

**ANALYSIS OF INBREEDING DEPRESSION IN WEST COAST TALL
COCONUT (*Cocos nucifera* L.)**

by

CHETHANA S

(2013 - 11 - 186)

THESIS

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requirement for the degree of**

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DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2016

DECLARATION

I, hereby declare that this thesis entitled “**ANALYSIS OF INBREEDING DEPRESSION IN WEST COAST TALL COCONUT (*Cocos nucifera* L.)**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled “**ANALYSIS OF INBREEDING DEPRESSION IN WEST COAST TALL COCONUT (*Cocos nucifera* L.)**” is a record of research work done independently by Miss. CHETHANA S (2013-11-186) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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“Drops of water hollow out a stone” this study is the outcome of help extended by many hands

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Introduction

1. INTRODUCTION

Coconut is a widely cultivated tropical species which gained importance in socio-cultural needs of our society and preferred as a potential source of employment and income generation to farmers. So, it is known by many names as "Tree of Life", "Kalpavriksha", "Tree of Heaven", "Tree of Abundance", "King of Palms" and "Gods Gift to Mankind". There are mainly two types: tall and dwarf. The Tall Variety, West Coast Tall is the superior coconut cultivar which has been extensively cultivated all over the west coast regions of India. It is having good productivity with simple cultivation practices. It is having high commercial importance of yield (40 to 100 nuts per tree), copra (176 g/nut) and oil (68 %). It is recommended for large scale cultivation in coastal regions of Kerala, Karnataka, Tamil Nadu, Gujarat, Bihar, Madhya Pradesh, Lakshadweep, Orissa and Tripura.

Botanically, it is a monocotyledon which belongs to a family Areaceae (Palmae) and known scientifically as *Cocos nucifera* L., diploid ($2n=2x=32$) species. As in nature, it is a monoecious species which begins to flower after 6 to 7 years. The male and female flowers are borne in the same inflorescence. However in contrast male and female flowers mature at different period leading to out-crossing. Being a cross pollinated crop, it influences high degree of variability which produces heterogeneous population. The genetically larger variable population are used to produce inbred lines. An inbred line is a nearly homozygous line obtained through continuous inbreeding, accompanied by selection. Inbreeding is a technique used in selective breeding. However, inbreeding can occur in plants *via* selfing or the union of haploid gametes produced by the same genetic individual also known as autogamy or self-pollination. Self-pollination is one of the preferable ways to develop inbred lines in a cross-pollinated species. Selfing reduces the level of heterozygosity and thereby, expose deleterious recessive loci for selection and simultaneously reduce the number of loci which shows heterosis.

The most important features of the cross pollinated species are inbreeding depression and heterosis. With some exceptions, inbreeding reduces offspring fitness in essentially all naturally outcrossing plants and to a lesser extent in selfing species which refers as inbreeding depression. The careful breeding studies of Darwin (1876) first empirically demonstrated the inbreeding depression in a wide variety of taxa. Examples of traits which shows inbreeding depression include pollen quantity, number of ovules, and amount of seed produced, germination rate, growth rate and competitive ability. Inbreeding depression is the vigour degradation for the generation resulted from self pollination (Stebbins, 1958). Falconer (1989) expressed inbreeding depression as the reduction of the mean phenotypic value shown by characters associated with reproductive capacity or physiological efficiency.

Molecular markers play an important role in characterizing and distinguishing germplasm at earlier stage. The marker RAPD (Random Amplified Polymorphic DNA) effectively brings out the variability at genetic level in many crops. This has the greater advantage because it does not require DNA- sequence information. So, it has been used to analyse the less studied genome.

With the intension of developing inbred lines in coconut, the programme has been started in 1924 by selecting six groups of WCT coconut palms. The programme initiated with selfing the eighteen West Coast Tall palms belonging to six groups with two coloured types and four different yield groups at the Coconut Research Station (now, Central Plantation Crops Research Institute), Kasaragod. The first generation and subsequent second generation of these families of West Coast Tall coconut was planted in Coconut Research Station (Now, Regional Agricultural Research Station), Pilicode in 1927 and 1961 respectively. Nair and Balakrishnan (1991) had reported from their studies made on first and second generation selfed and sibmated progenies of WCT coconut that the selfed progenies were inferior to their grandparents and sibmated progenies which clearly indicated that inbreeding depression existed.

In this context, present research programme was undertaken to analyse the inbreeding depression of WCT coconut with the following objective:

1. Characterization of second (S_2) and third (S_3) generations of WCT (West Coast Tall) coconut including open pollinated WCT and dwarfs, COD (Chowghat Orange Dwarf) and CGD (Chowghat Green Dwarf) coconut through morphological and molecular approaches.
2. Analysis of inbreeding depression in second generation of WCT palms and third generation WCT coconut seedlings
3. Variability analysis of third generation seedlings using morphological and molecular data

Review of Literature

2. REVIEW OF LITERATURE

Attempts were made for developing inbred lines in WCT coconut palms as early as 1924 to 1926 by selecting four different yield groups and two coloured types. Their first and second generation progenies were planted at CRS, Pilicode during 1926 and 1961 respectively (Anonymous, 1961). Harland (1957) reported as an analogy with other out-bred species, selfing of tall coconut palms would be expected to reduce vigour and possible to develop inbred lines which could be used for the production of hybrids for higher productivity. Nair and Balakrishnan (1991) have reported from their studies made on first and second generation selfed and sibmated progenies of WCT coconut that the selfed progenies were inferior to their grandparents and sibmated progenies. Further they opined that the hybrid vigour was met with when crossing was done between the progenies of the same parent indicating early development of inbreds in coconut by selfing.

Attempts has been made to collect the literature available for the present study entitled "Analysis of inbreeding depression in West Coast Tall coconut (*Cocos nucifera* L.)" with the main objective of characterization of S_2 and S_3 generations including dwarfs of coconut through morphological and molecular approaches. Since the work on inbreeding depression in coconut is scanty, literature information pertaining to other crops also has been included.

The available literature on various aspects is presented in this chapter under following subheads:

1. Coconut palm
2. Development of inbred lines
3. Inbreeding and selection
4. Inbreeding depression
5. Variability
6. RAPD analysis

2.1. COCONUT PALM

Cocos nucifera L. or coconut belongs to the palm family, Arecaceae (Palmaceae) which consists of 200 genera and over 2,000 described species (Child 1974). The term coconut is derived from the Spanish and Portuguese word, "coco", which means "monkey/grotesque face". The plant is known by their local names as "naryal" in India, the "nut of India" by Cosmos, the Egyptian traveller in AD 545. The tree has been known as, "man's most useful tree", "king of the tropical forest", "tree of life", "tree of heaven" and lazyman's crop (Woodroof 1970). Coconut varieties include two broad groups, Tall or *typica* and Dwarf or *nana*. Tall and Dwarf types may hybridize to produce intermediate forms (Woodroof 1970, Child 1974). The Tall variety is usually cross pollinated therefore shows high genetic variability. The coconut plant is monoecious, producing both male and female flowers. The male flowers are located distally while the female flowers are found proximally on each inflorescence. The pollination type is determined by the maturation times of the male and female flowers. In the Tall varieties the male flowers open before the female flowers, while in dwarfs an overlap of the opening phases of male and female flowers occurs. This indicated that self-pollination takes place in dwarf types where high tendency toward homozygosity is possible. Each coconut inflorescence emerges from the base of a leaf which is approximately 120° around from the previous inflorescence. After fertilization of the female flowers, each inflorescence develops into a cluster of fruits called a bunch. Occasionally the spikelet of an inflorescence is in direct contact with the spikelet remnants of an older bunch (Hall 1981, Moore and Alexander 1987). The tree survive for over 60 years and are adaptable to all range of soils which is fairly resistant to water stress and diseases (Woodroof 1970, Child 1974).

Bavappa and Sukumaran (1983) studied the coconut improvement by selection and breeding. The breeding systems vary widely from tall and dwarf cultivars. Selfing of WCT study confirms that the heterozygous for height whereas COD is heterozygous for colour, vigour and stature, while true breed type

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are also available for these characters. Relationship analysed between mean copra content per nut and the nut yield are negatively correlated in WCT and observed positive correlation between the yield of oil per palm and yield of nuts.

The genetic control of husked nut weight in coconut (*Cocos nucifera*) were analysed by Fernando (1996). ANOVA of mean husked nut weight confirms the presence of additive and non additive genetic variance. He observed that the magnitude of non additive component was higher than the additive component. The crossing and selfing both improves the genetic constitution of the trait. Inheritance studies on two distinct types of phyllotaxy indicate the segregation in FS1 for left and right whorls in 1:1 ratio (Louis and Chidambaram, 1976). Asymmetry in coconut palm does not have control by genotypic differences nor by a cytoplasmic effect.

Meunier *et al.* (1983) analysed the systematic study on the nuts produced over a period of three years by two populations of coconut with different variability. The nuts of four for every two months were observed for the characters like weight of nut, weight of husked nut, weight of husk, weight of water, weight of shell, weight of endosperm and weight of copra. Almost all the nut characters were studied out of which two characters showed too variable i.e. in weight of water and copra.

Growth rate and seedling vigour as measured by collar girth and leaf production from the time of germination of 16 selected high yielding families indicated significant differences in growth rate of progenies between families. The growth characters showed high and positive correlation from the fifth month seedlings with those of tenth month, which helps in identifying the palms of superior genetic value (Satyabalan and Mathew, 1983).

Satyabalan and Rajagopal (1985) studied 1190 open pollinated progenies of 43 high yielding mother palms and 224 open pollinated progenies of 10 high yielding mother palms for the two characters viz., collar girth and leaf production at the fifth month from germination. This shown that only five palms are superior

to others in nursery which are generally more vigorous irrespective of months of harvest and germination. The palm with the high rate of transmission of the growth characters to their progenies are known to be the prepotents. From another study, it was known that prepotency are not transmitted to all the open pollinated second generation progenies.

2.2. DEVELOPMENT OF INBRED LINE

The investigation on development of inbred lines in WCT coconut started as early as in 1924 (Anon, 1961) by selecting eighteen palm. Nair and Balakrishnan (1991) attempts were made to identify the prepotent mother palms of Arsikere Tall cultivar in Maidan tracts of Karnataka through extensive survey of coconut growing region. The results showed lot of variation in the nut yield and copra content (Indires *et.al*, 2010).

Chanthaworn *et al.* (1998) studied inbreeding for the development of inbred line and screening for the recessive traits of amylose-free starch in cassava. S₁ progenies showed inbreeding depression for germination, plant height, fresh root weight, total plant weight, harvest index, root starch content and dry matter content. The cross showing difference in inbreeding depression helped in the selection of parents for the development of inbred line. The screening to inbreds for amylose-free starch was conducted by KI test and no inbreds were free of amylase-starch.

Mallika *et al.* (2006) evaluated inbred generation of five genotypes of cocoa viz. M 12-21, GII 7.4, M 18.7, GII 7.2 and GIV 35.7 for yield and other economic characters using self-incompatible S1 genotypes. Result revealed that percentage of self-incompatible (14.54) and developed inbreds were less vigorous with low jorquette height, forked stem, delayed flowering, small flowers, more cherelles with reduced size in pods and beans. Small pods show low bean weight, bean number, size and dry bean weight and some possessed irregular shape, dark green colour and rough mesocarp. Inbreds showed varied inbreeding depression

and the crosses (S1 x S1) were less vigorous than hybrids of the same age in plant height and wet bean yield.

2.3. INBREEDING AND SELECTION

The highly cross pollinated nature of coconut poses difficulty in selection of seed nuts and seedlings in nursery. Progeny evaluation is possible only several years after field planting. Inbreeding in coconut was studied by Liyanage (1969) on some characters like endosperm weight, embryo weight, leaf production and flowering period. It was reported that inbreeding depression existed in these characters as compared to the open pollinated progenies and the intensity varied among families. He also found that the endosperm weight and embryo weight were related to the breeding values of the grandparents.

Kumar *et al.* (1993) suggested selection index to identify the superior seedlings from four ecotypes of tall and dwarf varieties and a hybrid VHC 1. The identified contributing characters for high index score were duration taken for germination, length and width of the embryonic leaves, along with collar girth and height of seedlings at the fourth month.

Wickramaratne *et al.* (1987) reported nut selection should not be based on the size, quantity of nut water or shape which has been evidenced by showing no relationship of any variable with the germination for the efficient seednut selection in coconut.

Cardoso (2004) analysed the self-pollination in squash (*Cucurbita moschata*, cv. Piramoita) for successive generations without the selection. Result showed the increase of homozygosity level with the linear reduction in mean weight of fruit length, seed production (number and weight) per fruit except for seed quality.

Fasoula (2008) explained that obtaining a good phenotypic data could be a limiting factor in molecular breeding. The principal factor which affected the single plant selection and the accurate whole plant field phenotyping equation was

explained to evaluate the large number of plants which maximizes the selection efficiency and speed up the release of improved cultivar.

Ferreira *et al.* (2005) evaluated the effects of inbreeding on selection of sugarcane clones which showed inbreeding depression for tons of brix per hectare (TBH), tons of stalks per hectare (TSH), length, diameter and mean stalk weight except for brix value and number of stalks. The influence of the inbreeding showed that selection was not preferable for TBH and TSH due to high genetic load for yield per hectare. So, suggested to use alternative form of inbred by utilizing S1 clones as parents.

The selection criteria of *Coffea arabica* were investigated using 39 progenies of the fourth generation selfing after the second backcross between 'Catuai' and 'Mundo Novo'. The four analytical procedures viz., index based on the sum of ranks of Mulamba and Mock (ISR), index of desired gains of Pesek and Baker (DGI), classic index of Smith and Hazel (CI) and base index of Williams (BI) of selection indexes showed superiority compared to the DIS (direct and indirect selection). Best results were observed with CI and BI in terms of gains in yield and grain size, and in the distribution of gains in the other characteristics (Rezende *et al.*, 2014).

Importance of population size for conservation of cultivated plants was studied in *Coreopsis grandiflora* on population of variants of 1, 5, 10, 30, 50 and 100 individuals (Urbanek and Benetka, 2013). From the parental population the traits studied were plant height, flower size, seed number and weight per infructescence, thousand seed weight and germination in five consecutive years. The germination percentage observed inbreeding depression from the first year while seed weight observed from second year. Significant differences were observed in fifth year among variants of 30 individuals. But 50 and 100 individuals showed stability for number of seeds produced and their weight. Result revealed that the mostly cross pollinated cultivars of population below 50 individual lead to impairment and the population of 10 individual or less is at

risk of extinction. So the result showed population size was influenced by the level of cross-pollination and degree of selection.

Cabangbang *et al.* (1978) observed that the effective selection criteria in cotton for resistant to seed rot and damping off, seedling vigour, maturity and plant height were large and fast germinating seeds than smaller or slower germinating seeds. In seedlings, seed size and germination rate were considered as effective selection indices.

Kumar and Singh (2014) found Moisture Stress Tolerance Index (MSTI) as selection criteria in a Indian mustard genotypes for moisture stress tolerance with higher yield. In irrigated conditions, genotypes PR-2008-13, RGN-281, PR 2007-1, KM-9201 and DRMR-IJ-31 performed better whereas in rainfed conditions, HJUM-07-06, SKM B 817, RH-658, DRMR-2010-4, DRMR-868-3, Parasmani-33, KMR-10-1, NPJ-146, RL-2010, RH-0830, RB-650 and RMM 09-3 performed better. Also in both irrigated and moisture stress conditions, PBR-375, RRN702, RH-0735, RH-0555-B, NDRS-2017-1, RGN-282, SKM-815 and Divya 44 showed uniform superiority where the identified genotypes can be utilized for the development of superior hybrids.

2.4. INBREEDING DEPRESSION

Inbreeding depression reduces the survival and fertility of offspring of related individuals. From the twentieth century it was known that the inbreeding depression plays role in the evolution of outcrossing mating system. Generally tall coconut is a cross pollinated plant i.e., the genotype of the plant is heterozygote so that the progenies produced will be heterogenous (Menon and Pandalai 1958; Fremond *et al.* 1966; Child 1974; Foale 1992). Homozygous parent of tall coconut can be developed through self-pollination breeding technique (selfing) for some generations. After some generations obtained from self-pollination will have high degree of homozygous lines would be obtained, where the vigour would be degraded as an effect of inbreeding depression.

2.4.1. Inbreeding depression in cross-pollinated perennial crops

Miftahorrahman and Luntungan (1991) showed the inbreeding depression in coconut variety Mapanget Tall 10. They obtained inbred lines for hybridization mainly by self – fertilization of open pollinated crop, the coconut for about three generations. The treatment of the experiments was designed for three families i.e., 10, 32 and 55 where they showed varied vegetative characters. In family 32, inbreeding depression was shown by all vegetative characters except plant height whereas in family 55, inbreeding depression was observed for diameter of trunk, leaf length and petiole, but not for plant height and number of leaflets. The family 10 showed the inbreeding depression for plant height alone.

Cytological consequence of inbreeding depression was studied by Nambiar *et al.* (1970) in a few distinct exotic varieties of coconut. Inbred and open pollinated progenies of Laccadives, Andaman, Philippines, New Guinea and Cochin China varieties of indigenous and Malayan origin were screened for meiotic behavior. The open pollinated varieties showed more chromosomal aberrations and higher percentage of pollen sterility than the self pollinated dwarf varieties. It is suggested that inbreeding depression in Cochin China and lack of that phenomenon in Laccadive varieties are due to the difference in the intensity of inbreeding, the latter generally being less susceptible to inbreeding.

Pandin (2009) determined the inbreeding depression of selfed Mapanget tall coconut No. 32 (DMT-32) based on the morphological characters of second, third and fourth generations. The results showed inbreeding depression in both vegetative and fruit component characters in DMT-32 S4.

Oliveira *et al.* (2012) selected 60 mother plants in castor, from each plant three types of progenies developed by self-pollination (AU), crosses (CR) and open-pollination (PL). These progenies have been evaluated in two locations which provided a strong interaction with progenies. Comparing the progenies of AU with PL, broad mean values with 6.7% and 13.4% inbreeding depression was observed.

Ram and Singh (2012) assessed 60 hybrids in egg plant which involves 15 female lines and 4 male testers for ten characters in RBD with three replications. Maximum positive heterosis (69.23 per cent) over economic parent and inbreeding depression of 32.39 per cent was observed. Out of 60 crosses, some crosses showed maximum heterosis along with inbreeding depression in days to flowering, days to marketable maturity, plant height, and number of branches per plant, number of fruits per plant, fruit weight and plant spread. This showed high heritability for all the characters except for length of fruit and width of fruit which exhibited additive gene effects.

Highly significant positive heterosis and low inbreeding depression for seed yield and its component traits has been reported by Singh *et al.* (2013) in castor. The result was significant for heterosis over better parent mainly for the traits of number of capsules on main raceme in JP 96 X JI 368; length of main raceme, effective length of main raceme and number of capsules on main raceme in JP 96 X JI 372; and oil content in JP 101 X SKI 215. Inbreeding depression was seen for shelling out turn, seed yield per plant and 100 seed weight in JP 96 X JI 368; length and effective length of main raceme, number of capsules per plant and oil content in JP 96 X JI 372; and days to maturity, plant height, number of nodes and 100 seed weight in JP 101 X SKI 215.

Virani *et al.* (2014) studied heterosis and inbreeding depression in castor by using generation mean analysis involving six generations namely P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2 of five crosses. Among this five crosses, JP101 X SKI 215 (86.51%) and JP 96 X JI 368 (25.90%) exhibited high heterobeltiosis for seed yield per plant. In this cross JP 96 X JI 368 (52.20%) showed high positive standard heterosis over the check hybrid (GCH 6) followed by JP 101 X SKI 291 (21.24%) and JP 101 X SKI 215 (9.0%) for seed yield per plant. And also low (-0.35%) to moderate (57.91%) amount of inbreeding depression was observed for most of the traits, which influence the genetic constitution of crosses. In most of the traits showed positive inbreeding depression indicating dominance gene

effects. They have also observed the association of high heterosis with high inbreeding depression for seed yield and some of its component traits.

2.4.2. Inbreeding depression in cross pollinated annual crops

Ahmad *et al.* (2010) evaluated the performance of S1 lines for inbreeding depression regarding different parameters in maize var. Azam. This conducted by self-pollination for one generation and in next season 99 S1 lines were sown with a parental line. The result showed severe inbreeding depression in yield (Av. of 362.08 kg/ha). Average values of inbreeding depression were seen for days to silking (2.02 days), pollen-shedding (2.21 days), plant height (21.50 cm), ear height (4.87 cm), ear length (1.80 cm), ear diameter (0.2 cm), number of ears per row (2.5 g), kernel rows per ear (2.1 g) and 100 grain weight (3.89 g). Positive significant correlation was seen in grain yield along with plant height, ear height and yield components. Similarly, maturity trait showed positive and significant correlation with each other. The lines severely affected by inbreeding were neglected and the tolerant lines were selected for further breeding purpose.

Agarwal and Shrotria (2005) studied the magnitude of heterosis and inbreeding depression of 50 hybrids (F1s) and their F2s using 10 sudan grass restorer lines and 5 cytoplasmic male sterile lines in line X tester mating design. Quantitative and qualitative traits *viz.*, plant height, leaf length, leaf width, fifth leaf area, stem diameter, total soluble solids (T.S.S.%), protein percentage, digestibility percentage, shootfly infestation, green and dry fodder yield were observed. ICSA 94 x Pant Chari-4, ICSA94 x SDSL92111, ICSA94 x SDSL92113, ICSA94 x SDSL92140, ICSA94 x Selection 984, ICSA95 x ICSV96062 and 296A x SDSL92111 crosses showed best heterotic response for green and dry fodder yield. Similarly significant positive inbreeding depression for these traits was seen except for TSS%, protein % and shootfly infestation. Bhatt (2008) in this experiment with the best five crosses of 2219 x UPMC504, 2218A X SDSL92102-2, ICSA363 X PC4, ICSA363 X PC5 and ICSA X UPMC504 observed heterotic response for green and dry fodder yield, and also

positive inbreeding depression for all the characters except stem diameter, TSS%, protein%, shootfly infestation and HCN content.

Heterosis for eight characters has been assessed in bhendi which says arka abhay x Punjab padmini cross showed significant positive heterosis over better parent and standard parent for number of nodes per plant (15.7 per cent, 26.25 per cent), single fruit weight (5.9 per cent, 14.36 per cent), fruit length (13.5 per cent, 16.75 per cent), fruit yield by number (6.77 per cent, 13.99 per cent) and weight (14.48 per cent, 13.40 per cent). The same heterotic effect has been seen in the F2 generation due to low inbreeding depression (Kumar *et al.*, 2005; Amutha *et al.*, 2007).

Anantharaju and Muthiah (2008) revealed no significant inbreeding depression for yield where dominance plays major role to additive genes. Result showed transgressive segregant from the F2 population in the combination of CO 5 x ICPL 87119 for yield and the yield contributing characters, the inheritance for photosensitivity and photo insensitivity were controlled by the complementary/ duplicate type of interactions.

Basamma *et al.* (2009) reported maximum heterosis in F1 and high inbreeding depression in F2 which was assessed for seed cotton yield per plant followed by bolls per plant.

Ghai and Arora (2006) observed significant heterobeltiosis and standard heterosis for fruit yield per plant in two seasons for Punjab Padmini x Hisar Unnat. Other crosses for fruit yield were NDO 10 x Hisar Unnat, Punjab 8 x Pant Bhindi-1 and IIVR-11 x Pusa-A-4 for spring season and Punjab Padmini x Punjab 8, Punjab 8 x Hisar Unnat and Pant Bhindi-1 x IIVR-11 for rainy season. There was also significant inbreeding depression for all the traits. The transgressive segregants was seen due to negative inbreeding depression for fruit yield in Punjab 8 x Hisar Unnat 7 and Punjab Padmini x Punjab 8.

Holtsford (1996) studied the relationship between the self-fertilization rates of a population and the severity of inbreeding depression among and within

two populations in *Clarkia tembloriensis*, *Cantua creek* and Idria. This was studied in glasshouse trials which showed different rates of self-fertilization (s) and inbreeding coefficients (F) (s= 0.74, F=0.77 in CC-1 and s=0.16, F=0.10 in I-1). Result showed self-fertilization in CC-1 which have fewer recessive lethal genes compared to the outbreeding I-1 population. Large variation of inbreeding depression among families was observed which shows five to seven times larger than between the populations.

Jain and Bharadwaj (2014) revealed high significant standard heterosis in grain yield (-27.15 to 38.80) with 4 component characters and also positive and negative inbreeding depression of F₂ progenies for 12 characters in 36 crosses of Quality Protein Maize.

Heterosis and inbreeding depression was studied in cotton using 45 cross F₁ hybrids, 45 F₂s and 10 parents in randomized complete block design replicated thrice during 2002. Results revealed that positive heterobeltiosis was seen in 27 F₁ crosses for seed cotton yield, whereas the cross F 1861 X RS 2115 showed high heterobeltiosis for seed cotton yield and number of bolls per plant, and also low heterobeltiosis for seed cotton yield (LH 1836 X Pusa 101) and bolls per plant (LH 1861 X H 1123). With this, negative inbreeding depression was also observed for yield and yield contributing traits which indicate the superiority of F₂ over F₁. This suggested the production of commercial hybrid cotton through heterosis (Kaushik and Sastry, 2011).

Kaushik and Kapoor (2013) revealed that positive heterobeltiosis was seen in 27 crosses for seed cotton yield per plant out of 45 crosses. Negative inbreeding depression was observed in ginning per cent (C 2602-WIR 6109 X LH 1836), lint index (F 1861 X RS 2115), 2.5 per cent span length (F 1861 X LH 1896), fibre fineness (C 2602-WIR-6109 X LH 1836), seed index (C 2602-WIR-6109 X Pusa 101) and seed cotton yield per plant (LH 1861 X H 1123). This negative inbreeding depression was observed for yield and fibre quality traits

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which indicate the superiority of F_2 over F_1 . So, he suggested to exploit heterosis for the production of commercial hybrid cotton.

Khanorkar and Kathiria (2010) studied about the heterobeltiosis, inbreeding depression and heritability in okra using P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2 generations of six crosses which showed heterobeltiosis in HRB-55 x AOL-05-4 for the yield and its contributing traits i.e. 94.06% for fruit yield per plant and 86.12% for fruits per plant. Moderate to high narrow sense heritability was seen in HRB-55 x AOL-05-4 (E1), VRO-6 x AOL-05-3 (E1) and Parbhani Kranti x AOL-03-6 (E2) for primary branches per plant, VRO-5 x Red Long (E1), VRO-6 x AOL-05-3 (E1), GO-2 x AOL-04-3 (E1) and Arka Anamika x AOL-03-1 (E1) for fruit girth, HRB-55 x AOL-05-4 (E2), VRO-5 x Red Long (E1) and GO-2 x AOL-04-3 (E2) for fruit weight. And highest heritability were observed in HRB-55 x AOL-05-4 for fruit length (99.35) which could be utilized for varietal development. Significantly positive inbreeding depression were observed for days to first flowering in GO-2 x AOL-04-3 (E1 and E2) and for days to first picking in HRB-55 x AOL-05-4 (E2), VRO-5 x Red Long (E2), GO-2 x AOL-04-3 (E2) and Parbhani Kranti x AOL-03-6 (E2).

Komal *et al.*, (2014) estimated the nature and magnitude of gene action for cotton yield and its contributing characters. Results revealed high magnitude of dominance effect for number of monopodia per plant, number of sympodia per plant, number of bolls per plant, boll weight and seed cotton yield. Epistasis were observed in the expression of number of monopodia per plant and seed cotton yield. The magnitude of heterobeltiosis was high for number of monopodia per plant, number of bolls per plant and seed cotton yield in all four crosses. Both positive and negative inbreeding depression (low to moderate) was observed for most of the traits.

Kumar *et al.* (2012) studied the gene effects, heterosis and inbreeding depression in pigeonpea crosses viz., P_1 , P_2 , F_1 , F_2 , B_1 and B_2 for 8 metric traits. Results showed the presence of additive, dominance and epistasis gene effects in

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seed yield and test weight. In all these four crosses heterobeltiosis was observed for seed yield per plant with most of its attributes. And also observed inbreeding depression in PRG 100 x ICPI 87119 for days to 50% flowering, days to maturity and number of clusters per plant.

Kyu (2011) analysed the heterosis, inbreeding depression and genetics of fertility restoration in pigeon pea. The study showed negative heterosis for earliness (ICPH 2671), maturity (ICPH 2671, ICPH 3461, ICPH 3762, ICPH 3763, ICPH 4022 and ICPH 4024) and positive heterosis for plant height (ICPH 2671, ICPH 3933 and ICPH 3759), number of primary branches (ICPH 2671, ICPH 2751 and ICPH 3759). In seed yield, wide range of heterosis was observed in ICPH 2671 (148.94-208.44%) followed by ICPH 2740 (49.89-121.45%), ICPH 3477 (48.54-119.45%), ICPH 3491 (50.99-134.17%), ICPH 4017 (55.82-184.90%) and ICPH 4022 (127.23-155.64%). Inbreeding depression was recorded for pod cluster per plant ranging from -64.50% (ICPH 3494) to 68.44% (ICPH 4012), seeds per pod and 100 seed weight (ICPH 3359- 19.61%). Fertility restoration observed in pigeon pea revealed that two genes govern the character with the epistatic interaction. Same restorers was observed in ICPH 2671 and ICPH 2740 while in ICPH 3359 observed two major genes govern the fertility restoration.

Mistry (2012) revealed that three crosses in okra exhibited significant heterosis and heterobeltiosis followed by inbreeding depression for yield and yield contributing traits in six inter varietal crosses. This indicates the role of dominance gene effects, which further helps for the production of commercial hybrid.

Studies on 28 maize open pollinated varieties in 10 environments in a diallele fashion along with their S1 population revealed about the occurrence of deleterious alleles. Results shown reduction in yield from S0 to S1 varied from 34.6% (CMS 01) to 59.2% (CMS-30) with an average of 49.1%. Mostly inbreeding depression was higher in the 7 population having wide genetic base

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never exposed to inbreeding, but the inbred lines with high means was obtained in BR-105, BR-111, CMS-01, CMS-03, BR-106, CMS-14 C, and CMS-28 population. The parameters used for analysing the inbreeding depression inferred about the frequency of deleterious allele, which helps in selecting the parents for inter and intra population breeding programme (Pacheco *et al.*, 2002).

Studies on hybrid vigour and inbreeding depression were conducted in okra, *Abelmoschus esculentus* (L.) Moench using six crosses (Patel *et al.*, 2010; 2013). The relative heterosis observed were positive and significant for number of nodes per plant, number of fruits per plant and fruit length in KS-404 x HRB-108-2 whereas for number of fruits per plant and fruit yield per plant in VRO-5 x GO-2. Significant positive heterobeltiosis were seen in KS-404 x HRD-108-2 and VRO-5 x GO-2 for fruit length and fruit yield per plant. Even for all trait observed moderate to high amount of inbreeding depression.

Ranjeet and Shweta (2007) observed significant positive heterobeltiosis, standard heterosis and high inbreeding depression in Rohini X Varuna followed by RK 9870 X Vardan and Rohini X Vardan for seed yield in Indian mustard which can be utilized for developing hybrids.

Experiment was conducted in sunflower for yield and its contributing characters revealing significant genetic differences among the parents, their F1 hybrids and F2 population. Significant negative heterobeltiosis and inbreeding depression were observed in ARG x RHA 587. Result concluded that RES 834-1, RHA 587 and PARRUN 1329 were utilized as a parent for the future studies (Reddy *et al.*, 2006).

Shivani *et al.* (2010) revealed positive standard heterosis for number of capitula per plant (4 hybrids), number of seeds per capitulum (7 hybrids), test weight (5 hybrids) and seed yield (3 hybrids) and negative standard heterosis for earliness (two hybrids) and short stature (4 hybrids) in 38 safflower genotypes. Even positive inbreeding depression was observed in F2 generation for all the character except for oil content.

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Experiment was conducted in cotton for heterosis, heterobeltiosis, inbreeding depression, combining ability, correlation and path coefficient analysis, genetic parameters and gene action for yield and yield component characters (Soomro, 2010). Positive correlation was observed for seed cotton yield per plant along with sympodia per plant, bolls per plant and boll weight. Positive heterosis and heterobeltiosis were observed for seed cotton yield per plant in the cross Reshmi x TH-3/83 and Reshmi x Mc-Niar-3150. Even maximum inbreeding depression was seen in F₃ generation (68%). The observed SCA and GCA variances for all the quantitative characters showed significant effects. Heritability in broad sense were revealed upto 73% in seed cotton yield which were controlled by dominance and over dominance type of gene action.

Soomro *et al.*, (2012) analysed the heterosis, heterobeltiosis and inbreeding depression in 5 x 5 F₂ and F₃ diallel cross of cotton. Result obtained in F₂ population shows variation in the extent of heterosis for all the characters studied. Positive heterosis and heterobeltiosis were observed in F₂ hybrids viz., Reshmi x TH-3/83, Reshmi x Mc-Niar-3150, CIM-109 x Mc-Niar-3150, CIM-109 x TH-3/83, Mc-Niar-3150 x CIM-109 for seed cotton yield along with its component character. Even reduction of heterosis in F₂ and F₃ was observed due to decrease in heterozygosity. However, expected inbreeding depression was quite higher than observed for all the triats except for seed index.

Vyas *et al.* (2014) analysed maximum heterobeltiosis in SPV 1329 x ICSV 272 of sorghum for two environments (95.38% in E1 and 94.74% in E2). High heterobeltiosis were observed in SV 248 x ICSV 272 and SV 248 x ICSV 272 for grain yield per plant, number of primaries per panicle, 100 grain weight, and biological yield per plant and harvest index. Most of the crosses revealed heterosis and showed high inbreeding depression too for yield and its components which can be exploited through hybrids.

2.5. Variability

Fernando et al (1991) studied the variation in seedling characters of three different coconut cultivars. Diagnostic traits are necessary to detect the illegitimates amongst hybrid coconut seedlings and for sorting out the types. Seedlings of dwarf, tall and the hybrid, dwarf X tall were studied to distinguish the types. The important indicators are the rate of leaf production, length and width of leaves and time to first leaf splitting only when used with the combination at 6 months from laying.

