in the table below.

Parental line	Duration of male phase (days)	Duration of female phase (days)	Duration of gap between male and female phase (days)	Intra-spadix overlapping (days)
West Coast Tall (WCT)	15-17	3-5	2-3	0
East Coast Tall (ECT)	19-20	4-5	2-3	0
Andaman Ordinary Tall (ADOT)	20-22	4-5	2-3	0
Straits Settlement Apricot Tall (SSAT)	19-20	5-6	0	2-3
Laccadive Ordinary Tall (LCT)	18-20	4-8	2-3	0
Gangabondam Green Dwarf (GBGD)	17-18	8-9	0	5-6
Malayan Yellow Dwarf (MYD)	17-18	9-10	0	4-5
Chowghat Orange Dwarf (COD)	14-16	10-12	0	4-5
Malayan Orange Dwarf (MOD)	14-18	7-8	0	5-7
Malayan Green Dwarf (MGD)	13-15	6-8	0	3-4

Pollination

There is so far no consensus on the major factor responsible for pollination in coconut. A number of insects *viz.* ants, honey bees, wasps etc. play an important role as carriers in pollination. Wind pollination is also an important factor in coconut.

Fig. 11. Insects visiting receptive female flowers

After fertilization, it takes about 11 to 12 months for the flower to develop into mature fruit. The unfertilized female flowers turn brown and fall from the inflorescence. A few fertilized flowers also fail to develop properly and they too are shed.

COMMERCIAL PRODUCTION OF HYBRIDS

a) Emasculation

The first step in hybridization is the removal of male flowers from the inflorescence of the female parent to avoid selfpollination. This is called 'emasculation'. To avoid chances

Fig. 12. Emasculation of coconut inflorescence

of pollen contamination it is better to do the emasculation in the initial few days after opening of the inflorescence. This is done either by removing the individual male flowers by hand or by cutting the spikelet (with knife or secateur) about 4 to 5 cm away from the upper-most female flower and removing the remaining male flowers by hand. Generally, 1 to 2 male flowers are found attached to the base of the female flowers and care should be taken to ensure that these male flowers are also removed at the time of emasculation.

Fig. 13. Emasculated inflorescence

b) Pollen collection and processing

Collection of pollen from an inflorescence between 6 to 8 days after opening is recommended. For collection of pollen, mature, unopened male flowers are collected from fully opened inflorescence. The mature male flowers can be easily identified by their bluish green tinge and are ideally collected from the middle portion of the spikelet. The male flowers are placed between two sheets of blotting paper (or old newspapers) and slightly crushed using a wooden rolling pin to separate the perianth parts, taking care not to damage the anthers. The crushed male flowers are then dried in an incubator at 40°C for 36 to 48 hours.

Fig. 14. Crushing mature male flowers

Alternatively, equipment called 'Fluid-bed drier' can be used for quick processing of pollen. In this instrument the fresh male flowers are dried by exposing them to hot air.

Fig. 15. Drying male flowers in incubator

The air temperature and the speed can be regulated. The crushed male flowers are fed to the fluid bed drier and dried at a temperature of 40°C. It is possible to collect coconut pollen, at rates of 55 to 60g in about three to four hours. This is especially useful in seed gardens where a large quantity of pollen is required every day.

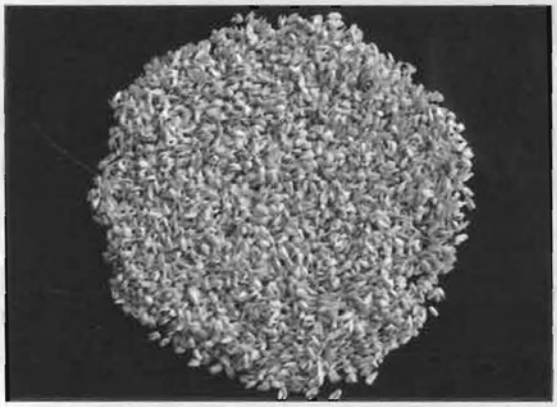

Fig. 16. Dried male flowers

The pollen is collected by sieving the dried male flowers using a sieve with mesh size of 0.2 mm. Pollen collected through fluid bed drying has viability similar to that of pollen from flowers dried for 40 hours on incubator shelves. The processed pollen is suitable for immediate use or it may be stored.

The collected pollen can be stored in desiccators over fused calcium chloride for 10 to 15 days. When longer duration of storage is required, the pollen can be stored in deep freezer (-20°C) for up to three months. Pollen can be mixed with neutral talc in a ratio of up to 1 part pollen to 9 parts of talc and used for pollination. However, if pollen is available in plenty, the pollen: talc ratio can be 1:6 or 1:7. When the pollen stored in deep freezer is to be used, it should be allowed to remain at room temperature for a few minutes before diluting.

Fig. 17. Drying male flowers in fluid bed drier

Fig. 18. Sieving of dried male flowers using shaker

Fig. 19. Manual sieving of dried male flowers

Fig. 21. Pollen and talc for preparation of pollen-talc mixture

Fig. 20. Sieved pollen in collection pan

Fig. 22. Processed pollen stored in desiccator

c) Pollination

The ideal time for assisted pollination is from 8 to 11 AM, coinciding with the receptivity of the female flowers. However, the pollination technique to be used in a garden depends on the type of plantation. When the female parents are scattered in a garden and planted with different types of tall cultivars, 'controlled pollination' technique is to be used. This method involves bagging of emasculated bunches for the entire period of the female phase and pollinating with desired pollen. The inflorescence is to be pollinated, generally, on the 1st, 3rd and 5th day starting from the day when the first female flower comes to receptivity. However, in the case of dwarf palms, having a longer female phase, it may be necessary to undertake 2 to 3 additional pollinations on the 7th, 9th and 11th day starting from the day when the first female flower comes to receptivity. When the stigmas turn brown and black the female flower is no longer receptive for pollination. Three days after the receptivity of the last female flower in an inflorescence, the protective cloth bag can be removed from the pollinated bunch.

Fig. 24. Pollen being dusted on receptive female flowers

Fig. 23. Bagged emasculated inflorescence

Fig. 25. Inflorescence with female flowers after pollination

Fig. 26. Fruit setting in a pollinated bunch

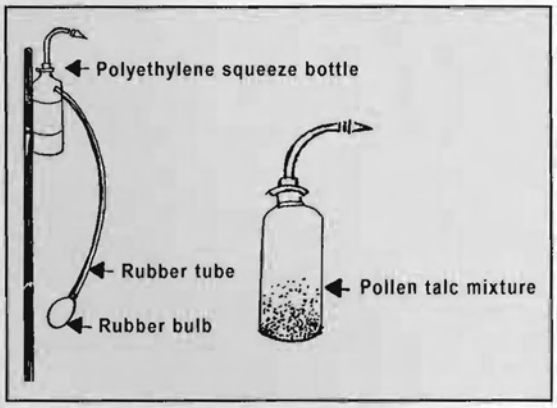

Fig. 27. Pole pollination device

The plantations of dwarfs inter-planted with a single tall cultivar are suitable for large scale commercial production of coconut hybrids. Such plantations should be established in isolated areas, ensuring that no coconut palms exist in the vicinity up to 500 m, to avoid pollen contamination through wind/insects. The isolated seed garden at Ambakelle, Sri Lanka is a famous example for this type of hybrid seed production. In this case, all the inflorescences in dwarf palms are to be emasculated so that only pollen from tall male parent is available in the garden. Emasculation is to be done on the first day of anthesis, immediately on splitting of the spathe, by removing all the male flowers and leaving only the female flowers for pollination. All the nuts collected from the dwarfs after emasculation will be hybrid nuts (D x T). However, the recovery of hybrids under natural pollination can be low as compared to the assisted pollination.