Experiment was conducted to determine the genetic variability and to select superior walnut genotypes within seedling population. The study indicated high heterozygotes for yield (lateral fruitfulness), tolerance to anthracnose, bacterial blight and nut quality parameters. The selected genotypes showed yield ratio of 50 to 80%, leafing time of medium group with nut weight, length, diameter, shell thickness, kernel weight (percentage), kernel fat and protein content which varies from 11.18 to 15.20g, 32.55 to 36.62mm, 31.58 to 36.15mm, 1.11 to 2.33 mm, 6.14 to 8.00g (47.08 to 58.57%), 55.18 to 65.70% and 14.70 to 20.10% respectively (Akca *et al.*, 2015).

Khan (2011) studied selected thirty F_1 and F_2 diallel cross combinations with six parental upland cotton genotypes where variation was observed among F_1 s, F_2 s and their parents. Cultivar CIM-1100 has exhibited exceptional performance when crossed as parental / maternal parent with other cultivars. Positive heterobeltiosis was observed in F_1 and F_2 hybrids. Even positive economic heterosis was recorded in F_1 hybrids for plant height (80%), sympodia (97%), boll weight (60%), bolls per plant (83%) and seed cotton yield (60%). In F_2 s population, inbreeding depression as seen for the above traits which has been performed better with the selection. This suggested the use of F_2 population as a hybrid to increase the yield.

Genetic variability, heritability and genetic advance were studied on Ethiopian mustard genotypes (Mekonnen *et al.*, 2014). Highly significant

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variation were recorded for maturity days, grain filling period, number of pod per plot, secondary branches per plant, harvest index, seed yield per plot, seed yield per hectare and oil content. Even significant differences were observed for flowering days, plant height, primary branches per plant, biomass per plot and oil yield per plot. High phenotypic coefficient of variation (PCV) and high genotypic coefficient of variation (GCV) were observed for grain filling period, plant height and secondary branches per plant while high PCV were observed for flowering days, grain filling period, plant height, secondary branches per plant, harvest index, oil yield per plot, seed yield per plot and seed yield per hectare. Even high heritability with high genetic advance were observed for plant height, grain filling period and secondary branches per plant whereas high heritability were observed for maturity days, grain filling period, flowering days, plant height, biomass per plot, secondary branches per plant, primary branches per plant, oil content and oil yield per plot. This study revealed about the variation in the traits which can be used in the selection for further improvement.

Ravindran and Sasikumar (1993) studied the variability in open pollinated seedling of three peppers for plant height, leaf number, leaf width, internodal length and stem girth. Maximum range and coefficient of variation were analysed from the Collection 1344 which has followed by the Panniyur-1 and a Karimunda Selection KS-27. And revealed the variants with eighteen distinct morphological in progeny of Coll. 1344.

The amount of variation of safflower was studied for all the characters in 20 population and 7 parents. Minor differences were observed between genotypic and phenotypic characters. High heritability with high genetic advance was observed in number of capsules per plant, number of seeds per capitulum, test weight and seed yield. Even maximum heterosis and inbreeding depression was also recorded (Shivani *et al.*, 2011).

Variability studies for the 151 cocoa trees in farmer field of Tamil Nadu (Velayutham *et al.*, 2013) among the tree, pod, bean and yield traits characters for

all the trees. Promising mother trees were identified from the trees with the following traits: Dry bean yield per tree (> 2.4 kg), number of pods per tree (60), number of beans per pod (> 35) and single dry bean weight (> 1 g). The total of 27 trees was screened for the economic traits.

Genetic variability within and among variety of white clover was observed for yield. Twenty varieties were used for experiment trial which were showing a wide range of origin and morphological structure. Result shows that the character seed weight per inflorescence and density of heads as effective criteria's for higher seed yield (Pacault and Huyghe, 2003).

Silva *et al.* (2015) studied the genetic variability among seventy one genotypes of *Coffea canephora* var. Kouilonensis and fifty six genotypes of *Coffea canephora* var robusta aimed to identify the higher divergent genotypes in intrapopulation crossings. Result showed the greater genetic variability between divergent populations which would be helpful for the selection of offspring in developing the superior genotypes.

2.6. RAPD ANALYSIS

RAPD is one of the most commonly used molecular techniques based on the polymerase chain reaction (PCR). RAPD is the modification of PCR where single, short and arbitrary oligonucleotide primer is used, which is able to anneal and prime at multiple locations throughout the genome. This also produces the spectrum of amplification products which are characteristic of the template DNA. The applications are in gene mapping, population genetics studies, molecular evolutionary genetics and plant and animal breeding. This technique, within the short period generates a large number of markers due to its speed, cost and efficiency (Kumar and Gurusubramanian, 2011).

Dasanayaka *et al.* (2005) studied about genetic diversity of seventeen germplasm accessions and three improved cultivars of coconut by RAPD which revealed 129 amplification products using 20 operon primers averaging about 6.5 bands per primer out of which 84 exhibited polymorphism averaging

4.2 polymorphic bands per primer. The genetic distance observed showed range from 0.036- 0.252 averaging of 0.145. Twenty coconut types formed two clusters where one include the dwarf forms viz. Aurantiaca form - King coconut and San Ramon and the second cluster include typica forms with three cultivars. Dwarf San Ramon exhibited the link with Philippines coconut and King coconut with South East Asian origin while tall types as expected had one tall parent.

Molecular analysis of East African tall coconut genotypes were conducted by different marker techniques which showed large number of DNA polymorphisms among genotypes and identified the genotypes by individual-specific fingerprints. Genetic relationship between genotypes were observed by clustering and association of individuals which were based on the known geographical origin and parental relationships (Duranl *et al.*, 1997).

Everard *et al.* (1996) analysed the inheritance of RAPD marker using thirty eight 10-12 mer primers to detect RAPDs among tall and dwarf parents and a F2 progeny of 18 individuals. Result showed 16 bands by 6 primers out of which 12 bands were able to distinguish the parents and one RAPD showed mendelian pattern of inheritance. So they suggested that RAPD can be used as a molecular marker for generating linkage map for the coconut palm.

The selection of coconut seedlings solely depends on the morphological markers at early stage of growth. Inorder to make this more efficient, molecular markers were used to distinguish between the tall or dwarf character using a pooled DNA approach (Rajesh *et al.*, 2013). The unique band of around 260bp in tall accessions has revealed from the primer OPA09. This primer was used for screening and validation in tall and dwarf coconut accessions.

Rajesh *et al.* (2014) investigated the coconut for the tall or dwarf trait by RAPD markers using a bulked DNA approach. Screening of tall and dwarf palms with 200 primers revealed RAPD primer OPBA3 which could be able

to differentiate between tall and dwarf palms. So, this primer was used to screen the parents and hybrids of dwarf x tall crosses.

Molecular markers were effectively used for diversity analysis in selected coconut palms by Renju (2012) showed that among the three morphological groups of coconut viz., talls, dwarfs and intermediate type of palms, the highest genetic diversity was found between talls and dwarfs (GD=0.14). Within talls Kuttiadi was found to be similar to Tiptur tall and Malapuram type. The dwarf population exhibited higher diversity index (GD=0.09). This was due to the coloured dwarf cultivar Malayan yellow dwarf. The intermediate type Gangabondam was genetically diverse from talls and grouped under cluster for dwarfs. The two cultivars Tiptur tall and Malayan yellow dwarf were most divergent among the cultivars selected.

Materials and Methods

3. MATERIALS AND METHODS

An experiment was carried out in College of Agriculture, Padannakkad and Regional Agricultural Research Station, Pilicode during December 2013 to January 2015. The material for the study consisted of S_2 progenies of six different groups of WCT palms in Pilicode which was planted in 1961. The first S_1 progenies had been developed in the Agriculture Research Station, formerly known (Central Plantation Crops Research Institute- CPCRI), Kasaragod in 1924, 1925 and 1926 by selfing the eighteen West Coast Tall palms by selecting from six different groups including two coloured types and four yield groups. The selfing was done in the West Coast Tall (WCT) coconut palms of these six groups for developing inbred lines. The S_1 progenies were planted in 1927 at the Agriculture Research Station, Nileshwar-I (at present known as Regional Agricultural Research Station- RARS, Pilicode) under replicated trial. In 1960, selfing and sibmating of the S_1 (first generation) palms were done and the second generation progenies (S_2) of the six groups of WCT planted in replicated trial. This constituted the experimental material. The objective of the experiment was characterization of S_2 palms and S_3 (seedlings) generations including dwarfs (COD and CGD) of coconut through morphological and molecular approaches.

The field experiment was conducted in Regional Agricultural Research Station, Pilicode to characterize and analyse inbreeding depression in S_2 (second generation) palms and study the effect of selfing in selfed and sibmated families of West Coast Tall (WCT) coconut palms of second generation (S_2 and S_3). Open pollinated WCT and dwarfs were used to examine the degree of inbreeding depression in the families each S_2 and S_3 generation.

3.1. FIELD EXPERIMENT ON S_2 MOTHER PALMS

The experiment material constituted two hundred and sixteen progenies of second generation (S_2) selfed and sibmated families, planted in

randomized block design with twelve treatments and three replications during 1961. At present, seventy two healthy palms in second generation (S_2) showing uniform characteristics of six families of WCT were selected. Six palms of selfed and sibmated progenies of six families in the field were utilized for conducting the experiment.

3.1.1. Design of the experiment

Variety : West Coast Tall

Design : RBD

Treatments : 15 (12 WCT families + WCT + COD + CGD)

Replications : 3

3.1.2. Observations

The morphological and reproductive characters of the second generation (S_2) palms (selfs and sibs) were recorded during 2014-2015. The details of the observations recorded are shown below

3.1.2.1. Morphological characters

The description of the morphological observations of the S_2 mother palms are given below.

3.1.2.1.1. Age of the palm (years)

Period in years from the date of planting in the main field to the time of recording the observation.

3.1.2.1.2. Height of the palm (m)

Measured in meter (m) from the base of palm above ground level to the base of crown.

3.1.2.1.3. *Girth of the palm (cm)*

Girth of trunk measured in centimetre (cm) at one meter above the base of palm.

3.1.2.1.4. *Internodal length (cm)*

Average length of 10 internodes measured at 1.5 m above from the base of palm in centimetre (cm) divided by 10 number of internodes.

3.1.2.1.5. *Number of Leaves (functional leaves)*

Number of leaves present on the crown at the time of recording the observation.

3.1.2.1.6. *Number of leaves per annum*

Total number of leaves produced on the crown per year.

3.1.2.1.7. *Petiole colour*

Colour of the portion of leaf without leaflets.

3.1.2.1.8. *Petiole length (cm)*

Length of the portion of leaf without leaflets measured in centimetre (cm).

3.1.2.1.9. *Leaf length (cm)*

Total length of leaf from petiole to the tip of leaf measured in centimetre (cm).

3.1.2.1.10. *Number of leaflets per leaf*

Number of leaflets on the right and left sides of the leaf.

3.1.2.1.11. *Spiralling of leaves on crown*

Position of succeeding leaf from the base portion to the left or right on the crown.

3.1.2.2. Observation on reproductive characters

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The observations on Reproductive characters of the seventy two second generation (S₂) WCT palms recorded were period of flowering phases, number of female flowers per inflorescence, setting percentage, stalk length, number of bunches per annum, number of nuts per bunches, nut yield per annum, size of nut, weight of unhusked and husked nut, diameter of eye, weight of the fresh mature kernel, kernel thickness at maturity, copra content, oil content, volume of tender nut water and visual recording of disease or pest attack on palms if any.

3.1.2.2.1. Number of female flowers per inflorescence

Number of female flowers produced per inflorescence. Average of the inflorescence produced per palm per year. Average of six years.

3.1.2.2.2. Period of flowering phases (days)

3.1.2.2.2.1. Length of male phase (days)

Average period in days between the opening of the first male flower and shedding of the last male flower in the inflorescence

3.1.2.2.2.1. Length of female phase (days)

Average period in days between the opening of the first female flower and opening of the last female flower in the inflorescence

3.1.2.2.3. Setting percentage

Ratio of the number of nuts set to the total number of female flowers. It is expressed in percentage.

3.1.2.2.4. Peduncle length (cm)

Distance between the point where the inflorescence is attached to the palm and the base of the first spike. It is expressed in centimetre (cm).

3.1.2.2.5. Number of bunches per annum

Number of bunches produced per palm per year. Average of six years per palm.

3.1.2.2.6. Number of nuts per bunches

Number of good nuts produced per bunch per year. Average of six years per palm.

3.1.2.2.7. Nut yield per annum

Total number of nuts produced per palm per year. Average of six years per palm.

3.1.2.2.8. Size of nut

Size of nut is measured at the broadest part of the unhusked nut as circumference. Average of three nuts/ bunch.

3.1.2.2.9. Weight of unhusked nut (g)

Weight of the nut before dehusking the fruit measured in gram (g). Average of three nuts.

3.1.2.2.10. Weight of husked nut (g)

Weight of the nut after dehusking the whole fruit measured in gram (g). Average of three nuts.

3.1.2.2.11. Diameter of the eye (cm)

Diameter of the endosperm eye measured in centimetre (cm). Average of three nuts.

3.1.2.2.12. Weight of the fresh mature kernel (g)

Weight of the kernel measured in gram (g) after separating it from the shell. Average of three nuts.

3.1.2.2.13. Kernel thickness at maturity (cm)

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Thickness of the kernel measured in centimetre (cm) of a matured fruit. Average of three nuts.

3.1.2.2.14. Volume of tender nut water (ml)

Volume of tender nut water measured in mililiter (ml) using measuring cylinder. Average of three nuts.

3.1.2.2.15. Copra content (g/nut)

Weight of copra (dried mature kernel) per nut measured in gram (g). Average of three nuts.

3.1.2.2.16. Oil content (%)

Oil content estimated using a soxlet instrument and expressed in percentage (%). Average of three nuts.

3.1.3. Selfing of S₂ palms

Selfing of seventy two S₂ palms was done in summer 2013 (November to May). The different steps for selfing are shown below.

3.1.3.1. Selection of parents for selfing

Uniform healthy and true to the different families of second generation (S₂) WCT palms were selected.

3.1.3.2. Pollen collection

Collection of mature, fertile and viable pollen was done after the male flowers open into 50 per cent.

3.1.4.3. Pollen storage

The pollens were collected from the male flowers, after drying of male flowers in shade and then rolling them in a white paper. The fine dust of pollen were sieved, packed in small sachets of paper and stored in dessicator containing CaCl_2 .

3.1.4.4. Pollination

Collected pollens were deposited on the receptive female flowers of the same inflorescence for selfing.

3.1.4.5. Bagging

Bagging was done with the cotton (kora cloth) bag immediately before opening of female flowers (when female flowers mature with a characteristic colour) and after each assisted pollination with pollen grains of same inflorescence to protect it from open pollination with other pollens. The bags were removed three days after pollination of the last female flower.

3.1.4.6. Tagging

The pollinated inflorescence was tagged designating the parents on removal of pollination bags.

3.1.4.7. Harvesting

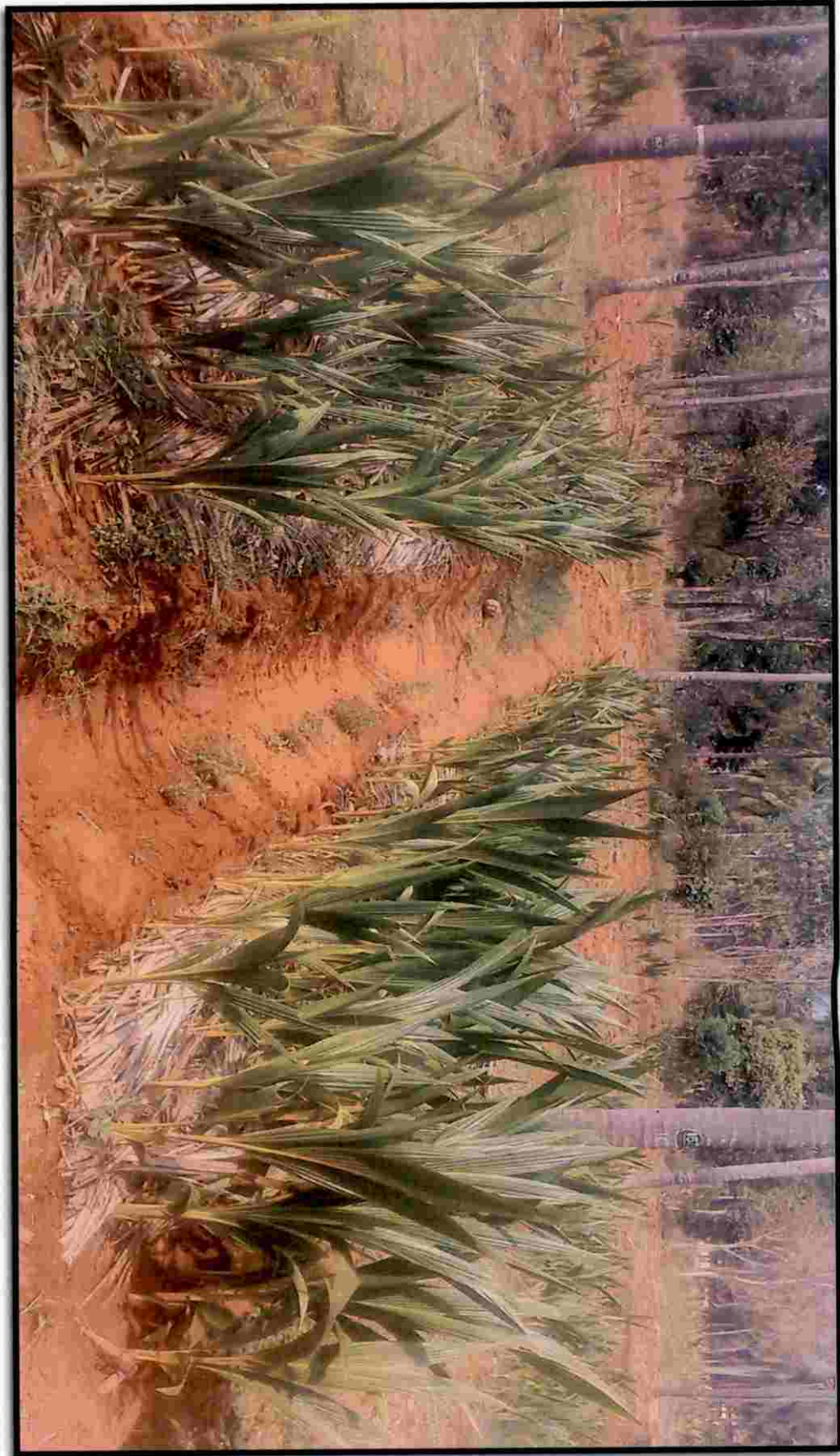
The mature nuts were harvested carefully after twelve months of maturity (S_3 seednuts).

3.1.4.8. Raising of third generation (S_3)

The mature third generation seed nuts collected were sown in the nursery for raising seedlings.

3.2. PLANTING OF SEEDLINGS

The selfed third generation (S_3) coconut seednuts were sown in the nursery in 2013. These seedlings observations were recorded from the seedlings.



Plates 1: Raised nursery of third generation seedlings

3.2.1. Seedling observations in nursery

Observations like germination percentage, seedling height, collar girth, number of leaves, splitting of leaves if any, petiole colour and length, breadth of petiole and leaf portions of fifth and seventh leaf were recorded.

3.2.1.1. Germination percentage

Ratio of sprouted nuts to the number of nuts sown expressed in percentage (%).

3.2.1.2. Seedling height (cm)

Measured in centimetre (cm) from the base of the seedling to the tip of topmost leaf.

3.2.1.3. Collar girth (cm)

Circumstances of seedling measured 5 cm above the base of seedlings. Measured in centimetre (cm).

3.2.1.4. Number of leaves

Number of leaves present in the seedling.

3.2.1.5. Splitting of leaves

Number of splitting leaves present in the seedlings.

3.2.1.6. Petiole colour

Colour of the petiole seen in the seedlings.

3.2.1.7. Length of third and fifth leaf (cm)

Table 1: Details of the experimental palms and seedlings

Sl. No.	Group	Generation	Family	Designation		Parents (Original) S ₀
				Palms	Progenies in third generation	
1	IA	S ₂	1	IAS ₂ -1	IAS ₃ -1	1/109 WCT green
2	IA	S ₂	2	IAS ₂ -2	IAS ₃ -2	
3	IB	S ₂	1	IBS ₂ -1	IBS ₃ -1	1/109 WCT orange
4	IB	S ₂	2	IBS ₂ -2	IBS ₃ -2	
5	II	S ₂	1	IIS ₂ -1	IIS ₃ -1	1/174 WCT Green
6	II	S ₂	2	IIS ₂ -2	IIS ₃ -2	
7	III	S ₂	1	IIIS ₂ -1	IIIS ₃ -1	V1/4 WCT Green
8	III	S ₂	2	IIIS ₂ -2	IIIS ₃ -2	
9	IV	S ₂	1	IVS ₂ -1	IVS ₃ -1	1/129 WCT Green
10	IV	S ₂	2	IVS ₂ -2	IVS ₃ -2	
11	V	S ₂	1	VS ₂ -1	VS ₃ -1	VIII/127 WCT green
12	V	S ₂	2	VS ₂ -2	VS ₃ -2	
13	WCT	S ₀	-	WCT	-	Green
14	CGD	S ₀	-	CGD	-	Green
15	COD	S ₀	-	COD	-	Orange

Note:

- 1- Selfed in second generation; 2- Sibmated in second generation
- S₂ – second generation; S₃- third generation
- In third generation, all palms were done with selfing only

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Length of fifth and seventh leaf counting from the top most leaf of seedling measured in centimetre (cm) from the base to the tip of leaf.

3.2.1.8. Breadth of third and fifth leaf (cm)

Breadth of fifth and seventh leaf measured in centimetre (cm) on either side from the middle portion of leaf.

3.2.1.9. Petiole length of third and fifth leaf (cm)

Length of the portion of leaf without leaflets measured in centimetre (cm). The measurement of fifth and seventh leaf was recorded.

3.4. MOLECULAR CHARACTERIZATION USING RAPD

The seedlings from the families showing inbreeding depressions are selected for the molecular analysis. RAPD was used to characterize the third generation (S_3) progenies which were found to be promising inbreds based on the morphological data, along with one tall cultivar, one dwarf with orange coloured nuts and one dwarf with green coloured nut.

3.4.1. Materials

3.4.1.1. Plant materials

The plant materials were collected from the RARS, Pilicode. The third generation seedlings from four WCT families viz., 1AS2-1, 1BS2-1, 3S2-1 and 5S2-1 were used for the study along with open pollinated WCT and two dwarfs viz., COD (Chowghat Orange Dwarf) and CGD (Chowghat Green Dwarf). The spindle leaves were collected for isolation of DNA from the above mentioned families. The tip and middle portion of newly emerged spindle leaf which is tender were used for DNA extraction.

3.4.1.2. Laboratory chemicals and glassware

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The chemicals used in the study were from Merck India Ltd. and SRL laboratories. The *Taq* DNA polymerase along with buffer, dNTPs, and molecular weight markers (λ DNA *Eco RI/ Hind III* double digest, 100 bp ladder, 50 bp ladder) were from Merck-Genei, Bangalore. The random decamer primers were selected based on earlier reports and the oligos were synthesized by Integrated DNA Technologies, New Delhi.

3.4.1.3. Equipments and machinery

The equipments available in the Department of Plant Biotechnology, College of Agriculture, Padannakkad were used for the study. DNA quantification was done using biophotometer (Eppendorf, German) and Polymerase Chain Reaction (PCR) was done in Mastercycler Eppendorf, German. BioRad imaging system was used for imaging and documenting the agarose gel.

3.4.2. Isolation of total genomic DNA

3.4.2.1. Standardization of genomic DNA extraction

Different manual protocols were tried for obtaining good quality genomic DNA from coconut leaves. The procedures of CTAB method with certain modifications reported earlier by Renju (2011) and Porebski et al. (1997) were tried as detailed below. However, the results were not satisfactory and hence kit method was used. Good quality DNA was extracted from the protocol of Origin Kit. The quality of DNA was observed by gel electrophoresis.

3.4.2.1.1. Protocol 1- CTAB method (Roger and Bendich, 1985)

Reagents:

1. Extraction buffer: (pH 8)
 - 2% CTAB
 - 100 mM Tris
 - 20mM EDTA

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- 1.4M NaCl
 - 2. β -mercaptoethanol
 - 3. Sodium metabisulphate
 - 4. Chloroform: Isoamyl alcohol (24:1)
 - 5. Isopropanol

Steps involved for DNA extraction:

1. CTAB buffer was preheated at 60-65⁰C. Remove the midrib and cut the young leaves into small pieces
2. Grind 1g of leaf tissue along with 2-mercaptoethanol 2-5 μ l, pinch of sodium metabisulphate in presence of liquid N₂ and add 4ml in a sterilized cold mortar and pestle
3. Transfer the ground material into the eppendorf tube (2ml)
4. Mix the sample by inverting the tube for several times and incubate in hot water at 65⁰C for 30 minutes. In between invert the tube occasionally
5. Add 2/3rd volume of chloroform: isoamyl alcohol (24:1) to the tube and invert the tube for several times and keep in a centrifuge for 10,000 rpm at 10 min.
6. Collect the upper aqueous phase in to the eppendorf tube, add 1/6th volume of cold isopropanol and invert the tube slowly for several times. Centrifuge for 10,000 rpm for 10 min
7. Decant the solution leaving the pellet and wash the pellet using 70% alcohol for 2 to 3 times
8. Air dry the DNA pellet in the room temperature, then dissolve in the sterile water.

Modified procedure:

PVP – 20%

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- In the second step, take 0.5 g of leaf tissue along with β -mercaptoethanol 20 μ l, sodium metabisulphate pinch, PVP 10 μ l in a sterilized mortar and pestle and grind by adding liquid nitrogen
 - The powdered material is obtained, to that add extraction buffer 7ml and mix by inverting for several minutes
 - The 4th, 5th and 6th step is followed as in above protocol but the centrifugation should be for 12,000 rpm for 15 min.
 - Rest of the step followed as in the above procedure

3.4.2.1.2. Protocol 2- Porebski et al. (1997) (modified)

Materials used:

1. β -mercaptoethanol
2. PVP 2%
3. Chloroform: Isoamyl alcohol (24:1)
4. 5M NaCl – 14.61 g/ 50ml
5. Alcohol 95%
6. Extraction buffer: pH 8
 - 10 % CTAB
 - 1M Tris
 - 0.5M EDTA
 - 4M NaCl

Steps involved in DNA extraction:

1. Collect the young leaf tissue of 0.5 g, remove the midrib and cut into small pieces. Wrap the sample in aluminium foil and freeze in liquid nitrogen
2. Take leaf tissue in cold pestle and mortar and add 25 μ l β -mercaptoethanol, 10 μ l PVP and liquid nitrogen
3. Immediately add extraction buffer 7ml and transfer to oak ridge centrifuge tube. Mix thoroughly by inverting the tube for several times and incubate

the tube in hot water for 65⁰C for 30 min. Occasionally mix by inverting slowly

4. Add equal volume of chloroform: isoamyl alcohol to the tube. Mix by inverting for several minutes to emulsify and then centrifuge for about 12,000 rpm for 15 min
5. Pipette out the upper aqueous phase, to that add 0.5 volumes of chilled 5M NaCl and 1.5 volumes of 95% alcohol
6. Invert the tube slowly for several times so that the DNA precipitates. Keep it in freezer for 10 min for complete precipitation. Centrifuge to 10,000 rpm for 5 min
7. Decant the solution leaving the pellet in tube and wash with the 70% alcohol. Air dry the DNA pellet in room temperature till alcohol evaporates.
8. Dissolve the pellet in the sterile water

Modified procedure:

- 70 µl PVP and 50 µl β-mercaptoethanol were used

3.4.2.1.3. Protocol 3- Origin kit

The kit used was the 'Origin' from Origin Diagnostics and Research, Karungapalli, Kerala.

Materials required:

- Buffer GP1
- Buffer GP2
- Buffer GD
- Buffer PW
- Buffer TE
- Spin columns CB3
- Collection tube (2 ml)

- Chloroform

Ensure that buffer GD and buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated in the bottle and shake thoroughly.

The steps involved in the process:

1. Place lyophilized 300 mg wet weight plant tissue and grind the samples thoroughly in liquid nitrogen
2. Add 2.1 ml 65⁰C preheated GP1 (β -mercaptoethanol must be added to buffer GP1 before use. The final concentration is 0.1%) to the powdered plant tissue. Vortex for 10-20sec to mix, make sure to disperse all clumps and then incubate for 20 min at 65⁰C, mix by inverting the tube for several times.
3. Add 2.1 ml chloroform, mix by inverting the tube for several times, centrifuge for 5 min at 12,000 rpm
4. Pipet the supernatant to a new tube, add 2.1 ml buffer GP2, mix by inverting the tube for several times
5. Pipet all the mixture from step 4, including any precipitate that may have formed, into the spin column CB3 (place the spin column CB3 in the collection tube). Close the CB3 lid and centrifuge for 30 sec at 12,000 rpm. Discard the filtrate and set the spin column CB3 into the collection tube. Since the capacity of CB3 is 700 μ l, centrifuge step should be repeated several times for processing all the mixture from step 4
6. Carefully open the column and add 500 μ l buffer GD, close the lid and centrifuge at 12,000 rpm for 30 sec then discard the filtrate and place the spin column into the collection tube
7. Add 700 μ l PW to the spin column CB3 to wash the membrane, and centrifuge for 30 sec at 12,000 rpm, discard the flow-through, replace the spin column CB3 in the collection tube
8. Add 500 μ l PW to the spin column CB3 to wash the membrane and centrifuge for 30 sec at 12,000 rpm, discard the flow-through

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9. Replace the spin column CB3 in the collection tube, centrifuge for 2 min at 12,000 rpm to remove residual wash buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5 ml or 2 ml microcentrifuge tube. Open the lid of the CB3 and incubate the assembly at room temperature (15-20⁰C) or 50⁰C for several minutes to dry membrane completely
 10. Pipet 50-200 μ l buffer TE directly onto the CB3 membrane, incubate for 2-5 min at room temperature and then centrifuge for 2 min at 12,000 rpm to elute

Note: ensure that the elution buffer TE is equilibrated to room temperature (15-20⁰C). Elution with small volumes (<50 μ l) will reduce the efficiency of the elution and the DNA yield. The pH value of eluted buffer will have some influence in eluting; Buffer TE or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in buffer TE and storing at -20⁰C is recommended, since DNA stored in water is subject to acid hydrolysis.

3.4.3. Electrophoresis of DNA

The quality of DNA was examined by the Gel electrophoresis.

3.4.3.1. Reagents and equipments

1. Agarose - 0.8 per cent (for genomic DNA)
- 1.2 per cent (for RAPD samples)
2. 50X TBE buffer (pH 8.0)
 - Tris buffer
 - Boric acid
 - 0.5 mM EDTA
3. Tracking/ loading dye (6X)
 - Bromophenol blue (Methylene blue)
4. Ethidium bromide

- 5. Electrophoresis unit, power pack, gel casting tray, comb
- 6. BIO-RAD Gel documentation and analysis system

3.4.3.2. Procedure

- 1. Swab the work area, gel tray and comb with 100% alcohol
- 2. Prepare gel tray by sealing the ends with tape. Comb should be placed in the gel tray about 1 inch from one end of the tray and positioned the comb vertically so that the teeth are about 1mm above the surface of the tray
- 3. 0.8% agarose gel was prepared by dissolving 0.45 g agarose in 40 ml 1X TBE buffer and kept in microwave until the agarose dissolved fully and the solution looks clear
- 4. Agarose solution was allowed to cool to 42 to 45⁰C and ethidium bromide was added at a concentration of 10 µl and mixed well. Bubbles should be avoided in the solution
- 5. Pour the warm gel solution into the tray. Allow it to solidify for about 30 – 45 minutes at room temperature
- 6. Comb and tape were removed without disturbing the gel and the gel was placed in the electrophoresis tank containing 1X TBE buffer
- 7. The tray was kept in a manner that the well side should be towards cathode
- 8. The samples were prepared by adding 5 µl of DNA sample with 2 µl of tracking dye, mixed well and was loaded into wells using micro pipette
- 9. Load suitable molecular weight marker (λDNA Eco RI/ Hind III) in one well
- 10. Electrophoresis done at 100 volts until dye has migrated two-third the length of the gel
- 11. Intact DNA observed as orange fluorescent bands

3.4.4. Gel documentation

The gel documentation was carried out using BIO-RAD imaging system. – A software package used for imaging, analysing and data basing 2-D

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electrophoresis gels. The gel picture was examined for intactness, clarity of band, presence of contamination such as proteins and RNA.

3.4.5. Determination of the quality and quantity of DNA by Biophotometer method

Biophotometer is a full spectrum and measures absorbance which was used for detecting the quantity and quality of DNA. It measures the concentration of nucleic acids in the samples based on Beer-Lambert law. Nucleic acid shows absorption maxima at 260 nm whereas proteins show peak absorbance at 280 nm. Absorbance recorded at both wavelengths indicated by the ratio OD_{260}/OD_{280} . A pure solution of double standard DNA at $\mu\text{g/ml}$ has an optical density of 1.0 at 260 nm and OD_{260}/OD_{280} ratio of 1.8. Contamination with protein or polyphenol will show OD_{260}/OD_{280} values significantly less than 1.8 and contamination with RNA shows a ratio greater than 1.8 (for pure RNA, OD_{260}/OD_{280} is 2.0). The value from 1.8 to 2.0 indicates that the DNA is pure which is free from proteins and other contaminants. The quantity of DNA in the pure sample was calculated using OD_{260} equivalent to 50 μg double stranded DNA/ml sample.

$$1\text{OD at } 260\text{ nm} = 50\ \mu\text{g DNA (ds)/ ml sample}$$

Therefore $OD_{260} \times 50$ gives the quantity of DNA in $\mu\text{g/ml}$.

3.4.6. RAPD assay of WCT families

3.4.6.1. Standardization of PCR conditions

PCR reaction mixture consists of DNA (20ng), *Taq* buffer with MgCl_2 , *Taq* DNA polymerase, dNTPs and primers showed effective amplification. DNA amplification conditions for PCR are conducted in Eppendorf Mastercycler. The PCR reactions were conducted in volumes of 20 μl containing 20 ng/ μl genomic DNA. The materials required for the reaction mixture are:

- Autoclaved distilled water – 13.1 μl

- *Taq* buffer with MgCl₂ (10X) – 2 µl
- dNTPs (10mM) – 1.6 µl
- Primer (10 µM) – 1 µl
- *Taq* DNA polymerase – 0.3 µl
- DNA – 2 µl

The amplification was done using the following thermal profile

Thermal profile for RAPD assay:

- Initial denaturation – 94⁰C for 5 min
 - Final denaturation – 94⁰C for 1 min
 - Primer annealing – 37⁰C for 1 min
 - Primer extension – 72⁰C for 2 min
 - Final extension – 72⁰C for 10 min
- } 39 cycles
- 4⁰C - hold the sample

3.4.6.2. Screening of RAPD primers

The extracted DNA was used to screen the thirty 10-mer oligonucleotide primers in the Polymerase Chain Reaction (PCR). The primers were purchased in the series of OPA, OPB, OPC OPE, OP-AW and OP-AU (Table 2). The PCR was conducted to amplify the genomic DNA of one plant sample. Details of the primers used for screening is given in Table 2. The amplified products were loaded to 1.2 per cent agarose gel using 1X TBE buffer.

3.4.6.3. RAPD assay of the selected genotypes

Based on amplification pattern, 10 primers were selected for final analysis of the four promising inbreds using the protocol standardized. Negative control (without DNA) was also used. The profile was visualized and documented using gel documentation system. The documented RAPD profiles were carefully examined and the bands were scored manually based on intensity and clarity. Presence of band was indicated as '+' and absence as '-'.

Table 2: List of decamer primers used for screening WCT families

SL. No	Primer	Sequence
1	OPA03	AGTCAGCCAC
2	OPA09	GGGTAACGCC
3	OPA10	GTGATCGCAG
4	OPA12	TCGGCGATAG
5	OPA16	AGCCAGCGAA
6	OPBA3	GTGCGAGAAC
7	OPB07	GGTGACGCAG
8	OPC07	GTCCCGACGA
9	OPC10	TGTCTGGGTG
10	OPC17	TTCCCCCAG
11	OPE12	TTATCGCCCC
12	OP-AW02	TCGCAGGTTC
13	OP-AW03	CCATGCGGAG
14	OP-AW05	CTGCTTCGAG
15	OP-AW06	TTTGGGCCCC
16	OP-AW07	AGCCCCCAAG
17	OP-AW08 ^a	CTGTCTGTGG
18	OP-AW09	ACTGGGTCGG
19	OP-AW11	CTGCCACGAG
20	OP-AW12	GAGCAAGGCA
21	OP-AW13	CTACGATGCC
22	OP-AW14	GGTTCTGCTC
23	OP-AW15	CCAGTCCCAA
24	OP-AW16	TTACCCCGCT
25	OP-AW17	TGCTGCTGCC
26	OP-AW18	GGCGCAACTG
27	OP-AW19	GGACACAGAG
28	OP-AU02	CCAACCCGCA
29	OP-AU03	ACGAAACGGG
30	OP-AU04	GGCTTCTGTC

3.5. STATISTICAL ANALYSIS

The data obtained from the mother palms and the planted seedlings were analysed statistically and tested for its variability and inbreeding depression for all the characters.

3.5.1. Inbreeding depression calculation

The level of inbreeding depression on vegetative characters were analysed using the following formula (Lande and Schemke, 1985).

$$ID = 1 - \frac{F_{ib}}{F_{ob}} \times 100\%$$

Where,

ID = inbreeding depression

F_{ib} = mean value of phenotypic characters of self pollination

F_{ob} = mean value of phenotypic characters of open pollination

The significance of inbreeding depression is tested with the help of 't' value. The general formula for estimation of 't' value are given below.

$$'t' \text{ value} = \frac{\text{mean of open pollinated} - \text{mean of self pollinated}}{\text{standard error}}$$

Results

4. RESULT

The study was conducted to analyse the inbreeding depression of WCT coconut of second generation (S₂) palms and third generation (S₃) seedlings with respect to various characters viz., morphological, reproductive, yield and nut characters. The twelve families in second generation WCT palms for 26 characters and third generation seedlings of WCT for 7 characters were studied with open-pollinated WCT and dwarfs viz., COD and CGD. The twelve families of second generation (S₂) WCT palms were the selfed and sibmated families of six groups of open-pollinated WCT coconut. The results obtained with respect to analysis of second generation (S₂) palms and third generation (S₃) seedlings are presented here under:

- 4.1. Analysis of variance of second generation palms
- 4.2. Performance of second generation WCT palms
- 4.3. Inbreeding depression of second generation WCT palms
- 4.4. Variability analysis of third generation seedlings
- 4.5. Inbreeding depression of third generation seedlings of WCT coconut
- 4.6. Molecular analysis- RAPD

4.1. ANALYSIS OF VARIANCE OF SECOND GENERATION PALMS

Analysis of variance (ANOVA) for all the characters of second generation WCT palms of twelve families is presented in the Table 3. Result revealed significant differences for plant height, collar girth, internodal length, number of functional leaves, number of leaves per annum, number of leaflets per leaf, petiole length, leaf length, number of female flowers per inflorescence, period of flowering phase (male and female phase), setting percentage, peduncle length, number of spadices per annum, number of yielding bunches per annum, number of nuts per bunches, nut yield per palm per annum, unhusked nut weight, husked

Table 3: Mean squares of WCT coconut families with dwarf (CGD and COD) for yield and its component characters.

Sl. No.	Character	Degrees of freedom	Mean square	F-value	Significance
1	Plant height	14	23.66	19.76	**
2	Collar girth	14	210.93	9.69	**
3	Internodal length	14	8.89	27.40	**
4	No. of functional leaves	14	125.96	15.69	**
5	No. of leaves/ annum	14	4.99	3.52	**
6	No. of leaflets/ leaf	14	2986.47	4.40	**
7	Petiole length	14	2644.96	21.29	**
8	Leaf length	14	0.77	6.29	**
9	No. of female flowers/ inflorescence	14	399.06	25.51	**
10	Period of female phase	14	3.75	27.55	**
11	Period of male phase	14	11.57	8.86	**
12	Setting percentage	14	119.91	3.44	**
13	Stalk length	14	171.90	9.88	**
14	No. of bunches/ annum	14	8.48	6.61	**
15	No. of yielding bunches/ annum	14	20.81	13.90	**
16	No. of nuts/ bunches	14	7.83	2.65	**
17	Nut yield/ annum	14	1293.50	10.30	**
18	Size of nut	14	31.31	1.58	NS
19	Unhusked nut weight	14	75502.16	6.93	**
20	Husked nut weight	14	16070.02	4.50	**
21	Diameter of eye	14	0.36	12.69	**
22	Fresh mature kernel weight	14	6588.99	2.65	**
23	Kernel thickness	14	0.04	8.90	**
24	Copra content	14	2102.64	4.29	**
25	Oil content	14	124.15	8.25	**
26	Volume of tender nut water	14	14220.058	7.72	**

** = Significant at both 5% and 1% level of probability

N.S. = Non Significant

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nut weight, diameter of eye, fresh mature kernel weight, kernel thickness, volume of tender nut water, copra content and oil content. At both 5% and 1% level of probability the size of nut didn't show any significant difference between the twelve families of the WCT coconut.

4.2. PERFORMANCE OF SECOND GENERATION OF WCT PALMS

Performances of twelve families of second generation (S_2) WCT coconut with open pollinated WCT, COD and CGD for vegetative, reproductive, yield and nut characters were recorded. The mean values with the range of the different parameters were recorded for the second generation (S_2) coconut palms are presented in tables 4, 5 and 6.

4.2.1. Vegetative characters

The range and mean values of the various morphological characters of selfed and sibmated families of second generation (S_2) WCT palms comprising six groups of WCT, open pollinated WCT, COD and CGD are presented in table 4.

4.2.1.1. Height of Palm

The height of second generation palms varied from 6.20m to 17.30m (Table 4). The mean height of tall palms recorded in sibmated family IVS_2-2 was 15.66m and mean of short palms recorded in selfed family IIS_2-1 was 10.20m. Among the twelve families, the mean tallest palms (13.45m) occurred in IAS_2-1 with a range of 12.30m to 14.50m in selfed family. The shortest mean height of 11.70m ($IIIS_2-2$) with a range of 6.25m to 14.00m was found in sibmated families. The open pollinated WCT palms exhibited a highest mean height of 15.87m while that of the dwarf palms were 8.51m and 7.12m respectively in CGD and COD. Even though mean height was higher, few palms in the family IBS_2-1 (8.0m), IIS_2-1 (6.25m) and $IIIS_2-2$ (6.0m) expressed low height which is similar as the dwarfs (COD and CGD).

4.2.1.2. Girth of the palm

Among selfed families, the maximum mean girth of stem were observed from the family IBS₂-1 (85.52 cm) with the range 60cm to 90cm and the minimum mean girth were observed from the family VS₂-1 (70.25 cm) with the range 65cm to 75cm (Table 4). The maximum mean girth of stem was recorded in the family IIS₂-2 (85.45cm) ranging from 80cm to 90cm and the minimum mean girth was observed in the family IBS₂-2 (77.17cm) with the range 70cm to 105cm among the sibmated family. The mean girth of open pollinated WCT was 79.83 cm with a range of 70 to 90 cm. Similarly, the mean girth of CGD was 62.16 cm and COD, 63.44cm with a range of 59 to 66 cm.

4.2.1.3. Internodal length

The internodal length varied from 5cm (4S₂-1 and 5S₂-1) to 9.5cm (1BS₂-1) in second generation palms (S₂) of WCT (Table 4). The lowest mean length of internode was recorded in the selfed family IVS₂-1 (6.10cm) and highest mean length was observed in family IIS₂-2 (7.88cm) with the range of 5cm to 6.5 cm and 7cm to 8.5cm respectively. Whereas in sibmated families the lowest internodal length was noticed in IVS₂-2 (6.50cm) with the range 5.4cm to 7.5cm and the highest recorded value was in IAS₂-2 (8.25cm) between 7.0 cm to 9.0cm. The WCT open pollinated had the internodal length of 8.15 cm which is on par with IAS₂-1, 1AS₂-2, IIS₂-1, IIS₂-2 and dwarfs showed 3.24 cm and 3.87 cm for CGD and COD respectively.

4.2.1.4. Number of functional leaves

The table 4 shows that among selfed families, the maximum mean numbers of functional leaves were recorded in the family IIS₂-1 (21.25) with range 17 to 28 and minimum in the family IAS₂-1 (14.63) with the range of 8 to 17. In sibmated families, the maximum mean number of functional leaves was found in the family VS₂-2 (24.00) with range 14 to 34 and the minimum in the family IVS₂-2 (16.08) with range of 15 to 25. The open pollinated WCT exhibited about 37.13 functional leaves which ranged from 35 to 39. The dwarfs had 24.56 and 22.44 functional leaves in CGD and COD respectively. The selfed family

Table 4: Mean and range of vegetative characters of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

Family	Plant height (m)			Girth of palm (cm)			Internodal length (cm)			Number of functional leaves		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Low	High		Low	High		Low	High		Low	High
IAS ₂ -1	13.45	12.30	14.50	82.46	70	90	7.78	7.0	8.5	14.63	8	17
IBS ₂ -1	13.29	8.00	17.30	85.52	60	90	7.71	6.5	9.5	18.42	14	27
IIS ₂ -1	10.20	6.20	12.40	73.75	70	80	7.88	7.0	8.5	21.25	17	28
IIIS ₂ -1	13.30	12.70	14.50	81.25	75	85	7.50	7.0	8.0	18.25	16	21
IVS ₂ -1	12.12	11.20	13.50	82.11	80	85	6.10	5.0	6.5	17.67	15	23
VIS ₂ -1	11.83	10.00	13.20	70.25	65	75	6.69	5.0	7.7	15.50	13	17
IAS ₂ -2	15.22	13.50	16.20	82.17	78	90	8.25	7.0	9.0	16.38	12	19
IBS ₂ -2	12.69	11.00	14.70	77.17	70	105	7.25	5.3	9.1	23.86	17	29
IIS ₂ -2	13.11	12.20	14.50	79.00	70	82	8.02	7.5	8.3	18.00	17	19
IIIS ₂ -2	11.70	6.25	14.00	85.45	80	90	6.80	5.3	8.0	21.33	17	26
IVS ₂ -2	15.66	10.00	16.00	81.42	80	95	6.50	5.4	7.5	16.08	15	25
VIS ₂ -2	12.70	10.40	15.00	77.50	65	90	7.34	5.9	8.8	24.00	14	34
WCT	15.87	15.60	16.20	79.83	70	90	8.15	7.0	8.6	37.13	35	39
CGD	8.51	8.00	9.20	62.16	59	66	3.24	2.9	3.4	24.56	23	27
COD	7.12	6.95	8.20	63.44	60	66	3.87	3.5	4.1	22.44	21	24
C D 5%	1.56			6.66			0.81			4.40		
C D 1%	2.09			8.90			1.09			5.40		
C V	8.78			6.01			8.29			13.73		

Table 4 Continued.

Family	Number of leaves/annum			Leaf length (m)			Petiole length (cm)			Number of leaflets/ leaf		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Low	High		Low	High		Low	High		Low	High
IAS ₂ -1	9.71	5	12	2.64	2.10	3.30	72.92	65	85	183.42	139	227
IBS ₂ -1	11.17	10	13	2.81	2.30	3.60	78.33	65	115	191.67	156	231
IIS ₂ -1	11.00	8	13	2.85	2.40	3.20	80.00	75	85	174.50	151	197
IIIS ₂ -1	10.25	9	12	2.47	2.10	2.65	72.50	60	80	204.50	159	249
IVS ₂ -1	11.34	10	13	2.15	1.90	2.85	62.78	50	90	154.33	131	207
V S ₂ -1	9.38	8	11	2.47	1.90	3.55	75.13	68	98	193.00	131	269
IAS ₂ -2	10.63	8	13	2.71	2.30	3.20	82.33	70	97	196.38	156	237
IBS ₂ -2	12.08	11	13	2.78	2.40	4.00	83.98	65	120	167.58	131	225
IIS ₂ -2	11.00	9	12	2.82	2.40	3.15	80.00	60	85	195.33	145	247
IIIS ₂ -2	11.25	9	13	3.17	2.85	3.60	85.00	80	90	209.00	179	251
IVS ₂ -2	10.17	9	11	2.65	2.15	3.80	76.33	70	120	172.50	165	249
V S ₂ -2	10.50	8	13	2.60	1.95	3.25	82.50	60	105	202.00	169	235
WCT	12.25	12	14	4.12	3.90	4.60	115.25	95	125	276.38	247	287
CGD	11.50	11	12	2.69	2.41	2.87	79.19	57	95	190.13	240	290
COD	11.00	10	12	3.05	2.74	3.45	83.28	80	95	197.63	260	380
C D 5%	1.70			0.50			15.90			37.16		
C D 1%	2.27			0.67			21.26			49.68		
C V	10.69			12.47			13.17			13.43		

IIS₂-1 and sibmated families IBS₂-2, IIS₂-2 and VS₂-2 had retained the functional leaves similar to the dwarfs. All other families had less number of functional leaves.

4.2.1.5. Number of leaves per annum

The mean value of number of leaves produced in open-pollinated WCT was 12.25 leaves per annum and in dwarfs, CGD and COD it were 11.50 and 11.00 respectively. Among the selfed families, the maximum mean number of leaves per annum was recorded in family IVS₂-1 (11.34) with a range of 10 to 13 and the minimum mean number of leaves per annum in family VS₂-1 (9.38) with a range of 8 to 11 (Table 4). The maximum mean number of leaves produced per year was observed in family IBS₂-2 (12.08) with the range 11 to 13 and minimum was observed in family IVS₂-2 (10.17) with the range 9 to 11. Family IVS₂-1 and IBS₂-2 produced less leaves per annum than WCT, but on par with dwarfs COD and CGD at 5% level of significance. Similarly the mean number of leaves produced per annum in IIS₂-1, VS₂-1 in selfed and IAS₂-2, IVS₂-2 and VS₂-2 in sibmated families were significantly lower than dwarf at 5% level of significance.

4.2.1.6. Leaf length

From the table 4 it is evident that the mean leaf length of open pollinated WCT recorded with 4.12m and dwarf CGD with 2.69m and COD with 3.05m. The maximum mean leaf length was observed in the family IIS₂-1 (2.85m) with the range 2.40m to 3.20m, the minimum mean leaf length was observed in the family IVS₂-1 (2.15m) with the range, 1.90m to 2.85m among selfed families (Table 4). While in sibmated families, the maximum mean leaf length was recorded in the family IIS₂-2 (3.17m) with range, 2.85m to 3.60m and the minimum in the family VS₂-2 (2.60m) with the range 1.95m to 3.25m. all the families had leaf length significantly less than the WCT palm leaf length. However, the leaf length of selfed and sibmated WCT families was on par with both the dwarfs except IVS₂-1.

4.2.1.7. Petiole length

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Among the selfed families, the highest mean petiole length was recorded in the family IIS₂-1 (80 cm) with range 75cm to 85cm and lowest in the family IVS₂-1 (62.78cm) with range 50cm to 90cm. From sibmated families the family IIS₂-2 (85 cm) had the highest mean petiole length with the range 80cm to 90cm and the lowest length was in the family IVS₂-2 (76.33cm) with the range 70cm to 120cm (Table 4). The open-pollinated WCT recorded mean petiole length of 115.25cm, while the dwarf CGD and COD observed the mean of 79.19 and 83.28 respectively. All the families produced shorter petioles and were highly significantly less than WCT, but on par with dwarf palm leaf petiole length.

4.2.1.8. Number of leaflets per leaf

Mean values of number of leaflets per leaf were presented in table 4. From selfed families, the highest mean number of leaflets per leaf was observed in the family IIS₂-1 (204.50) with range 159 to 249 and lowest mean was in the family IVS₂-1 (154.33) with range 131 to 207. The sibmated families exhibited the highest mean number of leaflets per leaf in family IIS₂-2 (209.0) with range 179 to 251 and the lowest in the family IBS₂-2 (167.58) with the range 131 to 225. The mean number of leaflets of open pollinated WCT, CGD and COD recorded 276.38, 190.13 and 197.63 respectively. The results show that none of families were on par with the WCT in leaflets production, but all produced highly significantly lower leaflets than WCT open pollinated. However, the leaflets produced by all the families were on par with dwarfs. The leaflets found in the family IVS₂-1, was significantly less than the dwarfs COD and CGD.

4.2.2. Reproductive characters

4.2.2.1. Number of female flowers per inflorescence

Mean values of number of female flowers per inflorescence presented in table 5 shows that the maximum mean number of female flowers per inflorescence was produced in the family IIS₂-1 (18.42) with range 12.12 to 24.05 and the minimum mean number of female flowers per inflorescence was observed in the family IVS₂-1 (9.23) with range 5.00 to 15.60 among the selfed families.

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The maximum mean number of female flowers per inflorescence was recorded in the family VS₂-2 (19.81) with range 10.58 to 29.05, whereas minimum mean number of female flower per inflorescence was recorded in the family IAS₂-2 (11.20) with the range 9.25 to 16.68 in the sibmated families. The family IIS₂-1 and IIIS₂-2 recorded on par with the dwarfs where CGD and COD showed 22.62 and 22.22 mean values and highly significantly less than WCT (20.97). The female flowers production per inflorescence was highly significantly less than dwarfs COD and CGD in IAS₂-1, IBS₂-1, IIS₂-1, IIIS₂-1 and IVS₂-1 in self pollinated and IAS₂-2 and IIS₂-2 in sibmated families of WCT studied

4.2.2.2. *Period of flowering phase (days)*

4.2.2.2.1. *Male phase*

From selfed families, the maximum mean period of male phase was observed in the family IIIS₂-1 (20.9 days) with range 20 to 23 days and the minimum mean period of male phase in the family IAS₂-1 (17.2 days) with range 16 to 20 days (Table 5). Similarly among the sibmated families, the maximum mean period of male phase recorded in the family IIIS₂-2 (21.7 days) with range 20 to 24 days and the minimum mean period of male phase was observed in the family IAS₂-2 (19.1 days) with range 18 to 21 days. Period of male phase of open pollinated WCT was only 20.8 days and the dwarfs CGD with 16.7 days and COD with 17.8 days.

4.2.2.2.2. *Female phase*

The maximum mean period of female phase was observed in the family VS₂-1 (4.6 days) with range 3 to 6 days and the minimum mean period of female phase in the family IIIS₂-1 (3.3 days) with range 3 to 4 days (Table 5). Similarly among the sibmated families, the maximum mean period of female phase recorded in the family IBS₂-2 (4.1 days) with range 3 to 6 days and the minimum mean period of female phase was observed in the family IIS₂-2 (2.3 days) with range 2 to 3 days. Period of female phase of open pollinated WCT was 3.3 days

which observed as same as family IIS₂-1 and the dwarfs CGD with 5.2 days and COD with 5.6 days.

4.2.2.3. *Setting percentage*

From the table 5 it is evident that the highest mean setting percentage was recorded in the family IIS₂-1 (53.17 %) with range 32.48 to 68.10 % and the lowest percentage was observed in the family VS₂-1 (34.34 %) with the range 23.67 to 40.22 % in selfed families. In sibmated families, the highest setting percentage was noticed in the family IAS₂-2 (44.83 %) with range 38.11 to 48.65 % and the lowest was observed in the family IIS₂-2 (37.30 %) with range 30.73 to 46.95 %. The mean of open-pollinated WCT showed setting percentage of 40.10% and dwarfs recorded with 36.43 in CGD and 30.75% in COD. The setting percentage was on par with the WCT open pollinated palms. The families IIS₂-1 and IVS₂-1 expressed significantly higher setting percentage than CGD. But the families IIS₂-1, IIS₂-1, IVS₂-1, IAS₂-2, IBS₂-2, IIS₂-2, and IVS₂-2 exhibited significantly higher setting percentage than COD.

4.2.2.4. *Peduncle length*

The highest stalk length was noticed in open-pollinated WCT with the mean of 56.38 cm (Table 5). The selfed WCT families recorded the maximum stalk length in the family IIS₂-1 (34.25 cm) with range 32 cm to 40 cm and the minimum stalk length was observed in the family IVS₂-1 (26.11 cm) with range 18 cm to 37 cm. From sibmated families the maximum stalk length in the family was observed in IVS₂-2 (37.87 cm) with the range 32 cm to 52 cm and the minimum was observed in the family IIS₂-2 (30.67 cm) with range 26 cm to 36 cm. The dwarfs observed with mean of 31.59 cm and 31.97 cm in CGD and COD respectively. The stalk length was maximum in WCT open pollinated is 56.38 cm. All the families, both selfed and sibmated families expressed highly significantly shorter stalk. The stalk length of IVS₂-2 was maximum and significantly longer than CGD at 5% level of probability, but on par with CGD at 1% significance, all other families had stalk length on par with dwarf.

Table 5: Mean and range of reproductive characters of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

Family	No. of female flowers/ inflorescence			Period of flowering phase (days)						Setting percentage (%)		
	Mean	Range		Male phase			Female phase			Mean	Range	
		Low	High	Mean	Range Low	High	Mean	Range Low	High		Low	High
IAS ₂ -1	10.34	7.10	15.82	17.2	16	20	3.4	3	4	34.91	26.25	51.06
IBS ₂ -1	13.93	8.87	22.62	17.6	16	21	4.2	3	5	36.41	23.53	45.70
IIS ₂ -1	18.42	12.12	24.05	15.6	14	18	2.4	2	3	42.53	37.79	46.24
IIIS ₂ -1	14.44	11.35	19.55	20.9	20	23	3.3	3	4	53.17	32.48	68.10
IVS ₂ -1	9.23	5.00	15.60	17.5	16	20	3.8	3	5	45.75	32.53	53.85
V _S ₂ -1	15.03	10.77	21.68	19.7	18	22	4.6	3	6	34.34	23.67	40.22
IAS ₂ -2	11.20	9.25	16.68	19.1	18	21	3.5	3	4	44.83	38.11	48.65
IBS ₂ -2	14.84	9.33	22.63	20.8	18	23	4.1	3	6	40.68	28.52	58.60
IIS ₂ -2	14.09	10.45	18.53	19.4	17	21	2.3	2	3	41.49	27.36	52.78
IIIS ₂ -2	16.57	13.83	27.95	21.7	20	24	3.5	3	4	37.30	30.73	46.95
IVS ₂ -2	14.89	9.62	25.98	20.3	17	24	2.8	2	4	39.60	24.71	49.77
V _S ₂ -2	19.81	10.58	29.05	19.6	18	21	2.7	2	3	37.67	35.28	40.07
WCT	20.97	14.00	29.00	20.8	19	22	3.3	3	4	40.10	38.00	43.18
CGD	22.62	19.00	27.00	16.7	15	18	5.2	5	6	36.43	24.00	42.85
COD	22.22	19.00	28.00	17.8	16	19	5.6	4	7	30.75	26.92	32.14
C D 5%	5.64			1.63			0.53			8.42		
C D 1%	7.54			2.18			0.70			11.26		
C V	22.07			6.03			10.03			14.86		

Table 5 Continued.

Family	Peduncle length (cm)			No. of spadices/ annum			No. of yielding bunches/ annum			No. of nuts/ bunches			Nut yield/ annum		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Low	High		Low	High		Low	High		Low	High			
IAS ₂ -1	29.58	24	34	8.75	8	9.5	3.39	1.5	5.4	3.67	1.90	6.98	14.41	3.37	37.69
IBS ₂ -1	30.67	23	45	10.28	9.4	10.8	3.72	2.0	6.0	5.22	2.56	10.0	22.33	6.40	50.00
IIS ₂ -1	33.00	23	40	9.75	9.2	10.1	3.97	2.5	5.4	7.98	4.58	11.12	30.99	18.30	55.60
IIIS ₂ -1	34.25	32	40	9.23	7.6	10.8	5.52	5.2	5.8	7.16	6.35	7.73	39.71	33.02	44.83
IVS ₂ -1	26.11	18	37	9.65	8.7	10.8	3.71	1.5	6.3	4.29	2.17	6.65	18.83	3.25	41.89
VS ₂ -1	33.88	18	48	8.55	7.5	9.78	4.84	2.75	6.0	5.71	2.97	8.72	25.38	8.17	52.32
IAS ₂ -2	34.63	30	39	9.13	7.5	10.8	3.15	1.0	4.8	5.00	4.43	7.75	16.46	4.50	37.20
IBS ₂ -2	32.50	21	50	10.95	10.2	11.2	4.26	2.3	6.8	6.07	3.02	11.38	27.49	7.55	61.45
IIS ₂ -2	30.67	26	36	9.43	8.9	10.6	2.63	1.5	3.6	6.10	3.33	9.78	18.24	4.99	35.21
IIIS ₂ -2	35.83	34	38	9.73	7.4	11.2	4.47	2.5	6.2	6.17	4.25	9.90	29.92	10.62	61.38
IVS ₂ -2	37.87	32	52	9.53	9.2	10	5.53	3.0	7.0	5.57	3.45	6.95	30.50	10.35	48.30
VS ₂ -2	35.00	30	40	9.40	7.0	12.10	4.60	2.4	7.0	7.24	4.24	10.25	40.96	10.18	71.75
WCT	56.38	56	65	12.03	11	14	9.36	9.00	12.0	8.41	6.68	11.68	78.79	69.40	84.50
CGD	31.59	25	35	12.75	11	14	8.18	6.00	11.0	7.15	6.00	9.00	50.28	39.00	68.00
COD	31.97	30	35	12.50	12	14	8.53	7.00	10.0	8.05	6.00	9.00	61.15	51.00	64.50
C D 5%	5.95			1.62			1.75			2.45			15.99		
C D 1%	7.96			2.16			2.33			3.28			21.38		
C V	12.12			11.14			23.76			27.48			33.27		

4.2.2.5. *Number of spadices per annum*

The maximum number of bunches or spadices produced per annum by the family IBS₂-1 (10.28) with the range 9.4 to 10.8 and the minimum number of bunches produced per annum in family VS₂-1 (8.55) with the range 7.5 to 9.78 in the selfed families (Table 5). In the sibmated families, the maximum number of bunches produced per annum was in family IBS₂-2 (10.95) with range 10.2 to 11.2 and the minimum number of bunches produced per annum was in the family IAS₂-2 (9.13) with range 7.5 to 10.8. The mean of open-pollinated WCT produced 12.03 spathes per year and dwarf CGD and COD developed 12.75 and 12.50 bunches per year respectively. The family IBS₂-2 produced number of bunches per annum and on par with COD. All other families produced highly significantly less number of bunches per annum than WCT open pollinated, COD and CGD.

4.2.2.6. *Number of yielding bunches per annum*

Mean values of number of yielding bunches produced per annum were presented in table 5. From selfed families, the highest mean number of yielding bunches per annum observed in the family IIS₂-1 (5.52) with range 5.2 to 5.8 and lowest mean value in the family IAS₂-1 (3.39) with range 1.5 to 5.4. The sibmated families exhibited the highest mean number of yielding spadices per annum in family IVS₂-2 (5.53) with range 3.0 to 7.0 and the lowest in the family IIS₂-2 (2.63) with the range 1.5 to 3.6. The mean values of bunches in WCT, CGD and COD recorded 9.36, 8.18 and 8.53 respectively. All the selfed and sibmated families produced highly significantly less number of yielding bunch per annum.

4.2.2.7. *Number of nuts per bunches*

Mean values of number of nuts produced per bunch were tabulated in 5. From selfed families, maximum number of nuts per bunches were produced in the family IIS₂-1 (7.98) with the range 4.58 to 11.12 and the minimum number of nuts per bunches were produced in the family IAS₂-1 (3.67) with the range 1.90 to 6.98. In sibmated families maximum mean number of nuts per bunches were produced by the family VS₂-2 (7.24) with range 4.24 to 10.25 and the minimum



Plate 2: Spadix containing male and female flowers of 12 families and WCT (open pollinated)

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were observed in family IAS₂-2 (5.0) with the range from 4.43 to 7.75. The open pollinated WCT produces the nuts per bunches with the mean of 8.41 and dwarfs of CGD and COD recorded with mean of 7.15 and 8.05. The selfed families IIS₂-1, IIS₂-2, IBS₂-2, IIS₂-2, IIS₂-2 and VS₂-2 were on par in nut production per bunches to WCT open pollinated and all other families were on par with COD except IAS₂-1, IBS₂-1, IVS₂-1, IAS₂-2 and IVS₂-2, which were significantly inferior. Similarly selfed family IAS₂-1 was significantly inferior to CGD in production of nuts per bunches.

4.2.2.8. Nut yield per annum

The open pollinated WCT recorded the highest nut yield per annum with the mean of 78.79 and the dwarfs with 50.28 and 61.15 in CGD and COD respectively. Mean values of nut yield per annum of different families along with WCT, CGD and COD were presented in table 5. As in number of yielding bunches per annum, nut yield per annum was highest in the family IIS₂-1 (39.71) with the range 33.02 to 44.83 and the lowest were observed from the family IAS₂-1 (14.41) with range 3.37 to 37.69. From sibmated families, highest nut yield per annum were obtained from family VS₂-2 (40.96) with range 10.18 to 71.75 and the lowest nut yield were from the family IAS₂-2 (16.46) with the range 4.50 to 37.20. The result of VS₂-2 and IIS₂-1 was on par with the nut yield per annum obtained from COD, but the nut yield were negatively significant with respect to CGD and WCT. The nut yield recorded was significantly low in all families compared to WCT, CGD and COD.

4.2.3. Nut characters

4.2.3.1. Size of nut

The mean values of size of nut were tabulated in table 6 where the sibmated families produced highest mean size of nut. In the family IVS₂-2 produced largest nuts with mean size of 47.43cm ranging from 40.29cm to 71.17cm and the lowest mean size were in the family IIS₂-2 (39.07 cm) with range 36.63cm to 43.96cm. While the selfed family had the largest nut in IIS₂-1

(42.86 cm) with the range value of 37.68cm to 48.46cm and lowest mean size were recorded in the family VS₂-1 (36.96 cm) with the range 31.92cm to 40.82cm. The character has not revealed any significance between treatments. The WCT, CGD and COD observed the mean size of 41.11cm, 38.05cm and 40.11cm respectively.

4.2.3.2. *Unhusked nut weight*

The highest mean weight of unhusked nut were observed in the open-pollinated WCT (922.50g) and lowest were in the COD (376.72g) followed by CGD (381.84g). Among selfed families, the highest mean weight of unhusked nut were recorded in the family IIS₂-1 (683.25g) with range 535g to 818g and the lowest mean nut weight were observed in family IVS₂-1 (461.45g) with the range 373g to 550g (Table 6). From sibmated families, the highest mean nut weight were obtained in family IBS₂-2 (676.42g) with range 375g to 1300g and the lowest weight were observed in family VS₂-2 (430.00g) with range 260g to 600g. The nut weight of WCT open pollinated was highest where none of the family produced nuts equal to WCT open pollinated nut weight. The nut weight of the selfed family IIS₂-1, IIS₂-1 and sibmated family IBS₂-2 and IVS₂-2 were highly significantly superior to the nut weight of dwarf palms CGD and COD. The nut weights of all other families were observed on par.

4.2.3.3. *Husked nut weight*

The mean values of husked nut weight presented in table 6 showed that among the selfed families, the highest mean weight of husked nut were observed in the family IIS₂-1 (384.50g) with the range 343g to 430g and the lowest mean weight were recorded in family IVS₂-1 (258.00g) with range 197g to 280g. From sibmated families, the highest mean husked nut weight was observed in the family IBS₂-2 (364.62g) with range 230g to 650g and the lowest mean weight observed in family IAS₂-2 (294.00g) with range 200g to 413g. The highest mean of unhusked nut weight was 500g in WCT and lesser mean weight were in CGD (261.19g) followed by COD (243.19g). Even the highest husked nut obtained in

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the family IIS₂-1 was highly significantly inferior to WCT open pollinated husked nut weight. However the performance of the families IIS₂-1 with respect to husked nut weight was significantly superior at 1% level. The husked nut weight of IBS₂-1, IBS₂-2, IVS₂-2 and VS₂-2 were superior to dwarfs at 5% level. All other families had the husked nut weight on par with dwarfs.