Fig. 28. Pole pollination of emasculated bunch

For effective and speedy assisted pollination, a simple device has been developed. It consists of a polythene squeeze bottle, a rubber tube and bamboo pole. The squeeze bottle is tied at the end of a bamboo pole (or aluminum rod) of 2 to 3 m length. A rubber tube with a rubber bulb at one end is connected to the bottle just below the neck. When the rubber tube is pressed, it injects air into the squeeze bottle and in turn, the pollen-talc mixture present inside the bottle is released as a cloud. When the receptive female flowers are to be pollinated, the nozzle of the bottle is placed near the inflorescence and the rubber tube is pressed.

The pollen-talc mixture released will cover the inflorescence effecting the pollination. The process is repeated on the 1st, 3rd and 5th day etc. (alternate days) starting from the day when the first female flower comes to receptivity. When the stigmas turn brown and black the female flower is no longer receptive for pollination. By this method most of the dwarf palms can be pollinated from the ground level. Even the few dwarf palms, which are taller, can be reached with the help of a small ladder. As the laborious process of tree climbing can be avoided, a single pollinator can attend to about 150 trees a day. The setting percentage is high (about 30-50%) when compared to that in nature (20-25%).

COMMERCIAL COCONUT HYBRIDS

In coconut, many T x D and D x T hybrids have been released for commercial cultivation, in view of their higher yield and early bearing nature. In most countries, production of D x T hybrids is preferred over T x D hybrids because of the ease with which they can be produced, since the climbing of tall palms for pollination is laborious and time consuming. The hybrids released for commercial cultivation in the country are indicated in the following table.

Released hybrids of Coconut

Hybrid	Parentage	Agency for release	Area for which recommended	Annual nut yield/palm	Copra content (g/nut)	Oil content (%)
Chandra Sankara	COD x WCT	CPCRI, Kasaragod	Kerala, Karnataka, Tamil Nadu	110	208	68
Kera Sankara	WCT x COD	CPCRI, Kasaragod	Kerala, Karnataka, Maharashtra, Andhra Pradesh	106	198	68
Chandra Laksha	LCT x COD	CPCRI, Kasaragod	Kerala, Karnataka	109	195	69
Laksha Ganga	LCT x GBGD	KAU	Kerala, Tamil Nadu	108	195	70
Kera Ganga	WCT x GBGD	KAU	Kerala	100	201	69
Kera Sree	WCT x MYD	KAU	Kerala	112	216	66
Kera Sowbhagya	WCT x SSAT	KAU	Kerala	130	195	65
Ananda Ganga	ADOT x GBGD	KAU	Kerala	95	216	68
Godavari Ganga	ECT x GBGD	APAU	Andhra Pradesh	140	150	68
VHC-1	ECT x MGD	TNAU	Tamil Nadu	98	135	70
VHC-2	ECT x MYD	TNAU	Tamil Nadu	107	152	69
VHC-3	ECT x MOD	TNAU	Tamil Nadu	156	161	64.5
Konkan Bhatye Coconut Hybrid 1	GBGD x ECT	Regional Coconut Research Station, Bhatye	Konkan Coastal region	122	180	67.1
Kahikuchi Coconut Hybrid 1	MYD x WCT	Horticultural Research Station, Kahikuchi	North Eastern region	103	172.8	67.4

SEED GARDEN

It is essential to establish a seed garden to supply the seed requirements of identified highly productive hybrids on a large scale. The basic requirement is that both the male and female parental lines are available within the same seed garden. The planting material for establishment of seed garden should be obtained from research organizations/certified farms to ensure that the populations are true to type. To ensure the legitimacy of the seed nuts, it is necessary to maintain an isolation distance of at least 500m between adjacent coconut stands/ seed gardens in the vicinity. As the performance of the mother palms are known, the hybrids can be produced from these palms right from their initial flowering. To increase the pollination, pollinating insects such as honey bee colonies may be maintained in the seed garden. Thus it can be ensured that all the seed nuts so produced by natural pollination are hybrids.

Fig. 29. A seed garden

System of planting

The earlier method adopted for establishing seed gardens for hybrid seed production, was to plant Talls and Dwarfs in alternate rows so that by simple emasculation of the female parent both D x T and T x D hybrids could be produced. This seed garden has been adopted at Konark (Orissa) and Navalok (Tamil Nadu).