4.2.3.4. Diameter of eye

The lowest diameter of eye was noticed in CGD followed by WCT and COD with mean values of 1.27cm, 1.28cm and 1.40cm respectively. Mean values of diameter of eye were presented in table 6. From selfed families, maximum mean diameter of eye were observed in family IIS₂-1 (2.01cm) with the range 1.83cm to 2.15cm and the minimum were observed in family IVS₂-1 (1.50cm) with range 1.25cm to 1.7cm. From sibmated families, maximum mean diameter were observed in family IBS₂-2 (2.08cm) with 1.55cm to 2.4cm and the minimum mean value was observed in family IIS₂-2 (1.35cm) with range 1.2cm to 1.9cm. The diameter of eye of COD, CGD and WCT were on par. The eye diameter was highly significantly higher in all the families except IVS₂-1, VS₂-1, IIS₂-2 and VS₂-2 which were having eye size on par with WCT, CGD and COD.

4.2.3.5. Fresh mature kernel weight

Among selfed families, the highest mean weight of kernel were observed in family IIS₂-1 (294.37g) with the range 287g to 358g and the lowest mean weight were observed in family IVS₂-1 (209.00g) with the range 155g to 232g. But in sibmated families observed the highest mean weight was recorded in family IBS₂-2 (296.50g) with the range 187g to 468g and the lowest mean weight were observed in family IIS₂-2 (208.33g) with range 135g to 310g. The weight of fresh mature kernel recorded the mean values of 267.67g, 190.40g and 186.22g in WCT, COD and CGD respectively (Table 6). The mature kernel weight of all the families were on par with that of WCT open pollinated. However, the kernel weight of the families IAS₂-1, IBS₂-1, IIS₂-1 among selfed and IBS₂-2, IIS₂-2 and IVS₂-2 among sibmated families were significantly higher to that of CGD at

Table 6: Mean and range of nut characters of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

Family	Size of nut (cm)			Wt. of unhusked nut (g)			Wt. of husked nut (g)			Diameter of eye (cm)		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Low	High		Low	High		Low	High		Low	High
IAS ₂ -1	37.52	31.40	46.05	537.04	360	725	318.08	250	430	1.73	1.30	2.10
IBS ₂ -1	40.26	33.49	48.15	560.33	353	825	358.25	223	550	1.92	1.54	2.25
IIS ₂ -1	42.86	37.68	48.46	683.25	535	818	384.50	343	430	2.01	1.83	2.15
IIIS ₂ -1	40.69	37.68	43.96	594.25	460	687	312.50	267	343	1.97	1.86	2.05
IVS ₂ -1	39.82	33.49	42.91	461.45	373	550	258.00	197	280	1.50	1.25	1.70
V _S -1	36.96	31.92	40.82	512.00	275	686	293.87	145	426	1.54	1.00	2.00
IAS ₂ -2	43.96	32.97	56.52	522.87	340	670	294.00	200	413	1.95	1.80	2.26
IBS ₂ -2	43.50	32.97	61.75	676.42	375	1300	364.62	230	650	2.08	1.55	2.40
IIS ₂ -2	43.44	31.40	47.10	492.83	320	616	302.67	240	340	1.35	1.20	1.90
IIIS ₂ -2	39.07	36.63	43.96	545.00	473	645	323.33	237	375	1.97	1.60	2.13
IVS ₂ -2	47.43	40.29	71.17	611.13	330	750	354.13	250	420	1.95	1.73	2.20
V _S -2	41.47	38.99	43.96	430.00	260	600	353.50	200	507	1.40	1.05	1.76
WCT	41.11	37.11	48.98	922.50	895	969	500.00	483	528	1.28	1.21	1.42
CGD	38.05	36.42	38.94	381.84	351	411	261.19	243	274	1.27	1.23	1.31
COD	40.11	36.42	42.70	376.72	365	390	243.19	217	284	1.40	1.25	1.60
C D 5%	NS			148.96			85.29			0.24		
C D 1%	NS			199.16			114.02			0.32		
C V	10.82			18.85			18.21			10.04		

Table 6 Continued.

Family	Wt. of mature kernel (g)			Kernel thickness (cm)			Copra content (g)			Oil content (%)			Volume of tender nut water (ml)		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Low	High		Low	High		Low	High		Low	High			
IAS ₂ -1	260.87	210	365	1.15	1.07	1.25	117.71	80.0	162.5	52.54	45.03	59.00	163.54	125	245
IBS ₂ -1	280.16	157	430	1.19	1.00	1.50	108.86	37.0	175.0	51.58	46.53	57.20	90.42	30	170
IIS ₂ -1	294.37	287	312	1.12	1.06	1.16	139.75	123.0	148.0	47.23	46.43	48.60	157.50	140	195
IIIS ₂ -1	253.25	227	282	1.16	1.13	1.26	141.25	132.0	153.0	51.42	42.67	59.20	115.00	95	150
IVS ₂ -1	209.00	155	232	1.08	0.90	1.20	105.56	65.0	130.0	44.25	43.34	47.65	131.67	115	150
V _S ₂ -1	223.31	90	342	1.10	0.90	1.30	100.06	57.5	142.0	46.88	36.90	56.03	163.75	71	355
IAS ₂ -2	233.37	160	308	1.07	0.90	1.16	117.60	52.5	155.0	52.94	41.60	56.40	125.62	35	160
IBS ₂ -2	296.50	187	468	1.13	0.95	1.30	128.02	73.0	170.0	44.86	39.23	58.80	152.08	85	225
IIS ₂ -2	208.33	135	310	1.07	1.00	1.13	93.78	57.5	136.6	47.26	44.53	52.20	82.83	55	95
IIIS ₂ -2	276.50	220	365	1.12	1.05	1.20	125.25	115.0	140.0	46.67	42.43	51.87	143.33	70	195
IVS ₂ -2	287.88	213	335	1.13	1.03	1.20	116.63	50.0	152.0	42.51	37.00	49.00	185.00	125	290
V _S ₂ -2	251.50	160	343	1.18	1.10	1.26	108.00	103.0	113.0	42.95	40.70	45.20	205.00	190	220
WCT	267.67	254	296	1.30	1.20	1.40	183.83	176.0	194.0	60.33	56.00	67.00	214.50	195	242
CGD	186.22	179	199	1.39	1.34	1.42	91.67	68.74	123.4	55.84	49.00	61.00	145.84	138	152
COD	190.40	159	198	1.38	1.26	1.52	123.59	112.0	141.0	58.59	54.00	64.00	329.62	300	365
C D 5%	71.20			0.09			31.58			5.53			61.26		
C D 1%	95.19			0.13			42.22			7.40			81.90		
C V	19.97			5.87			18.56			7.80			26.77		

5% level of probability. These families were also superior to COD in fresh kernel weight except the family IAS₂-1. All other families were observed on par.

4.2.3.6. Kernel thickness

The mean values of fresh kernel thickness presented in table 6 showed that among selfed families, the maximum kernel thickness were observed in the family IBS₂-1 (1.19cm) with the range 1.0cm to 1.5cm and the minimum were observed in the family IVS₂-1 (1.08cm) with range 0.9cm to 1.2cm. But among the sibmated families, the maximum mean kernel thickness was observed in family VS₂-2 (1.18cm) with the range 1.1cm to 1.26cm and the minimum thickness were recorded in the family IAS₂-2 (1.07cm) with 0.9cm to 1.16cm. The open-pollinated WCT recorded the mean values of 1.30cm, the dwarf COD 1.38cm and CGD 1.39cm. The fresh kernel thickness of all the families was lower than WCT open pollinated, COD and CGD and found to be negatively significant difference.

4.2.3.7. Copra content

The mean values of copra content recorded in open-pollinated WCT were 183.83g, COD was 123.59g and CGD was 91.67g (Table 6). The highest mean copra content per nut were recorded in the family IIS₂-1 (141.25g) with the range 132g to 153g and the lowest mean copra weight was observed in the family VS₂-1 (100.06g) with range 57.5g to 142g among selfed families. Among sibmated families, the highest mean copra content per nut were obtained in family IBS₂-2 (128.02g) with range 115g to 140g and the lowest mean copra content were observed in family IIS₂-2 (93.78g) with the range 57.5g to 136.6g. None of the families produced copra to the tune of open pollinated WCT and the copra content was significantly less than that of open pollinated WCT. Similarly all the families were on par with copra content with COD. However the copra content of the selfed family IIS₂-1 and IIS₂-1 were highly significant than CGD copra content and the family IIS₂-2 was significantly superior at 5% level of probability.

4.2.3.8. Oil content



Plate 3: Performance of nut characters in all the 12 families including the control



Plate 4: Unopened nuts produced in the families of selfed (below) and sibmated (above)

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From selfed families, the maximum mean oil content were observed in family IAS₂-1 (52.54 %) with 45.03 to 59.0 % and the minimum mean oil content observed in family IVS₂-1 (44.25 %) with range 43.34 to 47.65 % (Table 6). From sibmated families, the maximum mean oil content were observed from family IAS₂-2 (52.94 %) with the range 41.60 to 56.40 % and the minimum mean oil content was observed from family IVS₂-2 (42.51 %) with the range 37 to 49 %. The WCT, COD and CGD recorded the mean values of 60.33 %, 55.84 % and 58.59 % respectively. The oil content was significantly lower than WCT open pollinate and COD in all the selfed and sibmated families. However, the oil content of the coconut in the families IAS₂-1, IBS₂-1, IIS₂-1 and IAS₂-2 were on par with the oil content of CGD.

4.2.3.9. Volume of tender nut water

Mean values of volume of tender nut water were presented in table 6 showed that the maximum mean volume of tender nut water were obtained from the family VS₂-1 (163.75ml) with range 71ml to 355ml and the minimum mean tender nut water were obtained from the family IBS₂-1 (90.42ml) with the range 30ml to 170ml among the selfed families. As well in sibmated families, the maximum volume of mean tender nut water were recorded from the family VS₂-2 (205.00ml) with the range 190ml to 220ml and the minimum mean volume were obtained from the family IIS₂-2 (82.83ml) with the range 55ml to 95ml. The volume of tender nut water observed in WCT was 214.50ml, while in dwarfs of COD was 329.62ml and CGD was 145.84ml. none of the families produced tender nut water equal or on par with WCT and COD all the tender nut water quantity obtained from the different families were negatively significant to the quantity produced by COD. Even volume of tender nut water produced by IBS₂-1, IIS₂-2 was not on par with the CGD. It was significantly lower than CGD.

4.3. INBREEDING DEPRESSION OF SECOND GENERATION WCT PALMS

Inbreeding depression was studied for the selfed and sibmated families of second generation palms. The results are given in the table (7 to 9) and the percentage of inbreeding depression observed for various vegetative, reproductive and nut characters are presented below.

4.3.1. Inbreeding depression of vegetative characters

4.3.1.1. Height of the palm

All the families of selfed and sibmated S_2 palms recorded significant positive inbreeding depression for this trait where as in general highest inbreeding depression was expressed in selfed palms in contrast to sibmated palm. Table 7 shows that highest percentage of inbreeding depression recorded in selfed family was IIS₂₋₁ (35.73 %) followed by VS₂₋₁ (25.46 %), IVS₂₋₁ (23.63 %), IBS₂₋₁ (16.26 %), IIS₂₋₁ (16.19 %) and IAS₂₋₁ (15.25 %).

In sibmated S_2 palms, highest inbreeding depression was observed for height of palms in family IIS₂₋₂ (26.26 %) followed by IBS₂₋₂ (20.04 %), VS₂₋₂ (19.97 %), IIS₂₋₂ (17.39 %), IAS₂₋₂ (4.10 %) and IVS₂₋₂ (1.32 %). In general the S_2 palms of group V expressed high in percentage of positive inbreeding depression when selfed as well as sibmated.

4.3.1.2. Girth of the palm (cm)

As in palm height, highest inbreeding depression was observed in selfed than sibmated palms (Table 7). In selfed, positive inbreeding depression was recorded in VS₂₋₁ (12.00 %) and IIS₂₋₁ (7.62 %) whereas negative inbreeding depression in IBS₂₋₁ (-7.13 %), IAS₂₋₁ (-3.29 %), IVS₂₋₁ (-2.85 %) and IIS₂₋₁ (-1.78 %) for girth of palm.

In sibmated S_2 family positive inbreeding depression was seen in IBS₂₋₂ (3.34 %), VS₂₋₂ (2.92 %), IIS₂₋₂ (1.04 %) and negative inbreeding depression in IIS₂₋₂ (-7.04 %), IAS₂₋₂ (-2.92 %), IVS₂₋₂ (-1.99 %) for the trait. The family IIS₂₋₁ and VS₂₋₁ of self-pollinated and IIS₂₋₂, VS₂₋₂ and a coloured type IBS₂₋₂ of sibmated families expressed positive inbreeding depression. The IAS₂, IBS₂,

Table 7: Inbreeding depression in vegetative characters of selfed and sibmated WCT coconut, percentage (%)

Characters	Selfed					Sibmated				
	IAS ₂ -1	IBS ₂ -1	IIS ₂ -1	IVS ₂ -1	VS ₂ -1	IAS ₂ -2	IBS ₂ -2	IIS ₂ -2	IVS ₂ -2	VS ₂ -2
Palm height	15.25*	16.26*	35.73*	23.63*	25.46*	4.10	20.04*	17.39*	1.32	19.97*
Girth	-3.29	-7.13*	7.62*	-2.85	12.00*	-2.92	3.34	1.04	-1.99	2.92
Internodal length	4.60	5.42	3.35	7.98	17.95*	-1.23	11.02*	1.63	20.25*	9.91*
Number of leaves	60.61*	50.39*	42.76*	52.41*	58.25*	55.89*	35.74*	51.52*	56.68*	35.35*
No. of leaves/annum	26.74*	15.72	16.98	14.45	29.25*	19.81	8.81	16.98	23.26*	20.75*
No. of leaflets / leaf	33.64*	30.65*	36.86*	44.16*	30.17*	28.95*	39.36*	29.32*	37.58*	26.91*
Petiole length	36.73*	32.03*	30.58*	45.53*	34.81*	28.56*	27.13*	30.58*	31.16*	28.42*
Leaf length	35.92	31.80	30.83	40.05	40.05	34.22	32.52	31.55	35.68	36.89

* Significant at 5% level of probability

IIS₂ and IVS₂ had expressed negative inbreeding depression on selfing as well as on sibmating. But the orange coloured type IAS₂ expressed differently when selfed and sibmated that was negative inbreeding depression on selfing and positive when sibmating.

4.3.1.3. Internodal length

Positive inbreeding depression was recorded for selfed (S₂) palms in IVS₂-1 (25.28 %), VS₂-1 (17.95 %), IIS₂-1 (7.98 %), IBS₂-1 (5.42 %), IAS₂-1 (4.60 %) and IIS₂-1 (3.35 %) for internodal length (Table 7).

In sibmated (S₂) palms, positive inbreeding depression was seen in IVS₂-2 (20.25 %), IIS₂-2 (16.56 %), IBS₂-2 (11.02 %), VS₂-2 (9.91 %), IIS₂-2 (1.63 %) and negative inbreeding depression in IAS₂-2 (-1.23 %) for this vegetative character. The family IVS₂ expressed highest inbreeding depression in both selfing and sibmating. This was negative in IAS₂ on sibmating.

4.3.1.4. Number of functional leaves

Number of functional leaves showed significant positive inbreeding depression in all the families of selfed and sibmated (S₂) palms. Selfed (S₂) palms showed high inbreeding depression than the sibmated palms. The family IAS₂-1 (60.61 %) recorded highest inbreeding depression followed by the VS₂-1 (58.25 %), IVS₂-1 (52.41 %), IIS₂-1 (50.84 %), IBS₂-1 (50.39 %) and IIS₂-1 (42.76 %) in selfed palms (Table 7).

In sibmated (S₂) palms highest inbreeding depression was observed in IVS₂-2 (56.68 %) followed by IAS₂-2 (55.89 %), IIS₂-2 (51.52 %), IIS₂-2 (42.54 %), IBS₂-2 (35.74 %) and VS₂-2 (35.35 %) for the number of functional leaves.

4.3.1.5. Number of leaves per annum

Positive inbreeding depression was recorded for all the families of selfed and sibmated (S₂) palms for this trait. It is evident from Table 7 that the sibmated families showed highest inbreeding depression than selfed palms. In

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selfed, highest inbreeding depression was observed for VS₂₋₁ (29.25 %) followed by IAS₂₋₁ (26.74 %), IIS₂₋₁ (22.64 %), IIS₂₋₁ (16.98 %), IBS₂₋₁ (15.72 %) and IVS₂₋₁ (14.45 %).

Highest inbreeding depression was noted in IVS₂₋₂ (23.26 %) in sibmated palms followed by VS₂₋₂ (20.75 %), IAS₂₋₂ (19.81 %), IIS₂₋₂ (16.98 %), IIS₂₋₂ (15.09 %) and IBS₂₋₂ (8.81 %) for the number of functional leaves produced.

4.3.1.6. Leaf length

Positive and high inbreeding depression was observed in all families of selfed and sibmated S₂ palms for leaf length. Highest inbreeding depression was observed in selfed than sibmated families. In selfed, highest inbreeding depression was observed for IVS₂₋₁ (47.82 %) followed by VS₂₋₁ (40.05 %), IIS₂₋₁ (40.05 %), IAS₂₋₁ (35.92 %), IBS₂₋₁ (31.80 %) and IIS₂₋₁ (30.83 %).

In sibmated families, VS₂₋₂ (36.89 %) showed highest inbreeding depression than IVS₂₋₂ (35.68 %), IAS₂₋₂ (34.22 %), IBS₂₋₂ (32.52 %), IIS₂₋₂ (31.55 %) and IIS₂₋₂ (23.06 %) for leaf length (Table 7).

4.3.1.7. Petiole length

All the families of selfed and sibmated S₂ palms showed significant positive inbreeding depression where as selfed families showed highest inbreeding depression than sibmated families. In selfed S₂ palms highest inbreeding depression was recorded in IVS₂₋₁ (45.53 %) followed by IIS₂₋₁ (37.09 %), IAS₂₋₁ (36.73 %), VS₂₋₁ (34.81 %), IBS₂₋₁ (32.03 %) and IIS₂₋₁ (30.58 %) for the length of petiole (Table 7).

In sibmated S₂ palms highest inbreeding depression was observed in IVS₂₋₂ (31.16%) followed by IIS₂₋₂ (30.58 %), IAS₂₋₂ (28.56 %), VS₂₋₂ (26.25 %), IBS₂₋₂ (27.13 %) and IIS₂₋₂ (26.25 %) for petiole length.

4.3.1.8. Number of leaflets per leaf

It is evident from Table 7 that the selfed and sibmated families showed positive inbreeding depression where highest inbreeding depression was observed in selfed families than sibmated families. In selfed families, highest inbreeding depression was recorded in IVS₂-1 (44.16 %) followed by IIS₂-1 (36.86 %), IAS₂-1 (33.64 %), IBS₂-1 (30.65 %), VS₂-1 (30.17 %) and IIS₂-1 (26.01 %) for the trait.

Among sibmated families highest inbreeding depression was observed in IBS₂-2 (39.36 %) followed by IVS₂-2 (37.58 %), IIS₂-2 (29.32 %), IAS₂-2 (28.95 %), VS₂-2 (26.91 %) and IIS₂-2 (24.38 %) for number of leaflets per leaf (Table 7).

4.3.2. Inbreeding depression of reproductive characters

4.3.2.1. Number of female flowers per inflorescence

Positive significant inbreeding depression was observed for this trait in all families. Very high inbreeding depression was observed in selfed family IVS₂-1 (81.95 %) followed by IAS₂-1 (49.89 %), IBS₂-1 (32.14 %), IIS₂-1 (29.58 %), VS₂-1 (26.16 %), and IIS₂-1 (11.42 %) for this character (Table 8).

Similarly in sibmated families, inbreeding depression was IAS₂-2 (45.47 %) followed by IIS₂-2 (31.84 %), IVS₂-2 (29.75 %), IBS₂-2 (27.96 %), IIS₂-2 (19.23 %), VS₂-2 (1.92 %) for number of female flowers produced per inflorescence.

4.3.2.2. Period of flowering phase (days)

4.3.2.2.1. Male phase

Positive inbreeding depression was observed for this trait except for family IIS₂-1 (Table 8). In selfed families highest positive inbreeding depression for the character was observed for IIS₂-1 (24.49 %) followed by IAS₂-1 (16.79 %), IVS₂-1 (15.51 %), IBS₂-1 (14.92 %) and VS₂-1 (4.88%) whereas the family IIS₂-1 expressed negative inbreeding depression of -0.48%.

Table 8: Inbreeding depression in reproductive characters of selfed and sibmated WCT coconut, percentage (%)

Characters	Selfed						Sibmated					
	IAS ₂ -1	IBS ₂ -1	IIS ₂ -1	IIIS ₂ -1	IVS ₂ -1	VS ₂ -1	IAS ₂ -2	IBS ₂ -2	IIS ₂ -2	IIIS ₂ -2	IVS ₂ -2	VS ₂ -2
No. of female flowers/ inflorescence	49.89*	32.14*	11.42*	29.58*	55.76*	26.16*	45.47*	27.96*	31.94*	19.23*	29.75*	1.92*
Period of male phase	16.79	14.92	24.49*	-0.48	15.51	4.88	7.72	-0.07	6.49	-0.55	2.14	5.74
Period of female phase	-2.92	-27.06	26.48	-0.60	-13.04	-37.11	-4.80	-31.18	29.33	-4.42	16.72	19.64
Setting percentage	12.96*	9.20*	-6.05	-32.59*	-14.09*	14.36*	-11.78*	-1.44	-3.45	6.99	1.25	6.06
Peduncle length	47.53*	45.60*	41.46*	39.25*	53.69*	39.91*	38.58*	42.35*	45.60*	36.44*	32.82*	37.92*
No. of spadices/ annum	32.82*	21.11*	25.14*	29.17*	25.91*	34.36*	29.94*	15.93	27.64*	25.34*	26.87*	27.83*
No. of yielding bunches	68.44*	65.42*	63.05*	48.63*	65.49*	54.95*	70.70*	60.42*	75.51*	58.44*	48.53*	57.21*
No. of nuts/ bunches	56.34*	37.92*	5.11	14.89	48.97*	32.18*	40.59*	27.82*	27.55*	26.63*	33.82*	13.91
Nut yield /annum	81.71*	71.66*	60.66*	49.60*	76.10*	67.78*	79.11*	65.12*	76.85*	62.02*	61.29*	48.01*

In sibmated families highest positive inbreeding depression for period of male phase was observed for IAS₂₋₂ (7.72 %) followed by IIS₂₋₂ (6.49 %), VS₂₋₂ (5.74 %), IVS₂₋₂ (2.14 %) and negative inbreeding depression was in IBS₂₋₂ (-0.07 %) and IIIS₂₋₂ (-0.55 %).

4.3.2.2.2. Female phase

Both positive and negative inbreeding depression was observed in different selfed and sibmated S₂ families. High positive inbreeding depression observed in selfed family IIS₂₋₁ was 26.48 %. In selfed, negative inbreeding depression was recorded in VS₂₋₁ (-37.11%) and IBS₂₋₁ (-27.06 %), IVS₂₋₁ (-13.04 %), IAS₂₋₁ (-2.92 %) and IIIS₂₋₁ (-0.60 %) for female phase of palm (Table 8).

Positive inbreeding depression for female phase was observed for sibmated families in IIS₂₋₂ (29.33 %), VS₂₋₂ (19.64 %), IVS₂₋₂ (16.72 %), while negative inbreeding depression was recorded in IBS₂₋₂ (-31.18 %), IAS₂₋₂ (-4.80 %) and IIIS₂₋₂ (-4.42 %).

4.3.2.3. Setting percentage

In selfed S₂ families positive inbreeding depression was recorded for VS₂₋₁ (14.36 %), IAS₂₋₁ (12.96 %), IBS₂₋₁ (9.20 %) and negative inbreeding depression was observed for IIS₂₋₁ (-6.05 %), IVS₂₋₁ (-14.09 %), IIIS₂₋₁ (-32.59 %) for setting percentage (Table 8).

Positive inbreeding depression for setting percentage was observed for sibmated families in IIIS₂₋₂ (6.99 %), VS₂₋₂ (6.06 %), IVS₂₋₂ (1.25 %), while negative inbreeding depression was recorded in IBS₂₋₂ (-1.44 %), IIS₂₋₂ (-3.45 %) and IAS₂₋₂ (-11.78 %).

4.3.2.4. Peduncle length (cm)

In all the families of selfed and sibmated S₂ palms high positive inbreeding depression were recorded for stalk length (Table 8). Selfed families showed high

inbreeding depression in IVS₂-1 (53.69 %) followed by IAS₂-1 (47.53 %), IBS₂-1 (45.60 %), IIS₂-1 (41.46 %), VS₂-1 (39.91 %), IIS₂-1 (39.25 %).

In sibmated families high inbreeding depression was recorded for IIS₂-2 (45.60 %) followed by IBS₂-2 (42.35 %), IAS₂-2 (38.58 %), VS₂-2 (37.92 %), IIS₂-2 (36.44 %) and IVS₂-2 (32.82 %) for the stalk length (Table 8).

4.3.2.5. *Number of spadices per annum*

All the families recorded positive inbreeding depression for the number of bunches produced per annum (Table 8) where in selfed families observed high inbreeding depression for VS₂-1 (34.36 %) followed by IAS₂-1 (32.82 %), IIS₂-1 (29.17 %), IVS₂-1 (25.91 %), IIS₂-1 (25.14 %) and IBS₂-1 (21.11 %).

In sibmated S₂ palms high inbreeding depression in IAS₂-2 (29.94 %) followed by VS₂-2 (27.83 %), IIS₂-2 (27.64 %), IVS₂-2 (26.87 %), IIS₂-2 (25.34 %) and IBS₂-2 (15.93 %) were recorded.

4.3.2.5.1. *Number of yielding bunches per annum*

Positive inbreeding depression was recorded for all the families of selfed and sibmated S₂ palms for this trait (Table 8). Sibmated families S₂ showed highest inbreeding depression than selfed palms. In selfed S₂ palms, highest inbreeding depression was observed for IAS₂-1 (68.44 %) followed by IVS₂-1 (65.49 %), IBS₂-1 (65.42 %), IIS₂-1 (63.05 %), VS₂-1 (54.95 %) and IIS₂-1 (48.63 %) in the production of productive bunches per year.

Highest inbreeding depression was noted in IIS₂-2 (75.51 %) in sibmated palms followed by IAS₂-2 (70.70 %), IBS₂-2 (60.42 %), IIS₂-2 (58.44 %), VS₂-2 (57.21 %) and IVS₂-2 (48.53 %) for the number of yielding bunches produced per annum.

4.3.2.6. *Number of nuts per bunches*

The inbreeding depression observed was positive for all the families where as selfed S₂ palms were showing highest inbreeding depression in comparison to

sibmated. In selfed highest inbreeding depression for the trait was observed in IAS₂₋₁ (56.34 %) followed by IVS₂₋₁ (48.97 %), IBS₂₋₁ (37.92 %), VS₂₋₁ (32.18 %), IIS₂₋₁ (14.89 %), IIS₂₋₁ (5.11 %) in producing number of nuts per bunch (Table 8).

In sibmated S₂ families highest inbreeding depression was seen in IAS₂₋₂ (40.59 %) followed by IVS₂₋₂ (33.82 %), IBS₂₋₂ (27.82 %), IIS₂₋₂ (27.55 %), IIS₂₋₂ (26.63 %) and VS₂₋₂ (13.91 %) for number of nuts per bunches.

4.3.2.7. Annual nut yield per palm

The selfed and sibmated S₂ families recorded highest positive inbreeding depression for nut yield per annum. In selfed S₂ families, IAS₂₋₁ (81.71 %) recorded highest inbreeding depression which followed by IVS₂₋₁ (76.10 %), IBS₂₋₁ (71.66 %), VS₂₋₁ (67.78 %), IIS₂₋₁ (60.66 %) and IIS₂₋₁ (49.60 %).

In sibmated, highest inbreeding depression was recorded in IAS₂₋₂ (79.11 %) followed by IIS₂₋₂ (76.86 %), IBS₂₋₂ (65.12 %), IIS₂₋₂ (62.02 %), IVS₂₋₂ (61.29 %) and VS₂₋₂ (48.01 %) for nut yield (Table 8).

4.3.3. Inbreeding depression of nut characters

4.3.3.1. Size of nut

In selfed S₂ families, moderately positive inbreeding depression was recorded in families VS₂₋₁ (10.09%), IAS₂₋₁ (8.74%), IVS₂₋₁ (3.13%), IBS₂₋₁ (2.07%) and IIS₂₋₁ (1.02%) whereas negative inbreeding depression were recorded from family IIS₂₋₁ (-4.26%) for the nut size (Table 9).

In sibmated families, positive inbreeding depression was observed in family IIS₂₋₂ (4.96 %). Negative inbreeding depression were observed from family IVS₂₋₂ (-15.37 %), IAS₂₋₂ (-6.93 %), IBS₂₋₂ (-5.81 %), IIS₂₋₂ (-5.66 %) and VS₂₋₂ (-0.88 %) for the size of nut.

4.3.3.2. Weight of unhusked nut

In both selfed and sibmated S_2 families recorded positive inbreeding depression for weight of unhusked nut (Table 9). In selfed families, highest inbreeding depression were observed from family IVS_{2-1} (49.98 %) followed by VS_{2-1} (44.50 %), IAS_{2-1} (41.78 %), IBS_{2-1} (39.26 %), IIS_{2-1} (35.58 %) and IIS_{2-1} (25.93 %).

In sibmated S_2 families, highest inbreeding depression was observed from family VS_{2-2} (53.39 %), IIS_{2-2} (46.58 %), IAS_{2-2} (43.32 %), IIS_{2-2} (40.92 %), IVS_{2-2} (33.75 %) and IBS_{2-2} (26.67 %) for unhusked nut weight.

4.3.3.3. *Weight of husked nut*

All the S_2 families of selfed and sibmated recorded positive inbreeding depression for this trait. In selfed families, highest inbreeding depression were observed from families IVS_{2-1} (48.40 %) followed by VS_{2-1} (41.22 %), IIS_{2-1} (37.50 %), IAS_{2-1} (36.38 %), IBS_{2-1} (28.15 %) and IIS_{2-1} (23.10 %) for the weight of husked nut.

In sibmated S_2 families, highest inbreeding depression were observed from the families IAS_{2-2} (41.20 %) followed by IIS_{2-2} (39.47 %), IIS_{2-2} (35.33 %), VS_{2-2} (29.30 %), IVS_{2-2} (29.17 %) and IBS_{2-2} (27.08 %) for this trait (Table 9).

4.3.3.4. *Diameter of eye*

Negative inbreeding depression was observed for diameter of eye from both the selfed and sibmated families (Table 9). In selfed families, highest inbreeding depression for the trait were observed from family IIS_{2-1} (-56.42 %) followed by IIS_{2-1} (-53.39 %), IBS_{2-1} (-49.11 %), IAS_{2-1} (-34.79 %), VS_{2-1} (-20.08 %) and IVS_{2-1} (-16.73 %).

In sibmated S_2 families, highest negative inbreeding depression were observed from families IBS_{2-2} (-61.48 %) followed by IIS_{2-2} (-53.70 %), IAS_{2-2} (-52.14 %), IVS_{2-2} (-51.75 %), VS_{2-2} (-9.10 %) and IIS_{2-2} (-5.06 %) for eye diameter in coconut.

Table 9: Inbreeding depression in nut characters of selfed and sibmated WCT coconut, percentage (%)

Family	Selfed families						Sibmated families					
	IAS ₂ -1	IBS ₂ -1	IIS ₂ -1	IIIS ₂ -1	IVS ₂ -1	VS ₂ -1	IAS ₂ -2	IBS ₂ -2	IIS ₂ -2	IIIS ₂ -2	IVS ₂ -2	VS ₂ -2
Size of nut	8.74*	2.07	-4.26*	1.02	3.13	10.09*	-6.93*	-5.81*	-5.66*	4.96*	-15.37*	-0.88
Weight of unhusked nut	41.78*	39.26*	25.93*	35.58*	49.98*	44.50*	43.32*	26.68*	46.58*	40.92*	33.75*	53.39*
Weight of husked nut	36.38*	28.15*	23.10*	37.50*	48.40*	41.23*	41.20*	27.08*	39.47*	35.33*	29.17*	29.30*
Diameter of eye	-34.79*	-49.11*	-56.42	-53.39*	-16.73*	-20.08*	-52.14*	-61.48*	-5.06	-53.70*	-51.75*	-9.11
Weight of fresh mature kernel	2.54	-4.67	-20.81*	5.39	21.92*	16.57*	12.81*	-10.77*	22.17*	-3.30	-7.55	6.04
Kernel thickness	11.85*	8.85*	14.08*	10.62*	16.54*	15.38*	17.62*	13.08*	17.31*	14.15*	13.00*	9.23*
Copra content	35.97*	40.78*	23.98*	23.16*	42.58*	45.57*	36.03*	37.43*	48.98*	31.87*	36.56*	41.25*
Oil content	12.91*	14.50*	21.72*	14.76*	26.66*	22.29*	12.25	25.64*	21.67*	22.65*	29.54*	28.81*
Volume of tender nut water	23.76*	57.85*	26.57*	46.39*	38.62*	23.66*	41.43*	29.10*	61.38*	33.18*	13.75*	4.43*

4.3.3.5. *Weight of fresh mature kernel*

In selfed S_2 families, positive inbreeding depression were observed from families IVS_{2-1} (21.92 %) followed by VS_{2-1} (16.57 %), IIS_{2-1} (5.39 %) and IAS_{2-1} (2.54 %). Negative inbreeding depression were observed from the family IIS_{2-1} (-20.81 %) and IBS_{2-1} (-4.67 %) for fresh mature kernel weight (Table 9).

In sibmated S_2 families, positive inbreeding depression were observed from families IIS_{2-2} (22.17 %) followed by IAS_{2-2} (12.81 %) and VS_{2-2} (6.04 %). Negative inbreeding depression were observed from family IBS_{2-2} (-10.77 %) followed by the family IVS_{2-2} (-7.55 %) and IIS_{2-2} (-3.30 %) for the trait.

4.3.3.6. *Kernel thickness at maturity*

The kernel thickness of coconut in all the selfed and sibmated S_2 families recorded positive inbreeding depression (Table 9). In selfed families, highest inbreeding depression were observed from families IVS_{2-1} (16.54 %) followed by VS_{2-1} (15.38 %), IIS_{2-1} (14.08 %), IAS_{2-1} (11.85 %), IIS_{2-1} (10.61 %) and IBS_{2-1} (8.85 %).

In sibmated families, highest inbreeding depression were observed from S_2 families IAS_{2-2} (17.61 %) followed by IIS_{2-2} (17.31 %), IIS_{2-2} (14.15 %), IBS_{2-2} (13.08 %), IVS_{2-2} (13.00 %) and VS_{2-2} (9.23 %) for this nut character.

4.3.3.7. *Copra content per nut*

Positive inbreeding depression was observed from both selfed and sibmated S_2 families for this trait. In selfed families, highest inbreeding depression for the trait were observed from families VS_{2-1} (45.57 %) followed by IVS_{2-1} (42.58 %), IBS_{2-1} (40.78 %), IAS_{2-1} (35.97 %), IIS_{2-1} (23.98 %) and IIS_{2-1} (23.16 %) for copra coconut.

In sibmated S_2 families, highest inbreeding depression were observed from families IIS_{2-2} (48.98 %) followed by VS_{2-2} (41.25 %), IBS_{2-2} (37.43 %), IVS_{2-2}

(36.56 %), IAS₂-2 (36.03 %) and IIS₂-2 (31.87 %) for the copra content per nut (Table 9).

4.3.3.8. Oil content

In both selfed and sibmated S₂ families, positive inbreeding depression were observed for the character oil content (Table 9). In selfed families, inbreeding depression were observed from families IVS₂-1 (26.66 %) followed by VS₂-1 (22.29 %), IIS₂-1 (21.72 %), IIS₂-1 (14.76 %), IBS₂-1 (14.50 %) and IAS₂-1 (12.91 %).

In sibmated S₂ families, highest inbreeding depression were observed from families IVS₂-2 (29.54 %) followed by VS₂-2 (28.81 %), IBS₂-2 (25.64 %), IIS₂-2 (22.65 %), IIS₂-2 (21.67 %) and IAS₂-2 (12.25 %) for percentage of oil content.

4.3.3.9. Volume of tender nut water

All the S₂ families of selfed and sibmated recorded positive inbreeding depression for the volume of water in tender nut. In selfed S₂ families, highest inbreeding depression were recorded from the families IBS₂-1 (57.85 %) followed by IIS₂-1 (46.39 %), IVS₂-1 (38.62 %), IIS₂-1 (26.57 %), IAS₂-1 (23.76 %) and VS₂-1 (23.66 %) for this trait (Table 9).

In sibmated S₂ families, highest inbreeding depression were observed from the families IIS₂-2 (61.38 %) followed by IAS₂-2 (41.43 %), IIS₂-2 (33.18 %), IBS₂-2 (29.10 %), IVS₂-2 (13.75 %) and VS₂-2 (4.43 %) for volume of tender nut water.

4.4. SEEDLINGS VARIABILITY IN THIRD GENERATION

4.4.1. Germination percentage

Each of the selected six selfed and six sibmated families of second generation WCT palms were self pollinated and the third generation selfed seed nuts were obtained. The germination percentage recorded for the seed nuts of the twelve families were differed in third generation. The highest percentage of

germination (Table 19) was recorded in IBS₃-1 with 82.35% followed by 71.95% in IAS₃-1, 68.08% in IIS₃-1, 50% in IIS₃-1, 45.88% in IVS₃-1 and lowest germination in VS₃-1 (44.44%). Similarly the seed nuts of second generation sibmated families recorded highest germination percentage of 66.67% in IBS₃-2 followed by VS₃-2 (63.89%) and 50% or less number of seed nuts germinated in IIS₃-2, IVS₃-2 (41.89%), IIS₃-2 (41.67%) and IAS₃-2 (41.66%).

Further the third generation selfed seedlings (S₃) from the twelve families of selfed and sibmated second generation WCT palms (S₂) were used for variability studies with respect to morphological characters viz., seedling height, collar girth and number of leaves. The seedling characters were grouped into three classes as low, medium and high values.

4.4.1. Seedling height

The mean seedling height ranged from 1.12m to 1.84m in all the selfed progenies of twelve families (Table 10). The seedlings were classified into short (\leq 50- 100 cm), medium (101-150 cm) and tall (151-200 cm) based on the total range of the values for seedling height. The table 10 shows that the frequency of occurrence of shortest seedlings was more in VS₃-1. The shortest seedlings were not found in IVS₃-2 and IBS₃-2. Further the sibmated family IBS₃-2 had produced only tall seedlings. Generally the frequency of medium tall seedlings was found to be more in the third generation. The maximum percentage of medium tall occurred in IIS₃-1 (75%), IVS₃-1 (74.36%) and IAS₃-1 (72.88%). The frequency of medium tall was very low in IBS₃-1 (21.43%) and VS₃-1 (25%). The availability of tall seedlings was comparatively lower than the medium tall but more than short seedlings. After IBS₃-2 (100%), the tall seedlings were recorded in IBS₃-1 (64.29%) and IVS₃-2 (54.84%). The tallest seedlings were very less in family IIS₃-2 followed by IIS₃-2 (8%) and VS₃-2 (8.69%).

4.4.2. Collar girth

The mean collar girth of seedlings in twelve families ranged from 13.13 cm to 16.52 cm in general. The occurrence of third generation WCT seedlings of

the twelve families were classified into three based on their values for collar girth at one year of age (Table 11). The three different classes for collar girth were less (≤ 5 to 15 cm), medium (15.1 to 20 cm) and high (20.1 to ≥ 25 cm). Majority of the seedlings had lower girth at collar with a maximum frequency of 51 (96.44 %) in IAS₃-1 family followed by 2S₃-2 with a frequency of 20 and 86.96 %. The frequency of low collar girth seedlings were found in the family VS₃-1 (frequency 1 and percentage 25%), followed by IBS₃-2 (frequency 6 and percentage 33.33%). The percentage of seedlings having medium collar girth was highest in VS₃-1 (75% with 3 frequency) followed by IVS₃-2 (frequency 19 and percentage 61.29%), IVS₃-1 (frequency 21 with 53.85%) and IIS₃-1 (frequency 17 with 53.12%).

However the frequency of occurrence of third generation seedlings with higher collar girth was very less (totally 11 seedlings). The eight families didn't produce seedlings with higher range of collar girth (20 to 25 cm). The family IAS₃-2 produced 25% of the seedlings with higher collar girth followed by IBS₃-2 (16.67%) and IIS₃-2 (8%). Only one seedling had the higher range of collar girth (20 to 25 cm) with a percentage of 1.69%.

4.4.3. Number of leaves produced

The number of leaves produced in seedlings ranged from 3.39 to 7.00. The mean, frequency and percentage of seedling with different range of leaves produced by the third generation seedlings of the twelve families are presented in table 12. The three different ranges of classification of leaves produced were ≥ 2 to 4, 5 to 7 and 8 to 10 numbers of leaves per seedlings. Generally the frequency and percentage of occurrence of medium range of leaves produced were more in all the twelve families. The highest frequency and percentage of leaves were found in IIS₃-1 (96.87%) with 31 seedlings, followed by VS₃-2 (91.30%) with 21 seedlings in medium range of leaf in the seedling (5 to 7 leaves). The lowest number of seedlings (43.48%) with a frequency of 10 seedlings was recorded in IIS₃-2. However the sibmated family of IVS₃-2 didn't have seedlings with 5 to 7

Table 10: Mean, frequency and percentage of seedling height in selfed third generation WCT coconut seedlings

Families	Short >50- 100 cm			Medium tall 101-150 cm			Tall 150-200< cm		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	85.64	07	11.86	128.09	43	72.88	158.44	09	15.25
IBS ₃ -1	79.17	06	14.29	121.00	09	21.43	184.41	27	64.29
IIS ₃ -1	95.00	02	22.22	128.80	05	55.55	177.00	02	22.22
IIIS ₃ -1	94.00	04	12.50	126.37	24	75.00	156.25	04	12.50
IVS ₃ -1	88.40	04	10.26	132.69	29	74.36	155.50	06	15.38
VS ₃ -1	75.00	02	50.00	142.00	01	25.00	154.00	01	25.00
IAS ₃ -2	89.23	03	15.00	120.90	10	50.00	169.57	07	35.00
IBS ₃ -2	-	-	-	-	-	-	183.89	18	100.0
IIS ₃ -2	80.70	06	26.09	124.44	16	69.56	157.00	01	4.35
IIIS ₃ -2	90.80	05	20.00	122.94	18	72.00	186.50	02	8.00
IVS ₃ -2	-	-	-	129.12	14	45.16	170.76	17	54.84
VS ₃ -2	95.83	06	26.09	123.00	15	65.22	166.00	02	8.69

Table 11: Mean, frequency and percentage of collar girth in selfed third generation WCT coconut seedlings

Families	Less >10- 15 cm			Medium high 15 – 20 cm			High 20 – 25< cm		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	13.24	51	86.44	16.42	07	11.86	24.00	01	1.69
IBS ₃ -1	13.37	31	73.81	16.64	11	26.19	-	-	-
IIS ₃ -1	12.49	07	77.77	17.50	02	22.22	-	-	-
IIIS ₃ -1	12.97	15	46.87	16.59	17	53.12	-	-	-
IVS ₃ -1	13.44	18	46.15	17.61	21	53.85	-	-	-
VS ₃ -1	10.20	01	25.00	16.13	03	75.00	-	-	-
IAS ₃ -2	11.38	11	55.00	17.75	04	20.00	22.98	05	25
IBS ₃ -2	14.03	06	33.33	17.01	09	50.00	20.00	3	16.67
IIS ₃ -2	12.75	20	86.96	15.63	03	13.04	-	-	-
IIIS ₃ -2	13.22	18	72.00	16.62	05	20.00	20.60	02	8.00
IVS ₃ -2	14.58	12	38.71	16.35	19	61.29	-	-	-
VS ₃ -2	12.92	15	65.22	15.75	08	34.78	-	-	-

Table 12: Mean, frequency and percentage of number of leaves in third generation WCT coconut seedlings

Families	Less >2 - 4			Medium 5 - 7			High 8 - 10<		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	4.00	03	5.08	6.28	43	72.88	8.31	13	22.03
IBS ₃ -1	3.82	11	26.19	5.45	31	73.81	-	-	-
IIS ₃ -1	4.00	03	33.33	5.83	06	66.67	-	-	-
IIIS ₃ -1	-	-	-	6.03	31	96.87	8.00	01	3.12
IVS ₃ -1	-	-	-	6.03	36	92.31	8.67	03	7.69
VS ₃ -1	3.00	01	25.00	6.33	03	75.00	-	-	-
IAS ₃ -2	4.00	01	5.00	5.82	17	85.00	8.50	02	10.00
IBS ₃ -2	4.00	01	5.55	5.86	14	77.78	8.00	03	16.67
IIS ₃ -2	3.85	13	56.52	5.20	10	43.48	-	-	-
IIIS ₃ -2	3.67	03	12.00	5.54	22	88.00	-	-	-
IVS ₃ -2	3.64	31	100.0	-	-	-	-	-	-
VS ₃ -2	4.00	01	4.35	7.05	21	91.30	9.00	01	4.35

Table 13: Mean, frequency and percentage of third leaf length in third generation WCT coconut seedlings

Families	Short 30-90 cm			Medium long 90-150 cm			Long 150-210 cm		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	71.56	16.00	27.12	119.95	43.00	72.88	-	-	-
IBS ₃ -1	75.04	9.00	20.45	113.77	21.00	47.73	173.50	12.00	27.27
IIS ₃ -1	82.50	4.00	44.44	124.50	4.00	44.44	161.00	1.00	11.11
IIIS ₃ -1	78.57	6.00	18.18	116.52	23.00	69.70	166.33	3.00	9.09
IVS ₃ -1	81.78	6.00	15.38	122.47	32.00	82.05	161.00	1.00	2.56
VS ₃ -1	64.50	2.00	50.00	128.50	2.00	50.00	-	-	-
IAS ₃ -2	70.00	1.00	5.00	114.96	13.00	65.00	169.83	6.00	30.00
IBS ₃ -2	76.40	5.00	27.78	119.37	8.00	44.44	169.60	5.00	27.78
IIS ₃ -2	58.51	11.00	42.31	109.75	12.00	46.15	-	-	-
IIIS ₃ -2	70.50	4.00	16.00	109.79	19.00	76.00	165.00	2.00	8.00
IVS ₃ -2	88.00	4.00	12.50	120.10	20.00	62.50	176.50	2.00	6.25
VS ₃ -2	87.83	3.00	13.04	118.67	18.00	78.26	166.00	2.00	8.70

Table 14: Mean and frequency of third leaf breadth for second generation WCT coconut seedlings

Families	Short 7-17			Medium long 17-27			High 27-37		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	14.98	20.00	33.90	20.28	36.00	61.02	31.17	3.00	5.08
IBS ₃ -1	15.38	25.00	56.82	18.73	17.00	40.48	-	-	-
IIS ₃ -1	14.83	3.00	33.33	20.05	6.00	66.67	-	-	-
IIIS ₃ -1	16.37	4.00	12.12	21.97	27.00	84.38	28.20	1.00	3.03
IVS ₃ -1	14.62	6.00	15.38	22.56	27.00	69.23	29.12	6.00	15.38
VS ₃ -1	8.00	1.00	25.00	18.00	1.00	25.00	33.50	2.00	50.00
IAS ₃ -2	14.49	16.00	80.00	18.32	4.00	20.00	-	-	-
IBS ₃ -2	-	-	-	22.72	17.00	94.44	29.00	1.00	5.56
IIS ₃ -2	13.94	8.00	30.77	21.78	13.00	56.52	28.35	2.00	7.69
IIIS ₃ -2	13.43	7.00	28.00	19.52	17.00	68.00	32.00	1.00	4.00
IVS ₃ -2	16.32	11.00	34.38	18.53	15.00	57.69	-	-	-
VS ₃ -2	15.33	3.00	13.04	23.67	15.00	65.22	29.44	5.00	21.74

Table 15: Mean and frequency of third leaf petiole length for second generation WCT coconut seedlings

Families	Short 10-30			Medium long 30-60			Long 60-90		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	25.00	4.00	6.78	49.59	44	74.58	67.18	11.00	18.64
IBS ₃ -1	23.70	4.00	9.09	46.75	21.00	47.73	78.96	17.00	38.64
IIS ₃ -1	-	-	-	42.43	7.00	77.78	74.50	2.00	22.22
IIIS ₃ -1	-	-	-	45.11	25.00	75.76	63.34	7.00	21.21
IVS ₃ -1	29.00	1.00	2.56	47.44	35.00	89.74	62.83	3.00	7.69
VS ₃ -1	20.50	2.00	50.00	59.00	2.00	50.00	-	-	-
IAS ₃ -2	-	-	-	49.11	9	45.00	76.45	11.00	55.00
IBS ₃ -2	-	-	-	53.67	3.00	16.67	73.00	15.00	83.33
IIS ₃ -2	22.00	6.00	23.08	40.85	17.00	65.38	-	-	-
IIIS ₃ -2	28.00	1.00	4.00	49.30	20.00	80.00	77.75	4.00	16.00
IVS ₃ -2	-	-	-	50.19	16.00	50.00	73.80	10.00	31.25
VS ₃ -2	27.00	1.00	4.35	45.66	17.00	73.91	75.40	5.00	21.74

Table 16: Mean, frequency and percentage of fifth leaf length in third generation WCT coconut seedlings

Families	Short 25-75			Medium long 75-125			Long 125-175		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	57.26	42.00	71.19	91.50	13.00	22.03	-	-	-
IBS ₃ -1	57.79	9.00	20.45	93.18	17.00	38.64	-	-	-
IIS ₃ -1	60.25	4.00	44.44	84.50	2.00	22.22	-	-	-
IIIS ₃ -1	57.07	16.00	48.48	88.86	16.00	48.48	-	-	-
IVS ₃ -1	62.99	15.00	38.46	85.75	21.00	53.85	142.50	2.00	5.13
VS ₃ -1	70.50	2.00	50.00	78.00	1.00	25.00	-	-	-
IAS ₃ -2	62.00	1.00	5.00	144.57	7	35.00	-	-	-
IBS ₃ -2	58.5	6	33.33	93.80	10.00	55.56	142.00	1.00	5.56
IIS ₃ -2	54.12	8.00	30.77	86.50	4.00	15.38	-	-	-
IIIS ₃ -2	53.73	15.00	60.00	85.33	6.00	24.00	-	-	-
IVS ₃ -2	-	-	-	-	-	-	-	-	-
VS ₃ -2	69.60	5.00	21.74	91.81	16.00	69.57	142.00	1.00	4.35

Table 17: Mean and frequency of fifth leaf breadth for second generation WCT coconut seedlings

Families	Short 05-13			Medium long 13-21			Long 21-29		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	11.24	28.00	47.46	15.91	26.00	44.07	25.00	1.00	1.69
IBS ₃ -1	12.88	5.00	11.36	15.37	21.00	47.73	-	-	-
IIS ₃ -1	10.50	2.00	22.22	16.87	4.00	44.44	-	-	-
IIIS ₃ -1	11.22	8.00	24.24	17.52	22.00	66.67	26.00	2.00	6.06
IVS ₃ -1	11.63	7.00	17.95	17.06	26.00	66.67	23.46	5.00	12.82
VS ₃ -1	15.00	1.00	25.00	16.25	2.00	50.00	-	-	-
IAS ₃ -2	12.20	6.00	30.00	14.60	14.00	70.00	-	-	-
IBS ₃ -2	12.00	4.00	22.22	17.39	9.00	50.00	22.62	4.00	22.22
IIS ₃ -2	11.34	5.00	19.23	16.88	7.00	26.92	-	-	-
IIIS ₃ -2	10.65	10.00	40.00	15.25	10.00	40.00	24.50	1.00	4.00
IVS ₃ -2	-	-	-	-	-	-	-	-	-
VS ₃ -2	11.08	5.00	21.74	17.78	10.00	43.48	23.71	7.00	30.43

Table 18: Mean and frequency fifth leaf petiole length for second generation WCT coconut seedlings

Families	Short 10-35			Medium long 35-60			Long 60-85		
	Mean	F	%	Mean	F	%	Mean	F	%
IAS ₃ -1	26.63	38.00	64.41	42.29	17.00	28.81	-	-	-
IBS ₃ -1	27.30	10.00	22.73	45.12	16.00	36.36	-	-	-
IIS ₃ -1	26.80	5.00	55.56	38.00	1.00	11.11	-	-	-
IIIS ₃ -1	25.13	23.00	69.70	39.02	9.00	27.27	-	-	-
IVS ₃ -1	28.07	27.00	69.23	40.50	10.00	25.64	76.00	1.00	2.56
VS ₃ -1	24.50	2.00	50.00	36.00	1.00	25.00	-	-	-
IAS ₃ -2	31.33	3.00	15.00	47.89	9.00	45.00	68.75	8.00	40
IBS ₃ -2	33.50	2.00	11.11	46.73	15.00	83.33	-	-	-
IIS ₃ -2	21.04	11.00	42.31	37.00	1.00	3.85	-	-	-
IIIS ₃ -2	26.72	16.00	64.00	38.80	5.00	20.00	-	-	-
IVS ₃ -2	-	-	-	-	-	-	-	-	-
VS ₃ -2	27.35	13.00	56.52	43.14	7.00	30.43	69.75	2.00	8.70

leaves. Again the family IVS₃-2 didn't produce any seedling with higher number of leaves i.e., 8 to 10 nos. So, it had all the seedlings with only less number of leaves. About 56.52% (with a frequency of 13 seedlings) of the family IIS₃-1 had less number of leaves. The selfed third generation family viz., IIS₃-1 and IVS₃-1 didn't have seedlings with less number of leaves (≥ 2 to 4 leaves). There were six families with higher number of leaves with a maximum frequency or percentage (22.03%) in family IAS₃-1 followed by IBS₃-2 (16.67%). The family viz., IBS₃-1, IIS₃-1, IIS₃-2, IIS₃-2, IVS₃-2 and VS₃-1 didn't have any seedlings with higher range of leaves.

4.4.4. Number of split leaves

The number of split leaves present in selfed third generation seedlings of selfed and sibmated families together ranged from 1 to 3 leaves per seedlings. The split leaves were seen mostly in all the selfed seedlings of the six selfed families, but only one sibmated family could produce split leaves in IAS₃-2 with a mean of numbers 2.38 (split leaves). The lowest number of split leaf per seedling is only one which was recorded in IIS₃-1. The maximum number of split leaves of 3 was produced in two families i.e., IAS₃-1 and VS₃-1. Medium value of 2 was produced in the families IBS₃-1, IIS₃-1 and IVS₃-1.

4.4.5. Leaf length of third leaf

The mean leaf length of third leaf ranged from 85 cm to 129 cm in all the selfed progenies of twelve WCT families but the open-pollinated WCT measured 147cm. The third leaf were classified into short (≤ 30 - 90 cm), medium (91-150 cm) and long (151-210 cm) based on the total range of the values recorded for third leaf length. The table 13 shows that the frequency of occurrence of short leaf was more in IAS₃-1 followed by the IIS₃-2. Further the family IAS₃-1, VS₃-1 and IIS₃-2 had not produced the shorter third leaf. Generally the frequency of medium long leaf was more in the third generation. The maximum percentage of medium long leaf occurred in IVS₃-1 (82%), VS₃-2 (74.26%) and IAS₃-1 (72.88%). The frequency of medium long leaf length was very low in

IBS₃-2 (44.44%) and IIS₃-1 (44.44%). The availability of long third leaf was comparatively lower than the medium long and short third leaf length. The long leaf was recorded in IAS₃-2 (30%), IBS₃-1 (27.27 %) and IBS₃-2 (27.78 %). The longer leaf were very less in family IVS₃-1 (2.56%) followed by IVS₃-2 (6.25%).

4.4.6. Leaf breadth of third leaf

The mean leaf breadth of third leaf in twelve families ranged from 16.74 cm to 23.39 cm in general. The occurrence of third generation WCT seedlings of the twelve families were classified into three based on their values for leaf breadth of third leaf at one year of age (Table 14). The three different classes for leaf breadth were short (≤ 7 to 17 cm), medium (17.1 to 27 cm) and long (27.1 to ≥ 37 cm). Majority of the seedlings had shorter breadth of third leaf with a maximum frequency of 25 (56.82 %) in IBS₃-1 family followed by IAS₃-1 with a frequency of 20 and 33.90 %. The minimum frequency of short breadth of third leaf were found in the family VS₃-1 (frequency 1 and percentage 25%), followed by IIS₃-1 (frequency 3 and percentage 33.33 %) and VS₃-2 (frequency 3 and percentage 13.04 %). The percentage of seedlings having medium leaf breadth was highest in IBS₃-2 (94.44% with 17 frequency) followed by IIS₃-1 (frequency 27 and percentage 84.38%), IVS₃-1 (frequency 27 with 69.23 %) and IIS₃-2 (frequency 17 with 68 %). However the frequency of occurrence of third generation seedlings with longer leaf breadth was very less (totally 21 seedlings). The four families didn't produce seedlings with higher range of leaf breadth (27 to 37 cm). The family 5S₃-1 produced 50% of the seedlings with longer leaf breadth followed by VS₃-2 (16.67%) and IVS₃-2 (15.38%). The lesser leaf breadth (20 to 25 cm) was observed in 3.03 % (frequency 1) of seedlings.

4.4.7. Petiole length of third leaf

The maximum petiole length of 69.78 cm was recorded in IBS₃-2 followed by 64.15 cm in IAS₃-2. The shortest petiole was found in IIS₃-2 (35.93 cm). However, the WCT open pollinated seedlings produced longer third leaf petiole (79.0 cm). The mean values of petiole length of third generation seedlings were

grouped into short (10 – 30 cm), medium long (31 – 60 cm) and long (61 – 90 cm) (Table 15). The frequency of shortest petiole bearing seedling were more in VS₃₋₁ (50 %) followed by IIS₃₋₂ (23.08 %) and both the families didn't produce seedlings with long petioles. Similarly the families IAS₃₋₂, IBS₃₋₂, IIS₃₋₁, IIS₃₋₁ and IVS₃₋₂ didn't produce any short petioled third leaves in third generation seedlings. The medium long petiole was more in all the third generation selfed seedlings with a maximum frequency in IVS₃₋₁ and IIS₃₋₁ with 89.74 % and 80 % respectively. The longest petioles were produced by IBS₃₋₂ (83.3 %).

4.4.8. Leaf length of fifth leaf

The leaf length of fifth leaf ranged with a mean value of 63 cm to 113 cm in all the selfed progenies of twelve WCT families but the open-pollinated WCT measured 129 cm (Table 16). The fifth leaf were classified into short ($\leq 25-75$ cm), medium (76-125 cm) and long (126-175 cm) based on the total range of the values recorded for leaf length. The variability shows that the frequency of occurrence of short leaf was more in IAS₃₋₁ followed by the IIS₃₋₂. Further the family IVS₃₋₁ had not produced the short third leaf in third generation. Generally the frequency of medium long leaf length was more in the third generation. The maximum percentage of medium long leaf occurred in VS₃₋₂ (69.57%), IBS₃₋₂ (55.56%) and IVS₃₋₁ (53.85%). The frequency of medium long leaf length was very low in IIS₃₋₂ (15.38%) and IAS₃₋₁ (22.03%). The availability of long third leaf was comparatively lower than the medium long and short third leaf length. The long leaf were recorded in IBS₃₋₂ (5.56%), IVS₃₋₁ (5.13 %) and VS₃₋₂ (4.35 %) and not produced in other families.

4.4.9. Leaf breadth of fifth leaf

The mean leaf breadth of fifth leaf in twelve families ranged from 12.50 cm to 18.15 cm in general. The occurrence of third generation WCT seedlings of the twelve families were classified into three based on their values for leaf breadth of fifth leaf at one year of age (Table 17). The three different classes for leaf breadth were small (≤ 5 to 13 cm), medium (14 to 21 cm) and long (22 to

≥ 29 cm). Majority of the seedlings had smaller breadth of fifth leaf with a maximum frequency of 28 (47.46 %) in IAS₃₋₁ family followed by IIS₃₋₂ with a frequency of 10 and 40 %. The minimum frequency of small breadth of fifth leaf were found in the family IBS₃₋₁ (frequency 5 and percentage 11%), followed by IVS₃₋₁ (frequency 7 and percentage 17.95%) and IIS₃₋₂ (frequency 5 and percentage 19.23 %). The percentage of seedlings having medium leaf breadth was highest in IAS₃₋₂ (70 % with 14 frequency) followed by IIS₃₋₁ (frequency 27 and percentage 84.38%), IVS₃₋₁ (frequency 27 with 69.23 %) and IIS₃₋₂ (frequency 22 with 66.67 %) and IVS₃₋₁ (frequency 26 with 66.67 %). However the frequency of occurrence of third generation seedlings with longer leaf breadth was very less (totally 20 seedlings). The six families didn't produce seedlings with higher range of leaf breadth (27 to 37 cm). The family 5S₃₋₂ produced 30.43% of the seedlings with longer leaf breadth followed by IBS₃₋₂ (22.22%) and IVS₃₋₁ (12.82%). The lesser leaf breadth (20 to 25 cm) was observed in 1.69 % percentage (frequency 1) seedlings.

4.4.10. Petiole length of fifth leaf

The mean range of petiole length of seedlings varied from 22.38cm to 56.20cm. The longer petiole length was found in IBS₃₋₁ (33.50 cm). However, the WCT open pollinated seedlings produced longer third leaf petiole (68.76 cm). The mean values of petiole length of third generation seedlings were grouped into short (10–35 cm), medium long (36–60 cm) and long (61–85 cm). The frequency of shortest petiole bearing seedling were more in IAS₃₋₁ (64.41 %), IVS₃₋₁ (69.23 %) and IIS₃₋₁ (69.70%) (Table 18). Similarly the families IVS₃₋₂ didn't produce any short petiole third leaf in third generation seedlings. The medium long petiole was more in all the third generation seedlings with a maximum percentage in IBS₃₋₂ and IAS₃₋₂ with 83.33 % and 45 % respectively. The lesser percentage of medium long petioles were produced by IIS₃₋₂ (3.85 %) and IIS₃₋₁ (11.11) with one frequency. The availability of long petiole length was comparatively lower than the medium long and short third leaf petiole length. The long petiole were

recorded in IAS₃-2 (40%), VS₃-2 (8.70 %) and IVS₃-2 (2.56 %) and not produced in other families.

4.5. INBREEDING DEPRESSION OF S₃ SEEDLINGS

The inbreeding depression were analysed for vegetative characters in third generation (S₃) seedlings on self-pollination of twelve families of selfed and sibmated second generation WCT coconut palms (Table 20). The third generation seedlings of self-pollinated WCT coconut palms of second generation were used for studying inbreeding depression on various vegetative characters viz., seedling height, collar girth, number of leaves, split leaves and third leaf length, breadth and petiole length as well as fifth leaf length, breadth and petiole length with wide range of variation.

4.5.1. Seedling height

All the families expressed the positive inbreeding depressions in third generation (S₃) seedling height. The seedling height showed high positive inbreeding depression in the selfed family VS₃-1 (41.01%), IAS₃-1 (32.22 %), IIS₃-1 (30.35 %), IVS₃-1 (30.34 %) and IIS₃-1 (30.16 %). The family IBS₃-1 (2.70 %) exhibited low positive inbreeding depression.

The sibmated families on selfing expressed positive inbreeding depressions at third generation (S₃). The observed inbreeding depression in the different families was IIS₃-2 (39.45 %), IIS₃-2 (35.66 %), VS₃-2 (34.62 %), IAS₃-2 (29.53 %) and IVS₃-2 (16.16 %). As in selfed S₃ generation the selfing sibmated family IBS₃-2 (2.70 %) lead to positive inbreeding depression.

4.5.2. Collar girth

The table '20' showed that both positive and negative inbreeding depressions were expressed for collar girth of seedlings in third generation. The positive inbreeding depression was recorded in the family IIS₃-1 (8.54 %), IAS₃-1 (7.20 %) and VS₃-1 (1.48 %). Negative inbreeding depression were observed from the families IBS₃-1 (-11.06 %), IVS₃-1 (-5.48) and IIS₃-1 (-0.16 %).

The highest positive inbreeding depression were recorded in the family IIS₃-2 (11.73 %) followed by VS₃-2 (6.49 %) and IIS₃-2 (2.54 %), while the negative inbreeding depression in family IBS₃-2 (-11.06 %), IVS₃-2 (-6.10 %) and IAS₃-2 (-5.95 %).

4.5.3. Number of leaves

With regard to the number of leaves produced by the seedlings of third generation observed both positive and negative inbreeding depressions. High positive inbreeding depression was seen in the family IIS₃-1 (8.06 %) and VS₃-1 (.17 %). Negative inbreeding depressions were observed in the family IAS₃-1 (-15.78 %), IVS₃-1 (-9.70 %), IBS₃-1 (-7.59 %) and IIS₃-1 (-7.28 %).

More or less high positive inbreeding depressions recorded in the families IVS₃-2 (40.37 %), IIS₃-2 (21.83 %) and IVS₃-2 (6.34 %). The inbreeding depressions were negative in three sibmated families, VS₃-2 (-23.24 %), IBS₃-2 (-7.59 %), and IVS₃-2 (-5.63 %).

4.5.4. Third leaf length

Only positive inbreeding depressions were observed for third leaf length. The positive high inbreeding depression was observed from the family VS₃-1 (34.35 %), IAS₃-1 (27.33 %), IIS₃-1 (25.25 %), IIS₃-1 (22.40 %), IVS₃-1 (20.27 %) and lowest in family IBS₃-1 (17.42 %).

Sibmated families also showed only positive inbreeding depression. Highest positive inbreeding depression was observed in the family IIS₃-2 (42.01 %) followed by IIS₃-2 (26.59 %), VS₃-2 (24.19 %), and lowest inbreeding depression was noticed in IVS₃-2 (18.71 %), IBS₃-2 (17.42) and IAS₃-2 (12.13%).