Subsequently, in the seed gardens at Dharmaveera and Kidu (Karnataka), the double hedge system (paired rows) of planting has been adopted. In this method, a spacing of 5m within rows and 6m between rows is provided for the dwarf palms (female parent). From the second to third row the distance is 9m and the planting pits are taken in a staggered fashion so that plants in rows 1, 3, 5 etc. come in one line when seen across, while those in 2, 4, 6 etc. come in one line.

In the isolated seed gardens at the Ivory Coast under IRHO, France, one pollinator (male parent) is planted for five mother trees (female parent). In the isolated seed garden at the Nigeria under NIFOR, one male parent is planted for eight female palms. In Sri Lanka, newer isolated seed gardens have been planted with one pollinator for nine mother trees. These are more economical as seed recovery will be higher owing to the presence of higher proportion of mother trees in these gardens. The disadvantage in these systems, however, is that only a single type of hybrid seed can be produced under natural pollination.

A modified method is suggested for the layout of seed garden where, different types of hybrids can be produced. In this layout, compact block of the parental varieties is planted with few rows of border plants. Border rows can be planted with the same kind of parental palms or with other suitable tree species such as *Casuarina* etc. However, seed nuts

should not be collected from the border coconut palms, as the legitimacy of the pollination cannot be ensured. In this method by adopting the artificial pollination technique one can produce the desired T x D, D x T or T x T combinations.

Varieties/cultivars identified for hybrid seed production

Among the tall cultivars, WCT, LCT, ADOT, SSAT and ECT have been recommended as parents for hybrid production. The dwarf parental lines recommended for commercial hybrid seed production are COD, MYD, MOD, MGD and GBGD.

SOME USEFUL ESTIMATIONS

i) 1 kg of fresh mature male flowers after processing gives 18 to 20 g of pollen.

ii) 20 g of pollen mixed with neutral talc (1:9) is sufficient to pollinate 45 to 50 bunches.

iii) One person can emasculate about 50 to 60 dwarf palms in a day and a pollinator can pollinate about 150 dwarfs or 30 talls a day.

iv) One emasculator can look after 1 ha of seed garden while one pollinator can manage pollinations in 2 ha (assuming that on a single day only a quarter of the total palms in the garden need to be either emasculated and/or pollinated).

v) Generally, 5 to 6 climbing are required for hybridization work in a bunch. However, when dwarfs having a longer female phase, are used as the female parent, the required number of climbing may go up to 10.

IMPORTANT DO'S AND DON'TS

i) Do the emasculation carefully ensuring that the entire male flowers including those which lie in between the female flowers are removed.

ii) Each and every inflorescence in a seed garden (Dwarf block) should be emasculated. Even if a bunch is not to be pollinated don't leave it without emasculation, as this will contaminate other bunches.

iii) Pollinate bunches only after inspecting the accuracy of emasculation. Don't pollinate if the few male flowers left over by oversight have already opened when the female flowers are in receptive condition.

iv) Collect male flowers only from top and middle spikelets.

v) Never use the pollen without checking the viability. Use pollen only if the germination is over 50%.

vi) Clean properly (with alcohol) all equipments used for processing and collection of pollen before and after use to avoid contamination in the pollen.

vii) It is advisable to store different pollen in different desiccators.

viii) Pollen-talc mixture should be prepared just before use. Never store the mixture for the next day.

ix) Use only a single type of pollen in one squeeze bottle and label the bottle for easy identification.

x) Clean the pollen applicator (squeeze bottle) daily and keep dry.

xi) In a seed garden, it is always better to use a single type of pollen for all the trees at a given time. When another combination of hybrid is also to be produced in the same garden, give at least a gap of two days before the second pollen is used for pollination.

xii) It is advantageous to tag pollinated bunches with the date of pollination. This will help in identification of pollen batch used.

xiii) Keep a register for recording all details like palm number, bunch number, date of pollination, pollen source, number of female flowers, number of nuts harvested and the recovery of hybrids palm wise. This will help in identifying and eliminating poor palms from being used for seed production.

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Calendar of operations for hybridization technique in coconut from the day of inflorescence opening