Table 19: Mean values of germination ability and morphological characters of nursery seedlings of selfed third generation WCT families

Families	Germination %	Plant height (m)	collar girth (cm)	No. of leaves	No. of split leaves	3rd leaf			5th leaf			colour of petiole			total plants
						Leaf length (m)	Leaf breadth h (cm)	Petiole length (cm)	Leaf length h (m)	Leaf breadth (cm)	Petiole length (cm)	O	Y	G	
IAS ₃ -1	71.95	1.28	13.80	6.58	3.00	1.07	19.55	51.20	0.65	16.46	31.47	7	6	46	59
IBS ₃ -1	82.35	1.56	14.23	5.02	2.00	1.13	16.74	57.60	0.81	14.89	38.27	35	0	9	44
IIS ₃ -1	50	1.32	13.60	5.22	2.00	1.10	18.31	49.56	0.68	14.75	28.67	0	3	6	9
IIIS ₃ -1	68.08	1.32	14.89	6.09	1.00	1.14	21.47	49.10	0.73	16.48	29.03	5	12	14	33
IVS ₃ -1	45.88	1.32	15.68	6.23	2.00	1.17	22.34	48.16	0.80	16.90	32.60	0	10	29	39
VS ₃ -1	44.44	1.12	14.65	5.50	3.00	0.97	23.25	39.75	0.73	12.50	28.33	0	0	4	4
IAS ₃ -2	41.66	1.33	15.76	6.00	2.38	1.29	15.26	64.15	1.13	13.88	56.20	1	1	17	20
IBS ₃ -2	66.67	1.84	16.52	6.11	-	1.21	23.07	69.78	0.84	17.35	45.18	17	0	1	18
IIS ₃ -2	50	1.14	13.13	4.44	-	0.85	19.62	35.93	0.65	14.58	22.38	0	7	19	26
IIIS ₃ -2	41.67	1.22	14.49	5.32	-	1.08	20.08	53.00	0.63	13.50	29.60	0	10	17	25
IVS ₃ -2	41.89	1.58	15.78	3.39	-	1.20	17.60	59.27	-	-	-	0	0	32	32
VS ₃ -2	63.89	1.24	13.90	7.00	-	1.11	23.39	51.31	0.89	18.15	36.23	0	5	18	23
WCT	94.50	1.89	14.87	6.68	-	1.47	26.27	79.00	1.29	16.48	68.76	0	0	72	72
COD	78.10	0.93	18.72	5.57	-	0.86	23.46	36.42	0.67	15.94	32.44	15	0	0	15
CGD	75.50	1.26	17.45	5.86	-	1.17	24.83	64.39	0.93	16.04	47.68	0	0	15	15

Table 20: Inbreeding depression of nursery seedlings

Families	Germination percent tage (%)	Plant height (m)	collar girth (cm)	Number of leaves	Number of split leaves	3rd leaf			5th leaf		
						Leaf length (m)	Leaf breadth (cm)	Petiole length (cm)	Leaf length (m)	Leaf breadth (cm)	Petiole length (cm)
IAS ₃ -1	23.86	32.22	7.20	1.55	0.00	27.33	25.58	35.19	49.34	0.13	54.23
IBS ₃ -1	12.86	2.70	-11.06	8.52	0.00	17.42	12.19	11.67	34.75	-5.30	34.30
IIS ₃ -1	47.09	30.16	8.54	21.82	0.00	25.25	30.30	37.27	47.03	10.50	58.31
IIIS ₃ -1	27.96	30.35	-0.16	8.78	0.00	22.40	18.29	37.85	43.43	0.03	57.77
IVS ₃ -1	51.45	30.34	-5.48	6.73	0.00	20.27	14.95	39.04	38.17	-2.56	52.59
V _S ₃ -1	52.97	41.01	1.48	17.66	0.00	34.35	11.50	49.68	43.41	24.15	58.79
IAS ₃ -2	55.92	29.53	-5.95	10.18	0.00	12.13	41.93	18.80	12.29	15.78	18.27
IBS ₃ -2	29.45	2.70	-11.06	8.52	0.00	17.42	12.19	11.67	34.75	-5.30	34.30
IIS ₃ -2	47.09	39.45	11.73	33.53	0.00	42.01	25.31	54.51	49.68	11.56	67.46
IIIS ₃ -2	55.90	35.66	2.54	20.36	0.00	26.59	23.58	32.91	51.35	18.08	56.96
IVS ₃ -2	55.67	16.16	-6.10	49.29	0.00	18.71	33.02	24.98	-	-	-
V _S ₃ -2	32.39	34.62	6.49	-4.79	0.00	24.19	10.96	35.05	30.97	-10.11	47.31

4.5.5. *Third leaf breadth*

As in third leaf length all the twelve families exhibited positive inbreeding depressions with respect to third leaf breadth in selfed families. Positive inbreeding depressions were observed in the family IIS₃-1 (30.30 %) and IAS₃-1 (25.58 %). Lowest positive inbreeding depression was observed in the family IIS₃-1 (18.29), IVS₃-1 (14.95 %), IBS₃-1 (12.19 %) and VS₃-1 (11.50 %).

The sibmated families with highest inbreeding depression values in S₃ seedlings were IAS₃-2 (41.93 %), IVS₃-2 (33.02 %), IIS₃-2 (25.31 %), IIS₃-1 (23.58 %) and lowest in IBS₃-2 (12.19 %) and VS₃-2 (10.96 %) for the third leaf breadth.

4.5.6. *Third leaf petiole length*

In all families, only positive inbreeding depressions were observed. As in leaf length and leaf breadth positive inbreeding depressions were recorded in the S₃ seedlings of selfed families VS₃-1 (49.68 %), IVS₃-1 (39.04 %), IIS₃-1 (37.85 %), IIS₃-1 1 (37.27 %), IAS₃-1 (35.19 %) and lowest positive was seen in IBS₃-1 (11.67 %).

Highest positive inbreeding depressions were observed in the S₃ generations of sibmated family IIS₃-2 (54.51 %), VS₃-2 (35.05 %), IIS₃-2 (32.91 %) and IVS₃-2 (24.98 %). The lowest inbreeding depression in IAS₃-2 (18.80 %), and IBS₃-2 (11.67 %) were observed.

4.5.7. *Fifth leaf length*

In all the families, positive inbreeding depressions observed in third generation for fifth leaf length. The highest positive inbreeding depression were analysed in the selfed family IAS₃-1 (49.34%), IIS₃-1 (47.03 %), IIS₃-1 (43.43 %), VS₃-1 (43.41 %), IVS₃-1 (38.17 %) and IBS₃-1 (34.75 %).

The sibmated families on selfing expressed positive inbreeding depressions at third generation was observed in the family IIS₃-2 (51.35 %), IIS₃-

2 (49.68 %), VS₃-2 (34.75 %), IAS₃-2 (30.97%) and IBS₃-2 (12.29 %). In family IVS₃-1 no fifth leaf was found.

4.5.8. Fifth leaf breadth

Both positive and negative inbreeding depressions were observed for fifth leaf breadth of seedlings in third generation (S₃). The highest positive inbreeding depression was recorded in the family VS₃-1 (24.15 %) followed by the family IIS₃-1 (10.50 %), IAS₃-1 (0.13 %) and IIS₃-1 (0.03 %). Negative inbreeding depression were observed in the family IBS₃-1 (-5.30) and IVS₃-1 (-2.56 %).

The highest positive inbreeding depression were recorded in the family IIS₃-2 (18.08 %) followed by IAS₃-2 (15.78 %) and IIS₃-2 (11.56 %), while the negative inbreeding depression in family VS₃-2 (-10.11 %) and IBS₃-2 (-5.30 %).

4.5.9. Fifth leaf petiole length

With regard to the fifth leaf produced by the S₃ seedling observed positive inbreeding depressions. High positive inbreeding depression was seen in the family VS₃-1 (58.79 %) and IIS₃-1 (58.31 %), IIS₃-1 (57.77 %), IAS₃-1 (54.23 %), IVS₃-1 (52.59 %) and lowest in family IBS₃-1 (34.30 %).

More or less higher positive inbreeding depressions recorded in the S₃ seedlings of S₂ sibmated families IIS₃-2 (67.46 %), IIS₃-2 (56.96 %) and VS₃-2 (47.31 %). The inbreeding depressions were lowest in two sibmated families IBS₃-2 (34.30 %), and IAS₃-2 (18.27 %).

4.6. MOLECULAR CHARACTERIZATION USING RAPD

Molecular characterization of four selected promising inbreds (IAS₃-1, IBS₃-1, IIS₃-1 and VS₃-1) along with open pollinated WCT, CGD and COD was carried out using RAPD. Good quality genomic DNA with sufficient quantity was subjected to PCR using 30 random decamer primers. Based on the number of amplicons with good intensity primers were selected. Each DNA sample from the selected genotypes were amplified with the selected 10 primers using PCR.

4.6.1. Quantification of DNA samples

The extracted DNA was pure but the presence of polysaccharides slightly reduced the quality based on the absorbance ratio (Table 21). However, the electrophoresis revealed a single high molecular weight band without any degradation. The quantity of DNA samples showed variation from each sample. The highest produced were from the CGD of 966.9 $\mu\text{g/ml}$ and the quality ratio observed was 1.03.

4.6.2. Selection of RAPD primers

Thirty primers were screened using DNA from a single sample. The ten primers that produced the clearest bands with more number of amplicons were chosen for the study of the seven samples of coconut. The primers selected are OPBA 03, OPAW 19, OPAW 15, OPAW 14, OPAW 13, OPAW 12, OPAW 09, OPAW 08, OPAU 03 and OPAU 02. The total number of bands obtained from each primer varied from 8 to 17.

4.6.3. RAPD analysis

The analysis was done with the selected 10 random primers. The amplification pattern includes polymorphic and monomorphic bands (Table 22). The banding pattern yielded by the primers was unique for each sample. The results obtained from the 10 primers are detailed below.

4.6.3.1. OPBA3

The primer OPBA-03 identified a total of nine bands which involves the three monomorphic bands and the remaining six bands are polymorphic. The amplicons of 1000bp and 950bp were specific to dwarf and tall coconuts respectively. The locus of 700bp had observed specifically in WCT alone. Family IAS₃-1, IBS₃-1 and VS₃-1 had several identical bands which in turn, were similar to that of WCT except for the WCT specific band. Family IIIS₃-1 had four bands out of seven which is monomorphic in all WCTs and lacks three bands. Even the

bands specific to dwarf and tall palms were also lacking in family IIS₃-1. Hence, family IIS₃-1 seems to be unique.

4.6.3.2. *OPAW-19*

The primer produced 15 polymorphic bands and one monomorphic band out of 16 bands. There dwarfs showed a specific band with 500bp and 1000bp. However, the band of 1000bp was shared with the genotype IAS₃-1 and the band of 800bp found in CGD was shared with IAS₃-1 (which is also having green coloured petiole). The 400bp band was shared with IAS₃-1 and IBS₃-1. IBS₃-1 recorded the unique bands of 1300bp, 1250bp, 1100bp and 450bp. The primer also showed that the genotype IIS₃-1 was unique which possess only two unique bands of 1100bp and 450bp and resembles no other plant except for the single monomorphic band with 1200bp. Banding pattern of IAS₃-1 and IBS₃-1 were more alike and similar to that of WCT.

4.6.3.3. *OPAW-15*

Total amplicons obtained using this primer were 11 including two monomorphic bands and 9 polymorphic bands. The primer had amplified the locus of 1200bp which was present in the dwarfs COD and CGD, whereas the 300bp locus had been found only in the dwarf CGD. The inbreds IAS₃-1 and IBS₃-1 has shared the locus of 900bp which was unique to them. The plant IIS₃-1 was found unique with the absence of any other amplicons except for the two monomorphic bands (1100bp and 1050bp).

4.6.3.4. *OPAW-14*

There were total of 13 bands out of which 5 are monomorphic and the rest 8 are polymorphic bands. The 1050bp band was found only in the dwarf CGD, while the two bands of 1000bp and 430bp noticed in CGD were shared with the inbred IAS₃-1. The locus of 1100bp was shared between CGD, IAS₃-1 and VS₃-1. The inbreds IAS₃-1 and IBS₃-1 were found similar except for the three bands which were shared only between CGD and IAS₃-1. The family IIS₃-1 had least

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number of amplicons (6 nos.), including the 5 monomorphic bands produced by the primer.

4.6.3.5. *OPAW-13*

There were 15 amplicons out of which 2 were monomorphic and the remaining 13 were polymorphic. The loci of 500bp and 350bp were found specific to the dwarfs and inbred IAS₃-1 respectively. The dwarfs CGD and COD and the family IBS₃-1 had shared the locus of 400bp whereas the dwarf CGD shared the locus of 1200bp with the inbred IAS₃-1. The band of 1100bp was shared between CGD, IAS₃-1 and VS₃-1. The inbreds IAS₃-1 and IBS₃-1 had shared the locus of 450bp. Here also the inbred IIS₃-1 was having the 6 amplicons only while other inbreds have 10-12 amplicons.

4.6.3.6. *OPAW-12*

Out of 17 amplicons 4 were monomorphic and remaining 13 were polymorphic. The bands of 350bp and 300bp were present in dwarfs only, whereas the inbred IAS₃-1 had a specific band 700bp. The 550bp band was shared between IAS₃-1 and IBS₃-1. Apart from the specific 4 bands out of 13, family IAS₃-2 and IBS₃-2 were similar to each other and CGD in all the remaining 9 loci and COD in 8 loci. WCT had only 4 bands which were the 4 monomorphic bands produced by the primer.

4.6.3.7. *OPAW-09*

There were 17 amplicons out of which 6 were monomorphic and 11 were polymorphic. There were no specific bands for any of the genotype. The inbred IAS₃-1 had shared the locus of 300bp and 250bp with CGD and 350bp with COD. The inbred VS₃-1 had similarity to WCT with 12 amplicons except for the presence of an additional band of 870bp. The inbred IIS₃-1 had 7 amplicons out of which 6 were the monomorphic bands produced by the primer.

4.6.3.8. *OPAW-08*

The primer produced 14 amplicons out of which 5 were monomorphic and remaining 9 were polymorphic bands. A band of 600bp was found only in the two dwarfs, whereas 650bp and 500bp bands were unique to CGD. Amplicon with 700bp was present in IAS₃-1 alone and it shared a 1250bp band with the dwarfs. The amplicon 300bp was shared between the dwarf CGD and the inbreds, IAS₃-1 and VS₃-1. The locus with 1300bp was noticed in WCT and the inbred only, while it was absent in dwarfs.

4.6.3.9. OPAU-03

Out of total 11 amplicons 2 were monomorphic while the remaining 9 were polymorphic. This primer amplified two dwarf specific bands of 1300bp and 550bp and a unique band of 420bp specific to inbred IBS₃-1. The dwarfs shared the locus of 900bp with inbred IAS₃-1 and IBS₃-1 and a 450bp band with IAS₃-1. The inbred IBS₃-1 shared the a 650bp band with IAS₃-1. The 600bp and 300bp bands were found in WCT and its inbreds. Similar to the WCT had noticed in family IIS₃-1 with the 4 amplicons whereas family VS₃-1 also known as WCT except with the presence of one locus of 700bp which were shared between CGD.

4.6.3.10. OPAU-02

Total of 8 amplicons were observed from the primer out of which 5 were monomorphic bands and the rest 3 were polymorphic bands. The primer produced a 900bp band which was specific to the dwarfs, COD and CGD. The locus with 1300bp was shared between inbreds IBS₃-1 and VS₃-1. The amplification pattern in inbred IIS₃-1 showed only monomorphic bands.

Table 21: Quantity and the ratio of DNA of the third generation families of WCT coconut along with open pollinated WCT, CGD

FAMILIES	Quantity ($\mu\text{g/ml}$)	Ratio ($\text{OD}_{260}/\text{OD}_{280}$)
IAS ₃ -1	282.9	2.17
IBS ₃ -1	47.4	1.65
IIS ₃ -1	38.6	1.37
VS ₃ -1	44.5	1.57
WCT	53.6	1.67
CGD	966.9	1.03
COD	157.3	1.37

Table 22: Characterization of selected 10 RAPD primers

Sl. No	Primer code	No. of polymorphic bands	No. of monomorphic bands	Total bands	Band size range (bp)
1	OPBA-03	6	3	9	250-1300
2	OPAW-19	15	1	16	200-1300
3	OPAW-15	9	2	11	300-1200
4	OPAW-14	8	5	13	280-1200
5	OPAW-13	13	2	15	350-1300
6	OPAW-12	13	4	17	300-1200
7	OPAW-09	11	6	17	200-1500
8	OPAW-08	9	5	14	270-1300
9	OPAU-03	9	2	11	300-1300
10	OPAU-02	3	5	8	400-1300

Discussion

5. DISCUSSION

Genetic variation is a key factor in competition among individuals in ecological communities which provides an opportunity for plant breeder to develop new and improved cultivars with the desirable characteristics. The use of variability is the ultimate objective of the activities of breeding programme in selection as well as in crop improvement programme. Variation within the population is the basis for the selection and adaptation which makes it possible to continue and advance the adaptive process on which evolutionary success depends. The macro evolutionary concept of conversion was from outcrossing to self-fertilization. The self-fertilization in outcrossing plants would benefit about 50 percent of transmission because of the contribution of the pollens to self-fertilize their own ovules. As the crossing shift to selfing, the associated changes occur mainly in floral morphology and the reproductive investment and mating pattern (Loveless and Hamrick, 1984) which inturn changes the population size and their stability to survive. The possibility of shifting mating system may promote to speciation which can affect the inferences about speciation and extinction (Magnuson and Otto, 2012). With the increased selfing will leads to inbreeding depression indicates the reduced fitness. As a result, becomes homozygous which may be unfavourable or favourable recessive genes. The unfavourable recessive genes will be eliminated whereas the favourable genes with no injurious effects will be utilized.

Inbreeding depression is the lowered fitness of inbred individuals compared to outcrossed individual that can directly affect the intrinsic selective advantage of increased selfing. The magnitude of inbreeding depression in natural populations is expected to evolve with the mating system. Darwin (1876) reported that progeny obtained from self fertilization were weaker than those obtained from out crossing which published in his book *Cross and Self Fertilization in Vegetable Kingdom*. The genetic basis beyond the self fertilization will reveal how the increased homozygosity will lead to the inbreeding depression which is due to non-additive gene action. The rate at which deleterious alleles are

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eliminated from population will depend on the genetic parameters. The effect of deleterious recessive allele or partially recessive allele will result in phenotype appearance.

As the coconut is a perennial and cross-pollinated crops, limited availability of inbreds and self pollinated coconut varieties which is a basic reason for its insufficient exploitation of coconut breeding at present. The hybrid developed now in cross pollinated crops doesn't give the hybrid vigour to the full potentials of the crop, because the selected type is cross-pollinated one and hence it's a hybrid by itself. These crosses with open pollinated ecotypes will give only weak hybrids as well as show segregation for different characters in first generation itself. The cross between two diverse inbreds is only useful in developing hybrids to its full potential with high vigour, early flowering, high yield and other beneficial traits. This also segregates faster to lesser valued individuals. Hence inbreeding depression study was undertaken to develop inbred lines and then identify the diverse inbreds for developing good hybrids in coconut. On inbreeding open pollinated West Coast Tall (WCT) coconut, there will be possibility of obtaining variable forms by crossing two outcrossing coconut palms. The variability will be useful for selection of parents for developing hybrid with low segregation and high vigour, high yield and beneficial nut characters of commercial importance.

Selection for any breeding programme requires variability in characters between varieties. The variation between the varieties should be significantly differ from each other, then only we can select a variety / type for further breeding or commercial cultivation. thus analysis of variance will guide the plant breeder for selecting the variable type/ variety for further use.

Molecular marker i.e. RAPD study has done to gain large number of genetic markers efficiently with small amounts of DNA in a short period. This has been utilized for characterization the genotypes. The ability of RAPD technique is to reveal intra specific variation in screening for the degree of inbreeding. This is

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mainly followed to prevent the frequency of deleterious recessive alleles in population.

The study of inbreeding depression in coconut, a cross pollinated crop was taken up to assess the variabilities available for different characters of coconut palms in second generation (S_2) and seedlings of S_2 in third generation of twelve families of six groups of open pollinated WCT coconut palms. The experiment on developing inbreds started as early as 1924, when the first generation (S_1) was raised and followed the second generation (S_2) in 1967. The S_2 palms of twelve families planted in replicated trial were used for the analysis of morphological, reproductive and nut characters. These S_2 palms of twelve families were selfed to develop the third generation (S_3).

The results of the study under two stages of generation of WCT coconut (S_2 and S_3) are discussed in light of the following subheads:

A. Second (S_2) generation WCT palms

- 5.1. Variability
- 5.2. Inbreeding depression
- 5.3. Characterization of S_2 palms

B. Third (S_2) generation seedlings of WCT families

- 5.4. Variability
- 5.5. Inbreeding depression
- 5.6. RAPD analysis

5.1. VARIABILITY OF SECOND GENERATION WCT COCONUT PALMS

The analysis of variance revealed significant differences among the twelve families of six groups of open pollinated WCT selected for development of inbred lines for all the characters studied suggest the presence of substantial variations among the S_2 families. The wide range of variability noticed in most of the characters confirm the heterozygosity of different groups of open pollinated WCT palms selected for the study. The variability for different characters was reported

by Satyabalan (1982), Menon and Pandalai (1958) and Sathyabalan and Rao (1970).

5.1.1. Vegetative characters

The morphological characters like palm height, girth of palm, internodal length, number of leaves on crown, number of leaves per annum, leaf length, petiole length and number of leaflets per leaf were recorded from the twelve families of selfed and sibmated (S_2) palms and each traits was found to be significantly differ between different families.

5.1.1.1. Palm height

Coconut palms are broadly grouped into two viz. tall and dwarf types based on the plant stature. Analysis of variance revealed significant difference for palm height both at 5 per cent and 1 per cent level of probability for all the families. From the results of selfing of open pollinated WCT coconut palm to second (S_2) generation, it is evident that the selfing reduces the height of the palms to the range of 6.20m to 17.30m. Among coloured groups, the lowest height was observed in sibmated family IBS₂-2 and showed on par with yield group family IVS₂-1, IIS₂-2 and VS₂-1 (Fig 1). It is significantly lower than the open-pollinated WCT. Sibmated families showed less height in orange coloured type compared to the selfed families. From yield group, the lowest height was recorded in family IIS₂-1 from selfed families and IIS₂-2 from sibmated families. They are significantly shorter than open-pollinated WCT but significantly taller than the dwarf viz. COD and CGD. Tallest palms were observed from the family IVS₂-2 and IAS₂-2 which shows significant difference with other families and on par with open-pollinated WCT. The other families showed almost similar height for both selfed and sibmated families but revealed as significantly taller than the dwarf and shorter than the open-pollinated WCT. The character helps in differentiating the stature of palm as dwarf and tall coconut palms. The shorter and smaller stem will indicate the similar appearance of the dwarfs

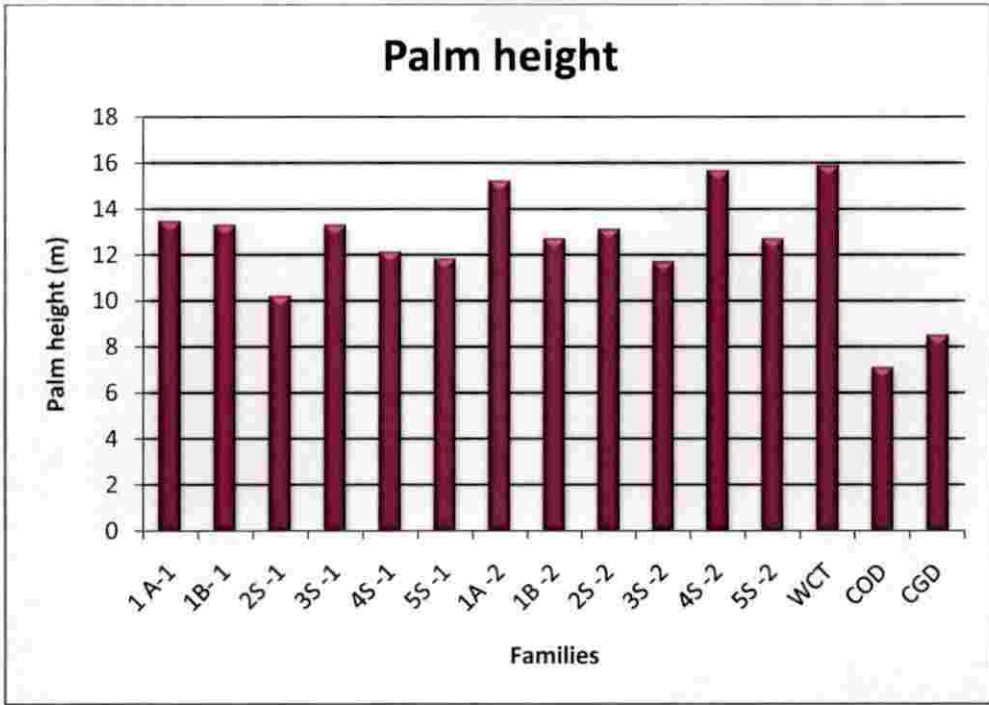


Fig 1: Mean of palm height of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

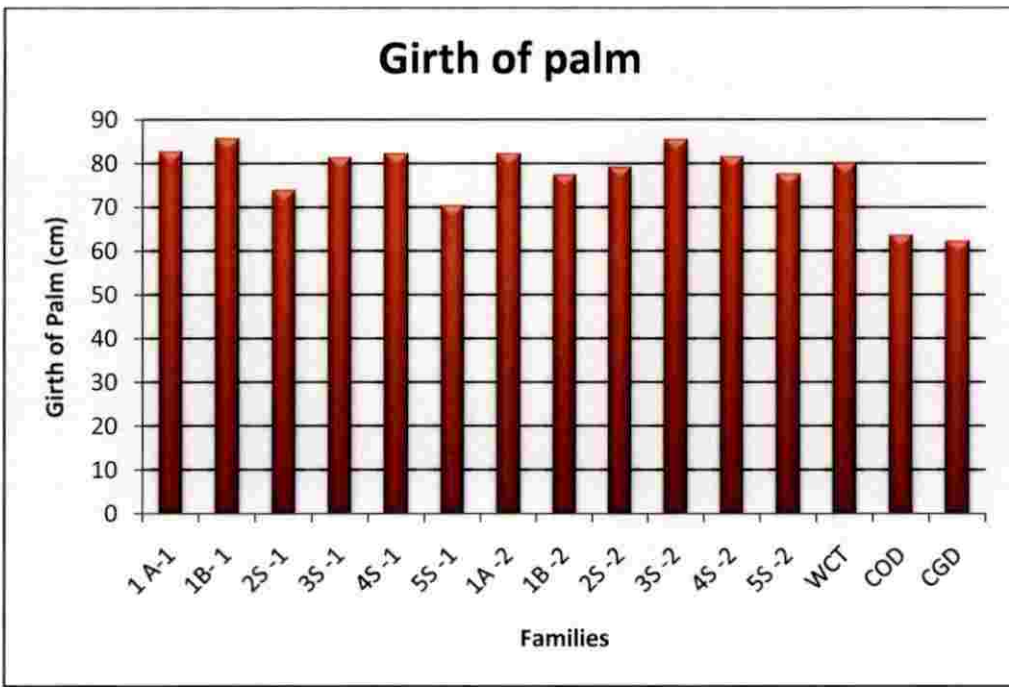


Fig 2: Mean of girth of palm of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

(Pandin,2009).Earlier studies conducted have revealed that the lengths of stem have effect on yield (Satyabalan et al., 1972 and Abeywardena, 1976).

5.1.1.2. *Girth of palm*

Girth of stem showed significant difference for 1 per cent level of probability which ranges from 60 cm to 105 cm. From coloured groups, shorter girth was recorded from family IBS₂-2 followed by IAS₂-2 (Fig 2). Similar to the height, girth also revealed lowest value in sibmated families. Mathew and Gopimony (1991) revealed the correlations of palm height with girth of stem and number of leaves. From the yield group, the lower girth of palms was observed in the family VS₂-1 in selfed families and which is significantly differed and on par with the IIS₂-1. In sibmated families, lowest were recorded from the family VS₂-2. The result indicated that the group VS₂ was lean in stature both in selfed and sibmated method of pollination. This VS₂ is in the positive side of achieving inbreeding in early generation. The family IIS₂-2 observed higher girth differing significantly and on par with seven families which is followed by IVS₂-1. The trunk girth of selfed and sibmated S₂ palms were significantly higher than the dwarfs, but on par with the open-pollinated WCT. The dwarfs and semitalls occurring were in nature as the products of several generations of inbreeding the tall (Thompson, 1993).

5.1.1.3. *Internodal length*

The internodal length varies from 5.0cm to 9.5cm and revealed significant difference at 1 per cent level of probability. In the coloured groups, shortest internodal length was observed from the family IBS₂-2 in sibmated families; whereas in selfed families it was in family IBS₂-1 (Fig 3). The shorter internodal length is a dwarf and recessive character in palms. Hence indicates a positive inbreeding depression. In yield groups, the lowest internodal length was recorded by group IVS₂ both in selfed and sibmated families which is significant and on par with other two families viz., VS₂-1 and IIS₂-2. The highest internodal length was recorded from the family IAS₂-2 which is significant and on par with the six

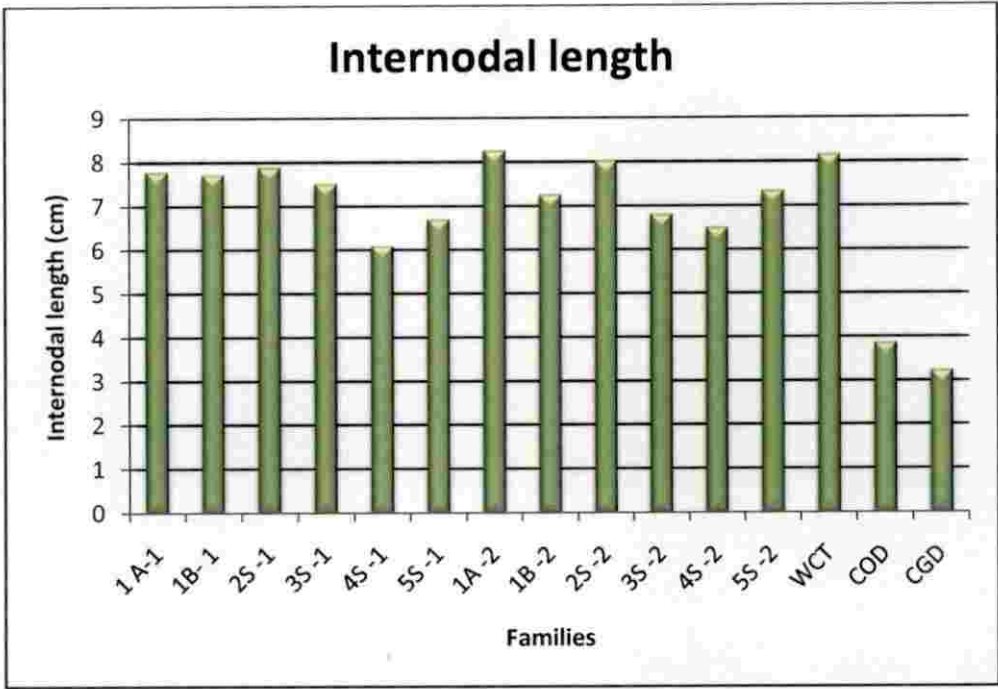


Fig 3: Mean of internodal length of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

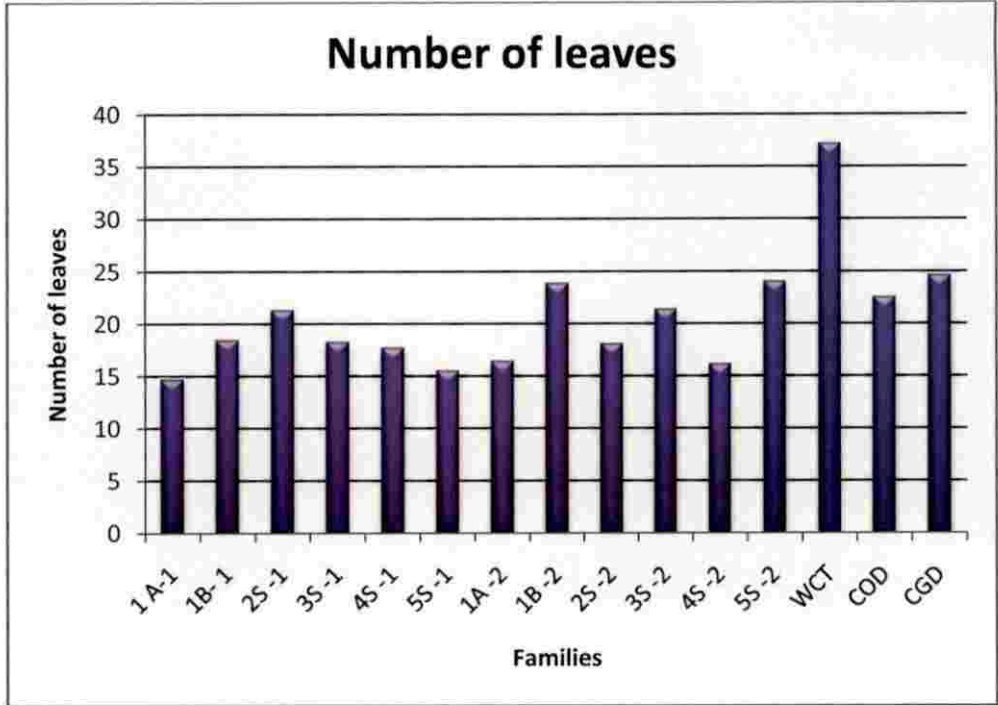


Fig 4: Mean of number of leaves of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

families (WCT, IIS₂-2, IIS₂-1, IAS₂-1, IBS₂-1 and IIS₂-1) which observed similar to the open-pollinated WCT. The lowest length of internodal length recorded were medium but more than the dwarf in IVS₂-1 and IVS₂-2.

5.1.1.4. *Number of functional leaves*

Analysis of variance showed significant difference at 1 per cent level of probability for functional leaves. It ranges from 8 to 34. The high numbers of functional leaves are associated with early bearing and higher productivity. The coloured group IBS₂ produced more number of leaves per annum on both selfed as well as sibmated families. Orange coloured groups showed more number of functional leaves compared to the green coloured groups. In yield groups, the more leaves were observed for family IIS₂-1 and in sibmated family IVS₂-2 (fig 4). The less leaves per annum were observed from the family IAS₂-1 followed by VS₂-1. The more number of leaves were from family VS₂-2 which showed similar result as COD. In family IIS₂-1 in selfed and VS₂-2, IIS₂-2 and IBS₂-2 in sibmated were similar to dwarfs with more number of functional leaves.

5.1.1.5. *Number of leaves per annum*

The number of leaves per annum referred as a marker for growth and vigour of the palm (Kutty and Gopalakrishnan, 1991). Number of leaves per annum reported significant positive association with yield components (Liyange 1967; Kalathiya and Sen 1991). ANOVA revealed that the character showed significant at 1 per cent level of probability. It ranges from 5 to 13. From the coloured groups, the more number of leaves were observed from the group IBS₂ in both selfed and sibmated families. Orange coloured type showed more number of leaves per annum from selfed and sibmated families. Among yield groups, the highest number of leaves was produced from the family IVS₂-1 in selfed families and IIS₂-2 in sibmated families. The leaves produced in all selfed and sibmated WCT families were less than the dwarfs indicating a low vigour and production on selfing or sibmating between selected open pollinated WCT (Fig 5).

5.1.1.6. *Leaf length*

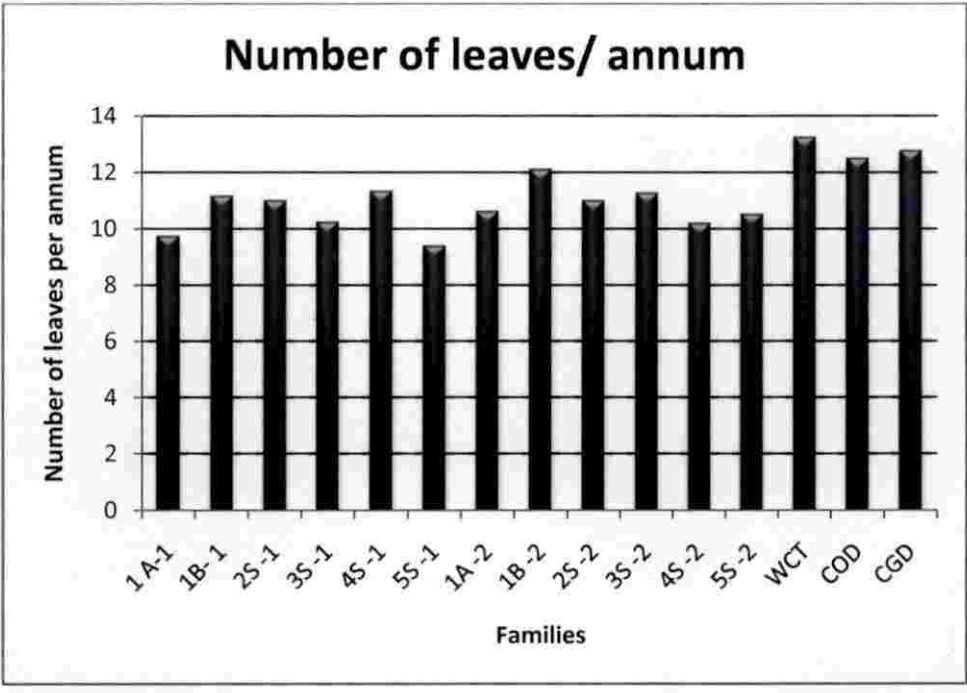


Fig 5: Mean of number of leaves per annum of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

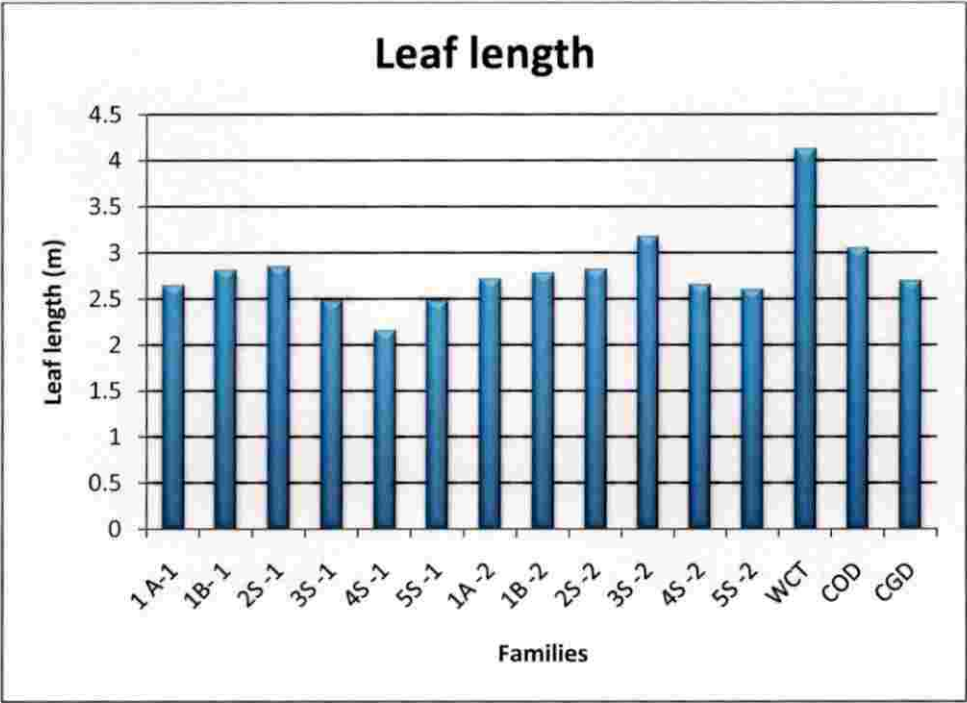


Fig 6: Mean of leaf length of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

ANOVA revealed that the leaf length varied significant difference in all the groups and family at 1 per cent level of probability. It ranges from 1.90 m to 4.0 m. From the coloured groups, the low leaf length was observed from the family IAS₂-1 in selfed families (Fig 6) and in sibmated families, lowest were recorded from family IAS₂-2. The green coloured type showed less number of leaves from both the families indicating low vigour and inbreeding compared to the orange type. From yield groups, the low leaf length was observed in both the family of IVS₂ group and revealed significant and was found on par with three families. This revealed the ability of IVS₂ group to reach homogeneity early. The observed leaf length was lesser than the dwarfs.

5.1.1.7. Petiole length

Petiole length of leaf should be shorter and wider in order to enable the palm to bear nuts. Length of petiole showed marked difference between tall and dwarfs (Pillai et al., 1991). ANOVA observed significant difference between families for this character at 1 per cent level of probability. The range varies from 50 cm to 120 cm. From the coloured types, the less petiole length was observed from family IAS₂-1 in selfed families and IAS₂-2 in sibmated families. Green coloured type showed short petiole compared to the orange coloured type (Fig 7). From yield groups, the short petiole was observed from the family IVS₂-1 and was found on par with the five families (IIS₂-1, IAS₂-1, VS₂-1, IVS₂-2 and IBS₂-1). In sibmated families, IVS₂-2 recorded low petiole length. This indicated a stout and short petiole bearing habit which is advantageous character, even though identifies as dwarf in IVS₂ group.

5.1.1.8. Number of leaflets per leaf

Number of leaflets/ leaf is an important character which enables the absorption of sunlight for photosynthesis (Awuy et al., 1999). The character showed a marked difference between tall and dwarfs (Pillai et al., 1991). ANOVA revealed the significant difference between the families for this trait at 1 per cent level of probability. The number of leaflets per leaf ranges from 131 to 269. From

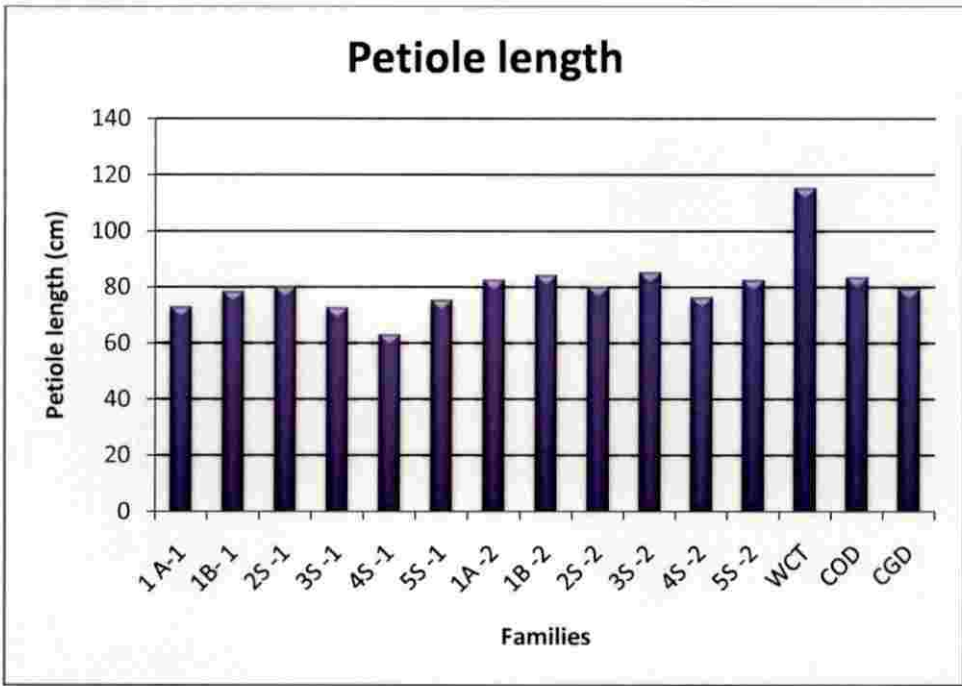


Fig 7: Mean of petiole length of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

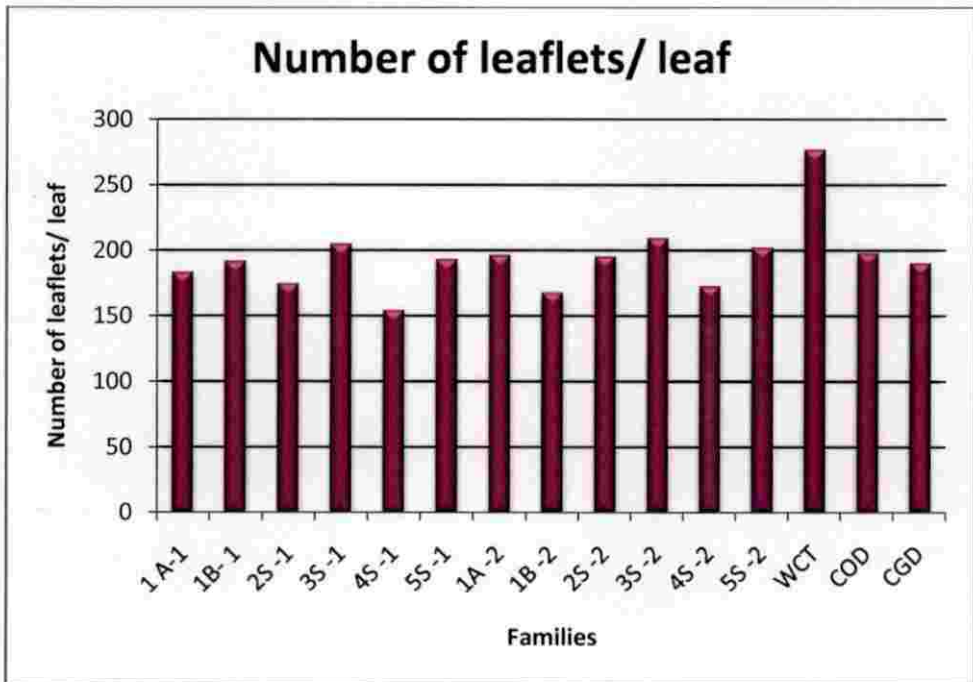


Fig 8: Mean of number of leaflets/ leaf of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

the coloured groups, the more number of leaflets were recorded from the green coloured family IAS₂-2 in sibmated families of orange coloured type. From the yield groups, the more number of leaflets were observed from the group IIIS₂ in selfed and sibmated families (Fig 8). Thus the yield groups IIIS₂ are expressing more number of leaflets than the coloured types in second generation. Minimum leaflet number in IVS₂-1 in selfed as well as in sibmated family IVS₂-2 which is less than the leaflet produced in dwarf. A close relationship has been noticed between the rate of apparent photosynthesis and chlorophyll content and yield in the variety WCT (Annual report, 1982).

5.1.2. Reproductive characters

The number of female flowers produced per inflorescence, period of male and female phase, setting percentage, number of spadices per annum, number of yielding bunches per annum, peduncle length, number of nuts per bunch and annual nut yield were studied under reproductive characters. All the twelve families of WCT were found to differ significantly at various degrees for these traits. The reproductive characters are the deciding factor for assessing the productivity of any crop and in coconut also it is true. There is high correlation with the reproductive parameters and final nut yield of coconut. The performances of each reproductive character in each of the selfed and sibmated families of S₂ generations of coconut are discussed below.

5.1.2.1. Number of female flowers per inflorescence

Coconut is a monoecious and unisexual producing both male and female flowers on the same inflorescence. The inflorescence bears a number of spikelets (usually 30 to 35) bearing flowers. The spikelets are densely set with male flowers. The female flowers are found on the base of spikelets. Highly significant difference between the twelve families S₂ palms of WCT coconut for the number of female flower per inflorescence. The number of female flowers in twelve families varied from 7.10 to 29.05. Of the twelve families, the orange coloured group recorded comparatively more number of female flowers when sibmated

(IBS₂-2) for second generation (Fig 9). However the green coloured groups produced comparatively less number of female flowers in second generation on both selfing and sibmating. The open-pollinated WCT produces with maximum number of female flowers (20.97). The dwarf COD and CGD had comparatively higher female flowers than the S₂ palm families. The selfed second generation (S₂) palms of yield group IIS₂ gave higher number of female flowers. The sibmated family of second generation yield group VS₂ gave higher female flowers among the twelve families studied. In contrast the family VS₂-1 recorded very less number of female flowers per inflorescence on selfing for S₂ generation. The considerable reduction in female flower production on selfing and sibmating to develop S₂ generation than the open pollinated WCT and dwarfs. The families attributes to the operation of phenomenon of inbreeding depression on selfing which leads to sterility.

5.1.2.2. Period of flowering phase

The flowering phase of WCT and dwarfs are quite distinct. In WCT coconut there is a gap between the male and female phases. Since the female flowers become receptive only after all the male flowers in the same spadix have shed their pollen. Thus making cross pollination obligatory for WCT. The intraspadix overlapping male and female phases is common in dwarfs usually leading to self pollination. Hence the tall are classified as cross pollinated and dwarfs as often self pollinated types of coconut.

5.1.2.2.1. Period of female phase

The female phase of WCT noticed were 3.3 while dwarf with 5.2 in CGD and 5.6 in COD. The days of female phase was expressed as same as WCT in IIS₂-1 (Fig 11). From coloured type, Orange coloured type showed more number of days for female flowers than the green coloured type in both selfed and sibmated families. From the yield group, VS₂-1 recorded more period of female phase which says that the family increased with the number of days as same as the

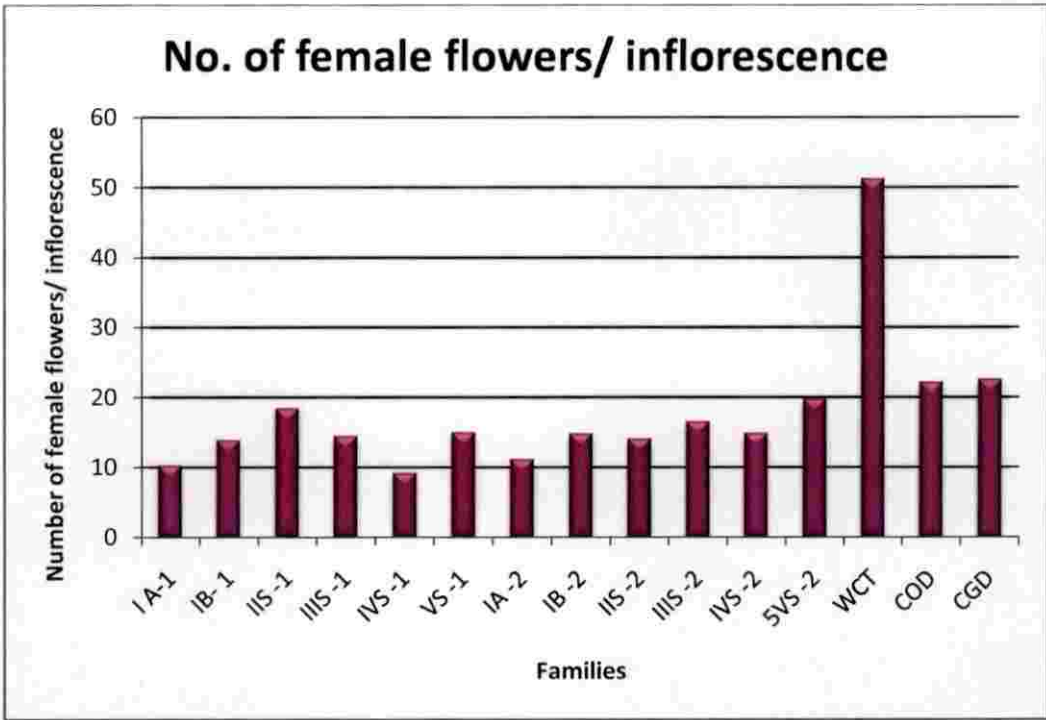


Fig 9: Mean of number of female flowers/ inflorescence of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

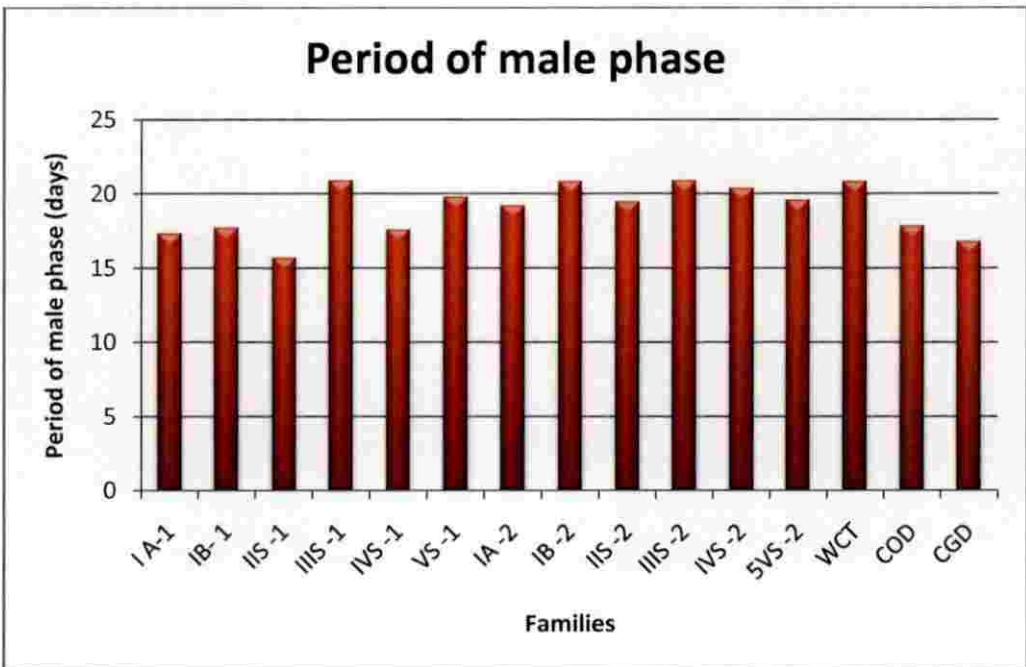


Fig 10: Mean of period of male phase of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

dwarf will coincide with the male phase. The S_2 families produced the female phase lesser than the dwarfs whereas greater than WCT except for the group IIS₂ and family IVS₂-2 and VS₂-2.

5.1.2.2.2. Period of male phase

The period of male phase revealed significant difference at 1 per cent level of probability. From the coloured groups, the less period of male phase were observed from the family IAS₂-1 in selfed families. In sibmated families, lesser male phase was recorded from family IAS₂-2. Green coloured type showed lesser male phase from selfed and sibmated families than the orange coloured type (Fig 10). From yield groups, the less period of male phase were observed from the family IIS₂-1 in selfed families revealed significant and on par with families IAS₂-1 and CGD. In sibmated families, IIS₂-2 recorded less period of male phase. The more period were from family IIS₂-1 showed significant difference and was found on par with seven families. The group IIS₂ showed less period of male phase for both selfed and sibmated families.

5.1.2.3. Setting percentage

The viable pollen, healthy female flower, fertile ovary and successful pollination and fertilization leads to high setting percentage and ultimately the nut yield. The S_2 palms (selfed and sibmated), WCT, COD and CGD exhibited significantly high difference for nut setting percentage. The nut setting percentage of the twelve families, WCT (open-pollinated), COD and CGD varied from 23.53 to 68.10. The families IIS₂-1, IVS₂-1, IAS₂-2 and IIS₂-1 recorded high setting percentage and were superior to all other S_2 families (Fig 12). The female flower production was higher and could convert more than 50% of the female flowers to nut yield. Even though the female flowers production in WCT (open pollinated), COD and CGD were moderately high, the setting percentage was not as good as selfed family of IIS₂-1, IIS₂-1 and IVS₂-1 and sibmated family IAS₂-2. Setting percentage was significantly higher than the dwarfs in S_2 palms except few of the S_2 palms (IAS₂-1, IBS₂-1 and VS₂-1), all others were showing dwarfing

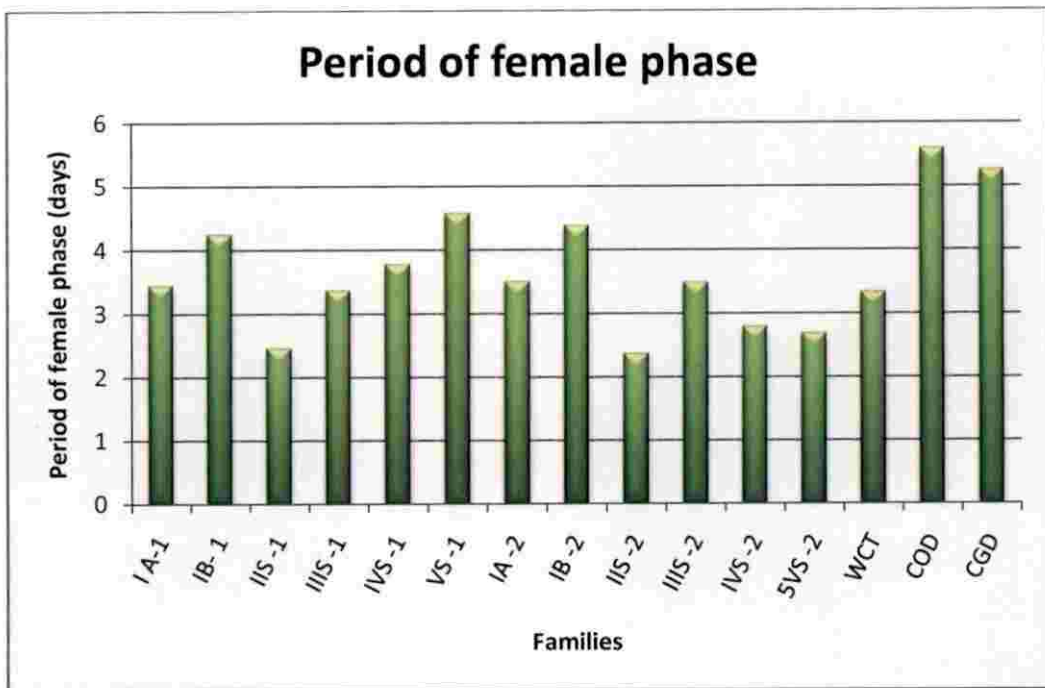


Fig 11: Mean of period of female phase of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

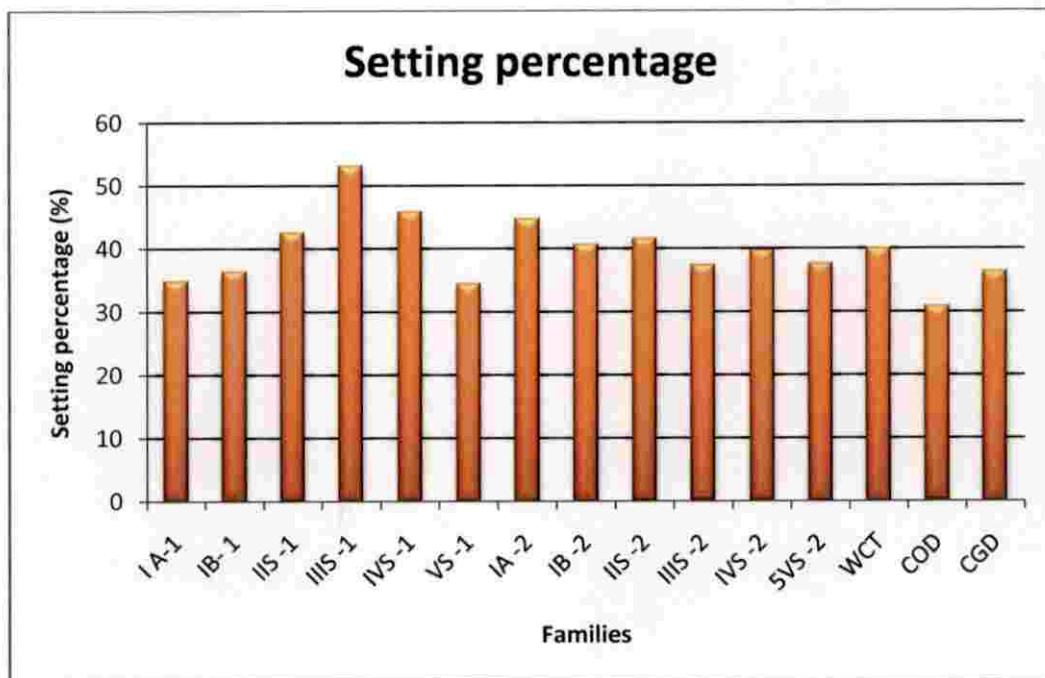


Fig 12: Mean of setting percentage of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

behaviour. In dwarfs, the female flowers will be high but setting percentage is low (Liyanage and Azis, 1983).

5.1.2.4. *Stalk length*

Higher stalk length is not an acceptable character for high yielding palms because it leads to buckling of bunches and breaking before the nuts reaching maturity incurring loss to farmers. However, none of the S₂ palms produced longer stalk as WCT (open pollinated) but ranges from 18cm to 52cm only (Fig 13). The stalk length is as good as dwarf and may be showing recessive nature. It is clear that the low yield in S₂ palms were not due to defective stalk, but due to some other traits.

5.1.2.5. *Number of spadices per annum*

This is a very important reproductive character contributing annual yield. The number of leaf production and bunches are associated (Menon and Pandalai, 1958; Mathew and Gopimony, 1991) because each leaf axil will bear a spadix or inflorescence in coconut. The average nut borne in each spadix or bunch decides the nut yield. The number of spadices produced in open pollinated WCT, COD and CGD were significantly higher than all the S₂ palms (Fig 14). Even though the open pollinated and dwarfs produced twelve bunch per annum on an average, the S₂ palms produced 9 per month. The family IAS₂-1 and VS₂-1 produced very less number of bunches. This is due to the reduced vigour of S₂ palms. Further the S₂ palms produced leaves regularly per month, but all the leaf axils didn't bear spadices due to the non formation or non conversion of leaf axils to spadices. Small rudimentary spadix like structure was observed (Duke, 1929). This was also reported earlier in weak and poorly managed plantations (Furtado, 1923).

5.1.2.6. *Number of yielding bunches per annum*

Even though the spadices are produced regularly in each leaf axil, the nut bearing branches were less. This is due to the defective bunches, spadices without female flowers, no setting of female flowers in the bunch, etc. This results in low yield of nut. This also explains the poor vigour of the palms. So actual bunches

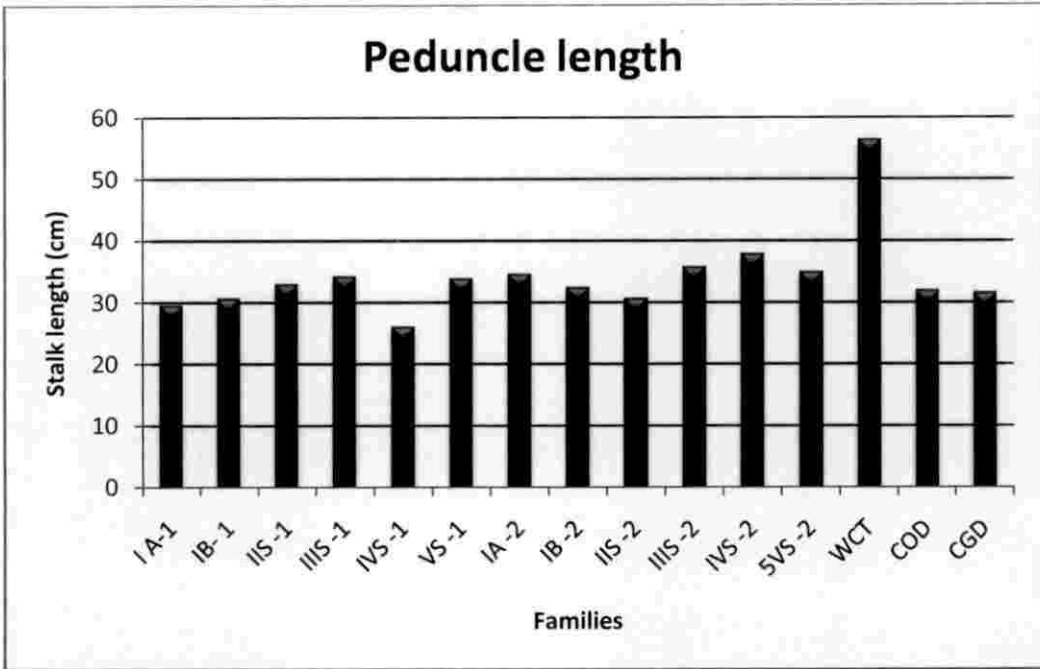


Fig 13: Mean of stalk length of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

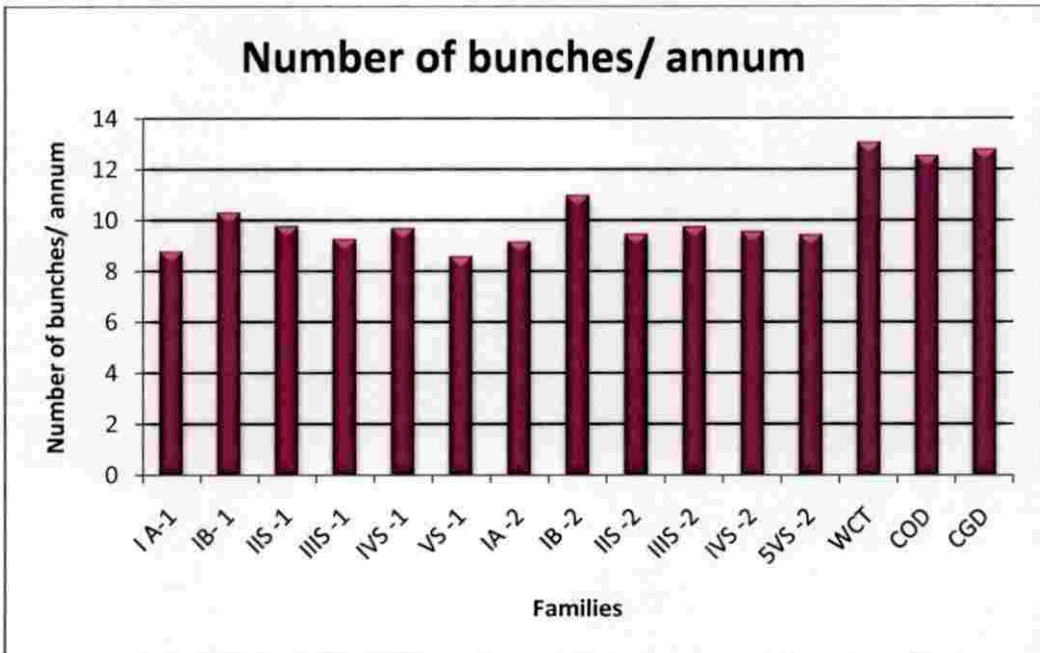


Fig 14: Mean of number of bunches/ annum of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

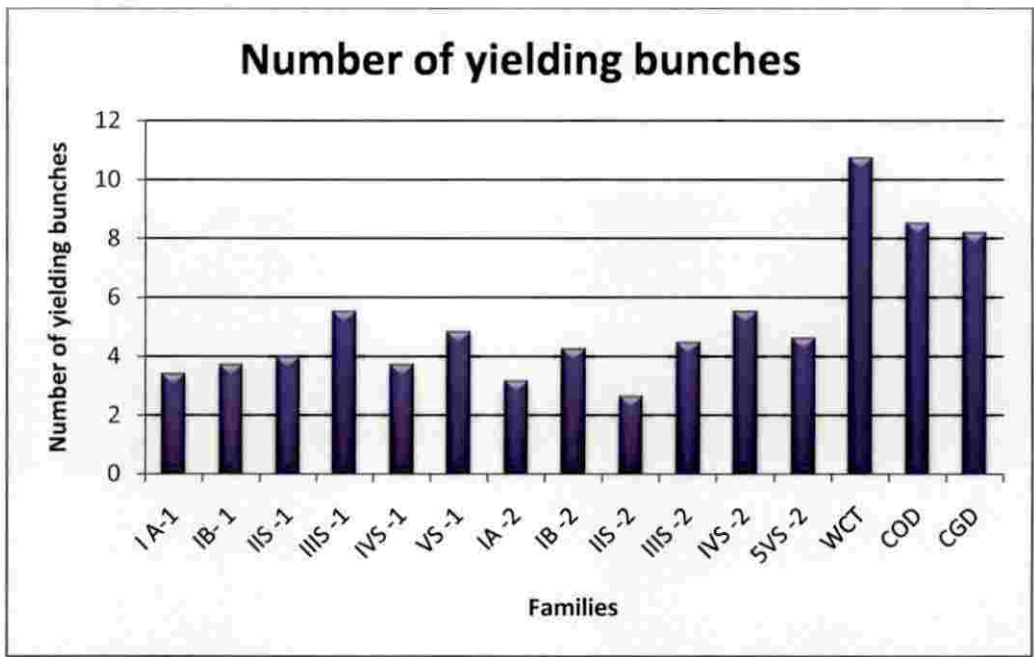


Fig 15: Mean of number of yielding bunches of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

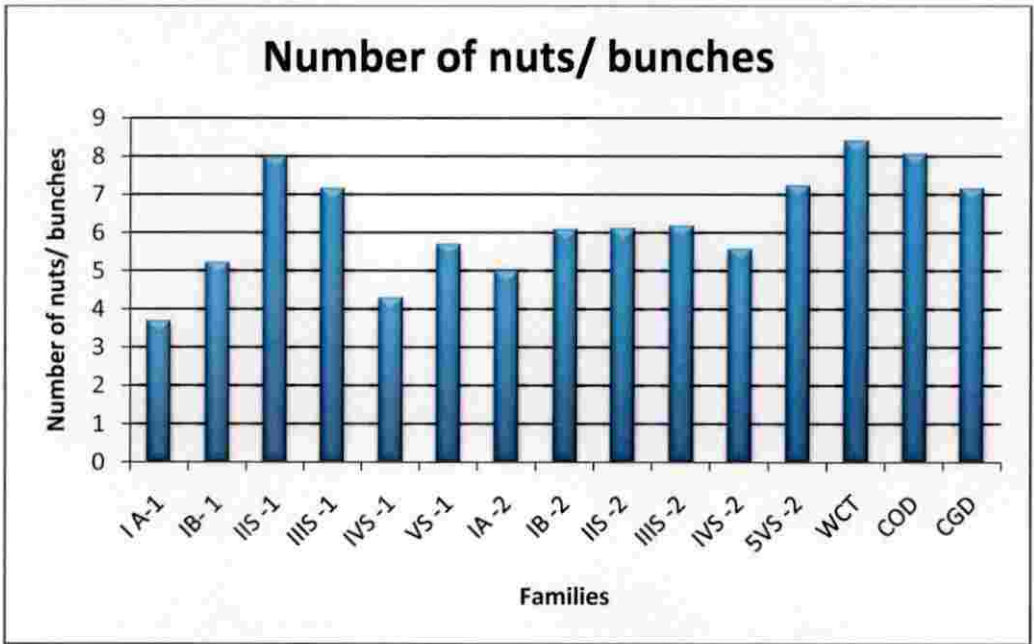


Fig 16: Mean of number of nuts/ bunches of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

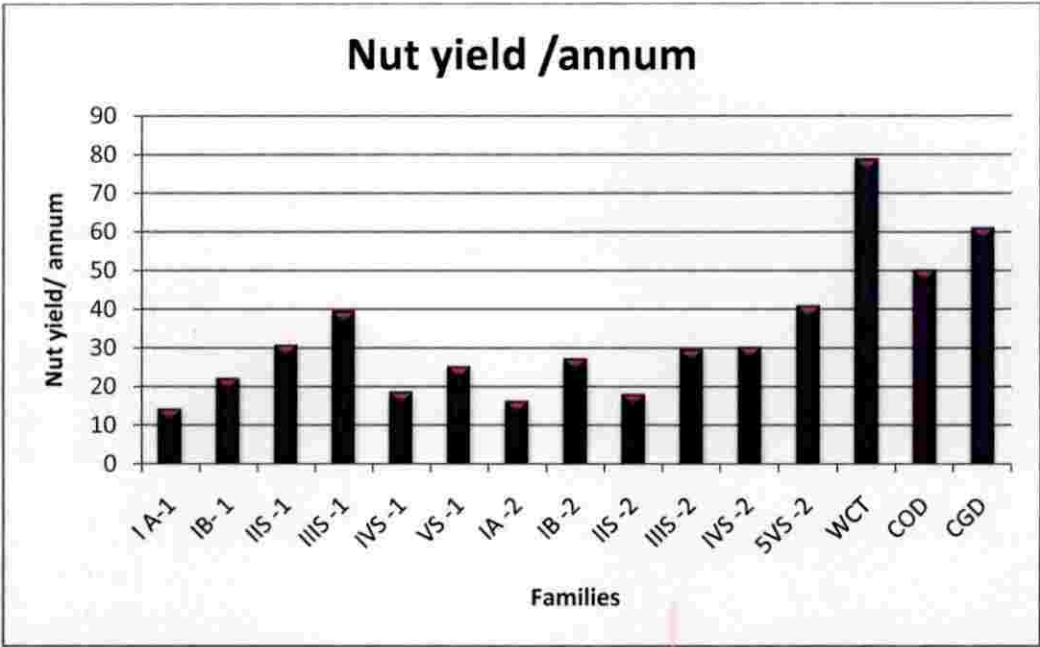


Fig 17: Mean of nut yield per annum of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

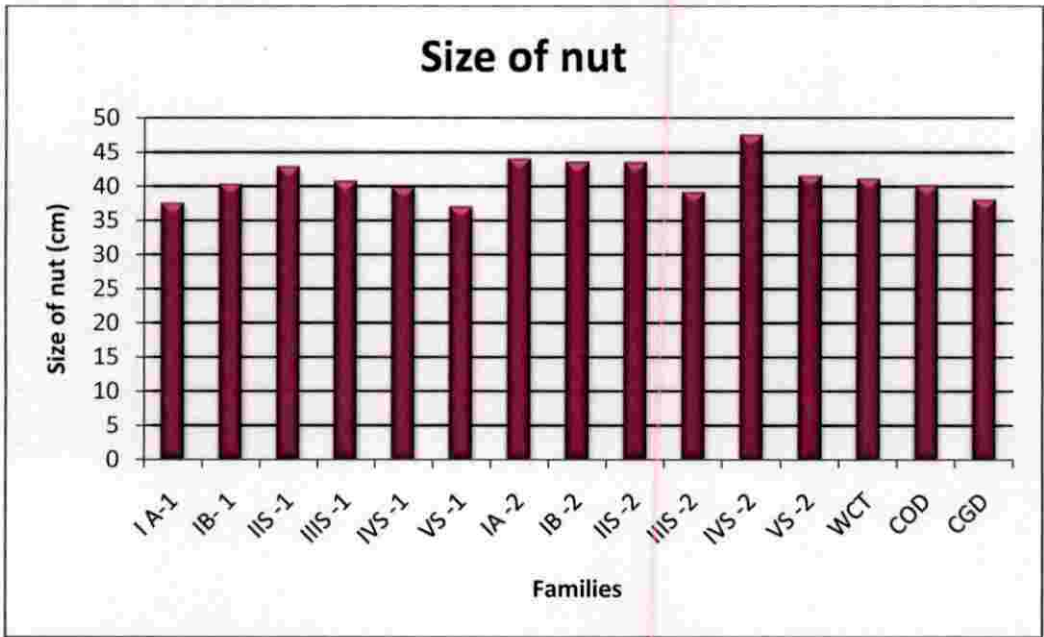


Fig 18: Mean of size of nut of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

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bearing the nuts were considered in this experiment. These bunches were very low in S_2 palm compared to open pollinated WCT, and dwarfs. It ranges from 1.0 to 7.0 (Fig 15).

5.1.2.7. *Number of nuts per bunch*

The nuts per bunch are an important character contributing to the yield of coconut. This also depends on the number of fertile female flower developed, set on fertilization and retention of good nuts in the bunch. The nuts per bunch borne in open pollinated WCT were high followed by COD. Similarly, the nuts per bunch produced in IIS₂-1 and VS₂-2 were highest (Fig 16). The selfed families IAS₂-1, IBS₂-1, IVS₂-1, IAS₂-2, VS₂-1 and IVS₂-2 produced nuts lesser. Thus the green coloured group IAS₂, IVS₂ and VS₂ were generally inferior in production of nuts per bunch, which would reflect in annual nut yield.

5.1.2.8. *Nut yield per annum*

The nut yield from open pollinated WCT was highest (78.79) and the dwarfs viz., COD (61.15) and CGD (50.28) Fig-17 shows that the family VS₂-2 and IIS₂-1 performed comparatively better even though the nut yield was not significantly superior. In general the nut yield was inferior to that of WCT (open pollinated). This may be due to the continuous selfing and sibmating, this may be due to infertility and low setting of nuts in general in selfed and sibmated families.

5.1.3. **Nut characters**

The ripe coconut is ovoid to oblong and three angled in shape. It has a smooth outside skin or exocarp which encloses the thick, fibrous mass called husk. Inner to this fibrous husk is the nut which is characterised by a hard covering or endocarp called shell. The endocarp encloses thick albuminous endosperm or kernel. Between the endocarp and kernel a thin brown testa which firmly adheres to the kernel or meat. At the top of nut there are three pores or depressions arranged in a triangular fashion with one larger compare to the other two. The larger one bears the embryo and embedded into the kernel. The cavity of

the endosperm is completely with water when young and partially filled when mature.

5.1.3.1. *Size of nut*

The size of nuts measured at the broadest (equatorial) part didn't show any significant difference S_2 palms of selfed and sibmated families. The size varied from 31.4cm to 71.17cm (Fig 18). The largest nuts were produced by IVS_2-2 the sibmated family of yield groups followed by IAS_2-2 and IBS_2-2 , the coloured groups. The nut size of the open pollinated WCT, COD and CGD were on par and relatively lower than the above selfed and sibmated families, even though statistically not significant. This was also reported by Balakrishnan (1991).

5.1.3.2. *Unhusked nut weight*

The whole mature nut weight at full maturity or ripe stage at 11 to 12 months is used as criteria for identifying different varieties. The dwarfs generally have small nuts with less weight (Pillai et al., 1991). Mathew and Gopimony (1991) reported that the weight of unhusked nuts had positive correlation with weight of husked nut, kernel weight, and germination of nuts and percentage recovery of number of quality seedlings. This trait showed a significant with all the families of S_2 palms and the weight ranges from 260g to 1300g (Fig 19). The open pollinated WCT had exhibited maximum weight. The orange coloured IBS_2-2 (676.42g) and IIS_2-1 (683.25g) recorded maximum weight in S_2 families. The nut size and unhusked nut weight were low in group VS_2 (in both selfed and sibmated families). Again the large size nuts of IVS_2 had less weight indicating more of husk than shell and kernel content. The percentage of husk in the fruit was considered as a important characters in distinguishing varieties (Harris, 1978). The family has more percentage of husk compared to the other families in this experiment.

5.1.3.3. *Husked nut weight*

In removal husk, which comprises about 50% of the total weight, the endocarp, endosperm and nut water makes the husked nut weight. The husked nut

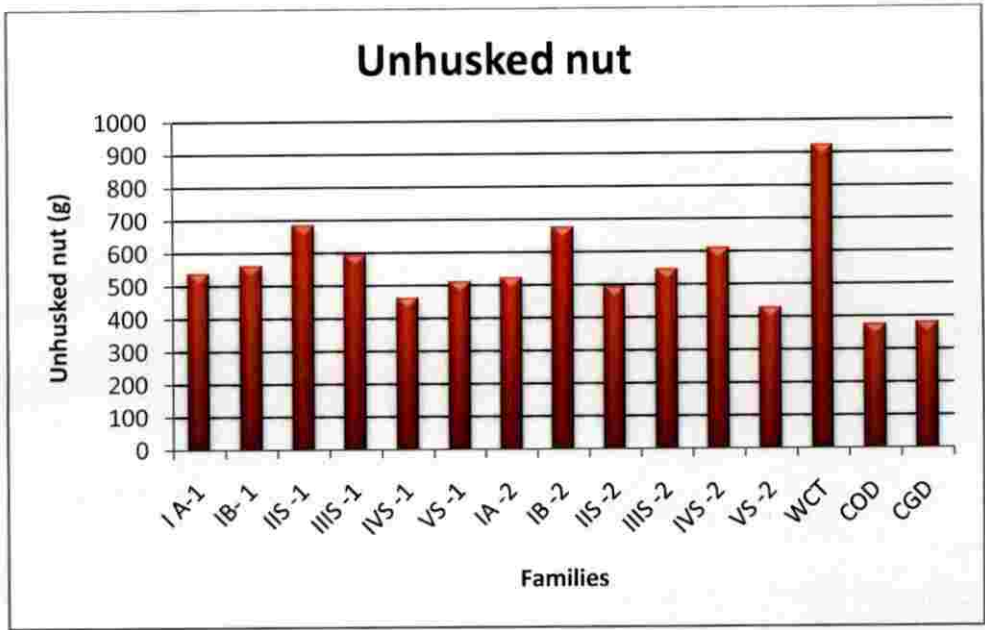


Fig 19: Mean of unhusked nut of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

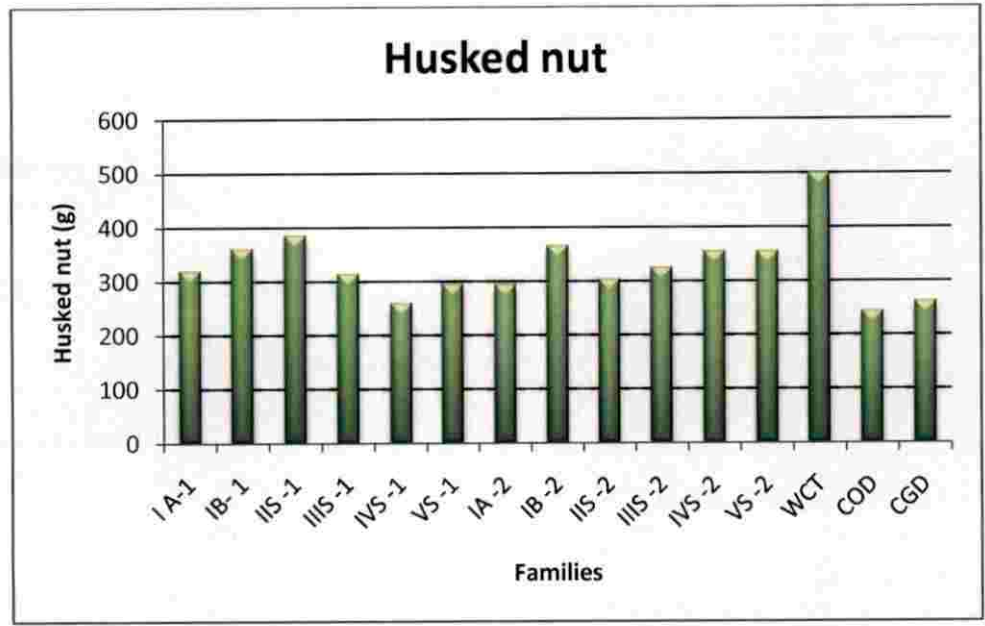


Fig 20: Mean of husked nut of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

weight reported to be exhibiting high correlation with weight of fresh kernel, diameter of eye, seedling height, collar girth and number of leaves (Mathew and Gopimony, 1991). The husked nut varies highly significantly among all the families. It ranges from 145g to 650g. The husked nut weight of open pollinated WCT was highest (500g), it was highly superior to all other families indicating selfing and sibmating reduced the husked nut weight (Fig 20). The next in rank on husked weight after WCT (open pollinated) was IIS₂-1, IBS₂-1 in selfed and IBS₂-2 in sibmated families. The importance of both additive and non additive genetic variance was reported by Fernando (1996). He suggested that the possibility of crossing among individuals and selfing the selected individuals for improving the character. All the traits, viz. Unhusked nut weight, husked nut weight and size of nuts were less in self pollinated IVS₂-1 family. The family IIS₂ produced large nuts with more weight when unhusked and husked condition, but were less than WCT. Pillai et al. (1991) reported that naturally evolved types of coconut had high percentage of husk than introgressed forms having lesser percentage of husks.

5.1.3.4. Diameter of eye

It is an important character bearing embryo of the seed nuts in coconut. It may have relation with the germination and vigour of the seedlings. The diameter of eye showed highly significant variation among the selfed and sibmated families of S₂ palms of six groups (Fig 21). It ranged from 1.00cm to 2.4cm. All the S₂ families differed significantly from the WCT (open pollinated) and dwarfs in this experiment. The maximum mean size of diameter of eye was recorded in IBS₂-2 family followed by IIS₂-1, IIS₂-2, IIS₂-1 and IVS₂-2. The diameter of eye exhibited a high heritability indicating additive gene action in its expression (Mathew and Gopimony, 1991).

5.1.3.5. Fresh mature kernel weight

This is an important heritable character for classification of coconut ecotypes as reported by Harris (1978). It had positive correlation with seedling

vigour and recovery of quality seedlings. The fresh mature kernel weight showed a highly significant difference between different families of S₂ palms. It ranged from 90g to 468g. The mean kernel weight was highest in IBS₂-2 (Fig 23). However, the meat weight of IBS₂-2 was on par with other families of S₂ palm viz. IBS₂-1 (selfed) and IVS₂-2 (sibmated). The kernel weight of all the families were on par indicating no much difference between families. This is an important character associated with copra and oil yield, the two commercially valuable products of coconut. The kernel weight by itself will not bring down economic yield in coconut on inbreeding.

5.1.3.6. *Kernel thickness*

Kernel is embedded to the endocarp as a thick layer of albuminous endosperm. The thickness of endosperm varies from 0.90cm to 1.50cm in the present study. The thick kernel was produced by the dwarf CGD followed by COD and open pollinated WCT. All S₂ palms families significantly differ for this trait (Fig 22). Among the S₂ families IBS₂-1 exhibited high meat thickness followed by VS₂-2. Lowest kernel thickness was recorded in IAS₂-1. The orange coloured S₂ palm had produced thick meat than the green and other yield groups. Harris (1978) had laid importance on meat weight and husk weight, the fruit components for classification of coconut ecotypes. Pillai et al. (1982) confirmed that the meat characters were not influenced by environment and ideal characters for classification of coconut.

5.1.3.7. *Copra content*

Copra is the dried kernel or endosperm of coconut which is high valued product in coconut. The WCT had highest mean copra content of 183.83g and the dwarfs had less with 91.67g in CGD and 123.59g in COD (Fig 24). The copra content per nut of the family members was highly variable with 37g to 175g with a mean of 108.86g. The mean lowest copra content was in IIS₂-2 (93.78g) and highest in IIIS₂-1 followed by family IIS₂-1. All the families were on par with dwarf COD in copra content. The IIS₂-2 and VS₂-1 were highly significant than

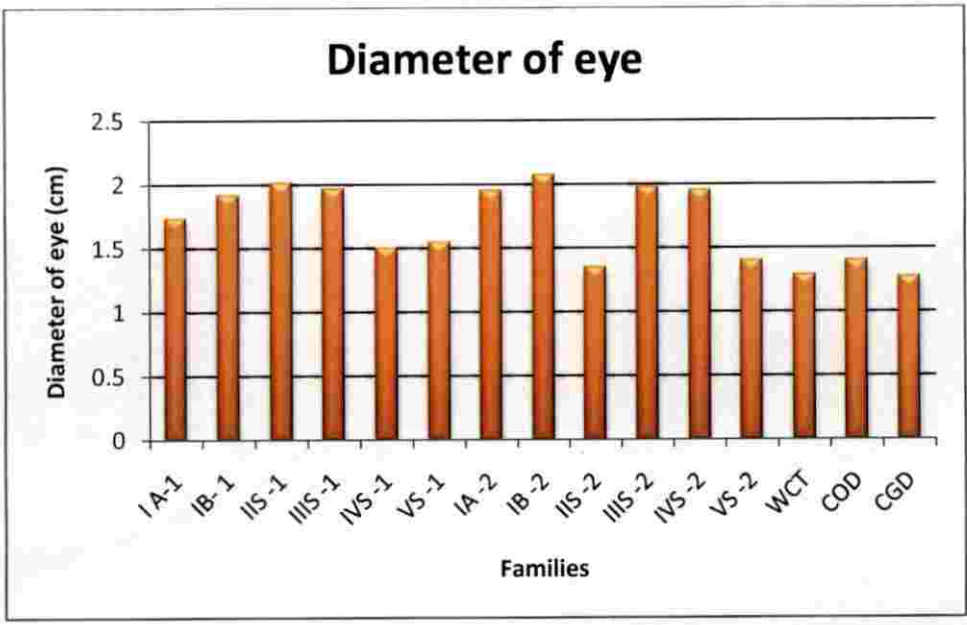


Fig 21: Mean of diameter of eye of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

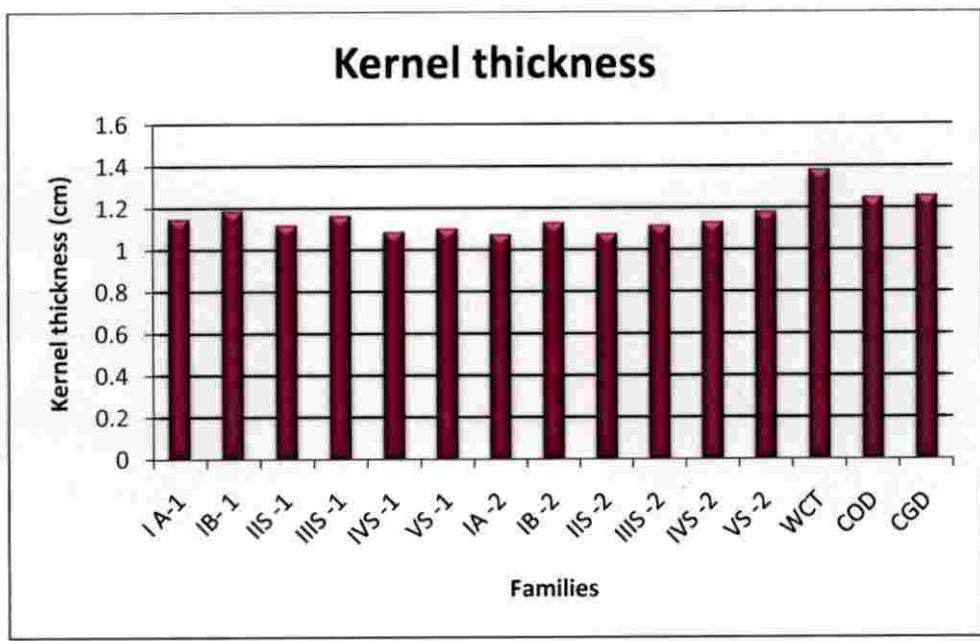


Fig 22: Mean of kernel thickness of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

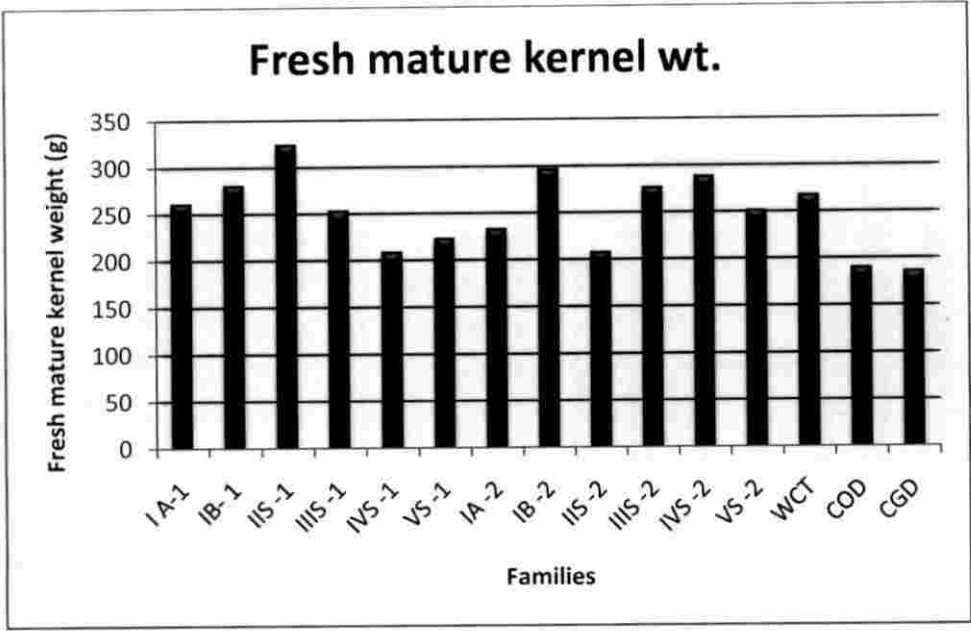


Fig 23: Mean of fresh mature kernel weight of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

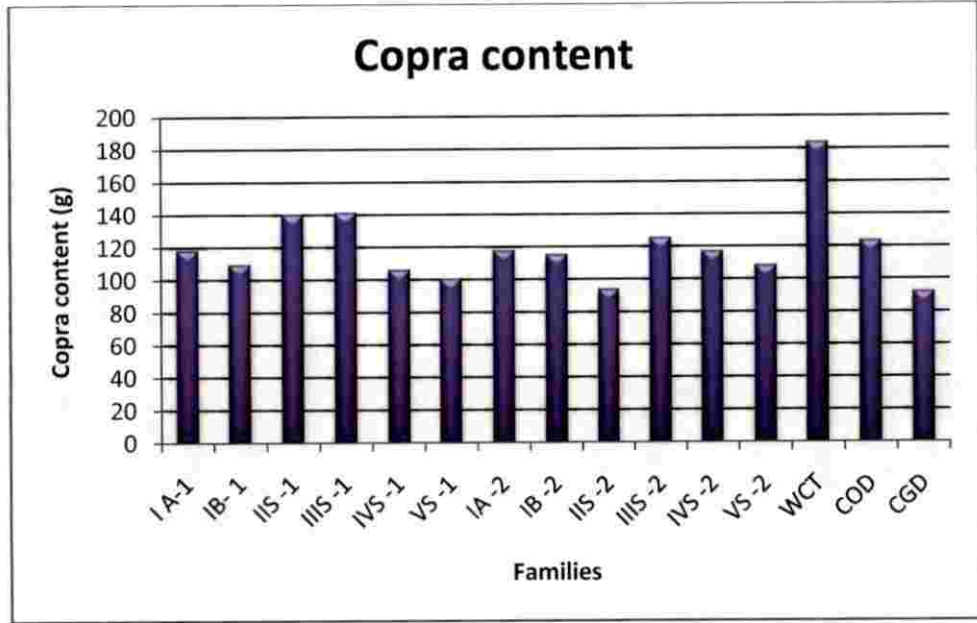


Fig 24: Mean of copra content of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

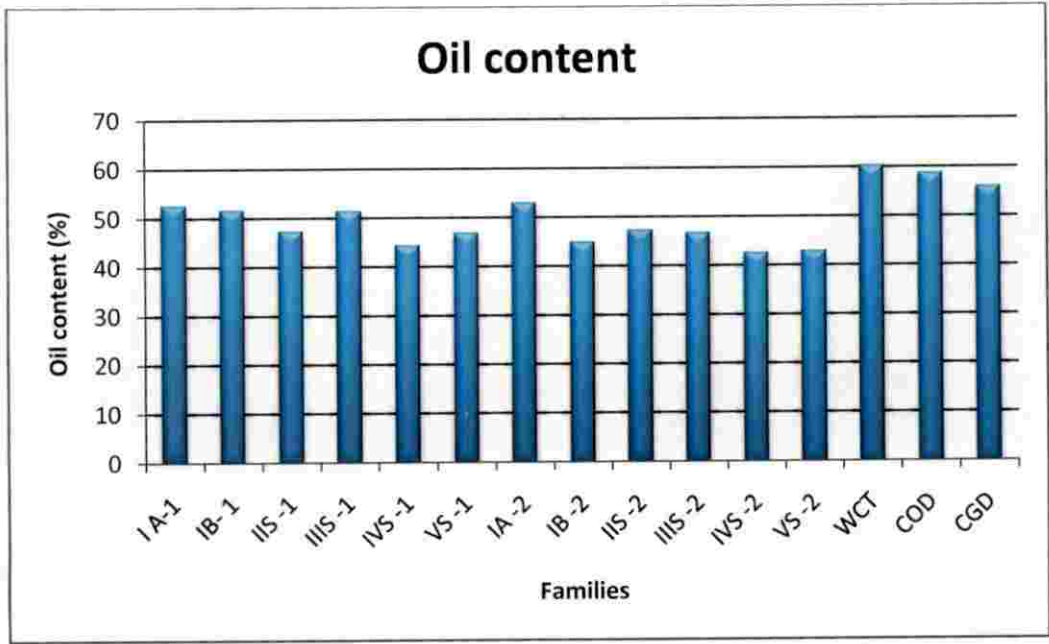


Fig 25: Mean of oil content of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

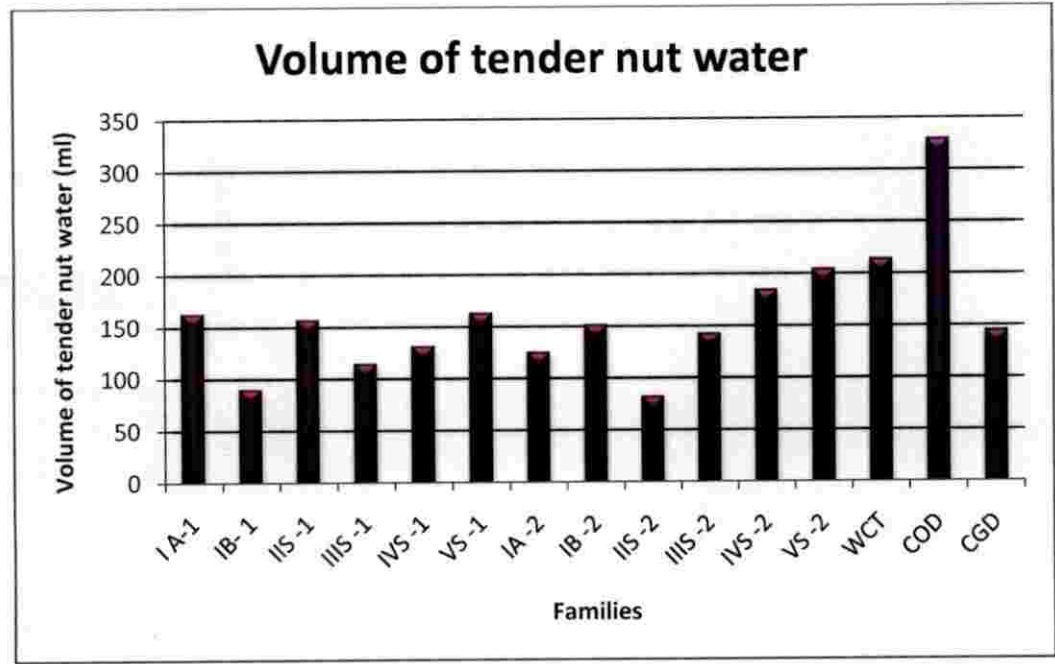


Fig 26: Mean of volume of tender nut water of selfed and sibmated second generation families of WCT, open pollinated WCT, COD and CGD palms

CGD in copra content and the family IIS₂-1 was significantly superior at 5% level. This is the first report on copra content in selfs and sibs of WCT S₂ palms. Copra content is being a heritable character shows that WCT has separate identity as a separate genotype from dwarf.

5.1.3.8. Oil content

The oil content of S₂ palms varied from 36.9% to 59.2% (Fig 25). The green coloured group IBS₂-1 yielded highest oil content even though it is lower than open pollinated WCT but on par with COD and CGD (Fig). The oil content had reduced in all the S₂ palm families and on par with COD and CGD. This also shows that the selfing and sibmating made the palms inferior than open pollinated. The relation of oil content with selfing and open pollinated palms was not reported so far.

5.1.3.9. Volume of tender nut water

The volume of tender nut water obtained from S₂ population ranged from 30ml to 290ml per nut in twelve families (Fig 26). The highest mean was 205ml in VS₂-2 followed by IVS₂-2 which were on par with open pollinated WCT and CGD but inferior to COD in quantity. It may be noted that the cavity bearing nut water was also less in all the families than COD and WCT as examined the quantity of tender nut water in seventh month, when the nut water would be full to the cavity of the nut. This also indicates that the nuts have become smaller as well due to selfing or sibmating WCT palms.

5.2. INBREEDING DEPRESSION OF SECOND GENERATION PALMS

The present investigation of inbreeding depression was studied for nut yield and its attributes in second generation WCT coconut. In S₂ generation WCT coconut palms, inbreeding depression was noticed for vegetative, reproductive and nut characters.

5.2.1. Vegetative characters

5.2.1.1. Palm height, stem girth and internodal length

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The three morphological characters viz. height of palm, stem girth at 1m above ground level and internodal length on stem are the interrelated characters contributing for stature i.e. tallness or dwarfness of palm. The result of these characters on self pollination and sibmating of six groups in second generation (S_2) exhibited varying level of inbreeding. The selfed family II in S_2 generation expressed positive inbreeding depression for palm height and internodal length where as in stem girth expressed less positive inbreeding depression. That is no inbreeding depression for stem girth in the second generation selfed families, IAS_2-1 , IBS_2-1 , IIS_2-1 , IVS_2-1 , where as the two groups IIS_2-1 and VS_2-1 showed positive inbreeding depression. The inbreeding depression was highest in family IIS_2-1 for palm height (Fig) followed by VS_2-1 and IVS_2-1 when selfed. Thus all the families of six groups on selfing showed a tendency to become short with short internodal length and more or less thin stem. Similar results of inbreeding depression in vegetative characters like height, stem girth and internodal length was reported earlier by Miftahorrahman and Luntungan (1991) in Phillipines and Pandin (2009) in Indonesia when he had carried out inbreeding depression studies by selfing for four generations in Mapanget Tall Coconut. Both the two coloured type (Green and Orange) in group IS_2 of WCT also expressed inbreeding depression for palm height and internodal length, but negative or no inbreeding depression for girth of stem.

When the six groups were subjected sibmating positive inbreeding depression was recorded for the palm height in all the six families in S_2 generation. Except for green coloured group in family IAS_2-2 of WCT, all other five families of sibmated expressed positive inbreeding depression for internodal length. The highest inbreeding depression was expressed in the family IIS_2-2 . The stem girth showed negative inbreeding depression in family IAS_2-2 , IIS_2-2 and IVS_2-2 . Thus it is evident that sibmating of S_1 palms for S_2 had contributed inbreeding depression these traits. The study on sibmating of S_1 families of WCT was done for the first time in coconut.

5.2.1.2. Leaf characteristics

The inbreeding depression studied in leaf characteristics viz. number of leaves produced per year, number of functional leaves, leaf length, petiole length, number of leaflets by both selfing and sibmating S_1 families for S_2 generation. All these vegetative leaf characters expressed positive inbreeding depression at varying degrees (Fig). The functional leaves had shown high inbreeding depression in all the six selfed families of WCT in S_2 generation. Similarly leaf length also showed high inbreeding depression followed by number of leaflets per leaf, petiole length and number of leaves produced per annum. The less number of functional leaves, short petiole length, short leaves, short leaflets and less production of leaves per annum are dwarfing nature of palms. These characters expressed in dwarfs. The inbreeding depression in number of leaves produced in selfing was reported by Pandin (2009). The desirable leaf characters on coconut are the wider, thicker and short petiole so as to sustain the nut bunches having more nuts and weight (Tampake, 1987; Novarianto et al., 1999). A long leaf stalk or petiole is not able to sustain the fruit bunches so that the petiole will sad or break from the stem (Mahmud et al., 1990).

Number of leaves produced is correlated positively with number of bunches. Generally each leaf produces one spadix. Hence more number of leaves with thick and short petiole are the required characters for obtaining high yield in coconut. The number, width and length of leaf and leaflets are desirable in coconut as more sunlight can be absorbed for photosynthesis (Awuy et al., 1990).

In sibmated families of S_2 palms also exhibited positive inbreeding depression for all the leaf characteristics. The highly responsive family for inbreeding depression were IAS_2-2 , IIS_2-2 and IVS_2-2 while sibmating. More or less all the families exhibited equal response in inbreeding depression while selfing S_1 families. The leaf characteristic study also reported for the first time with sibmating in WCT palms.

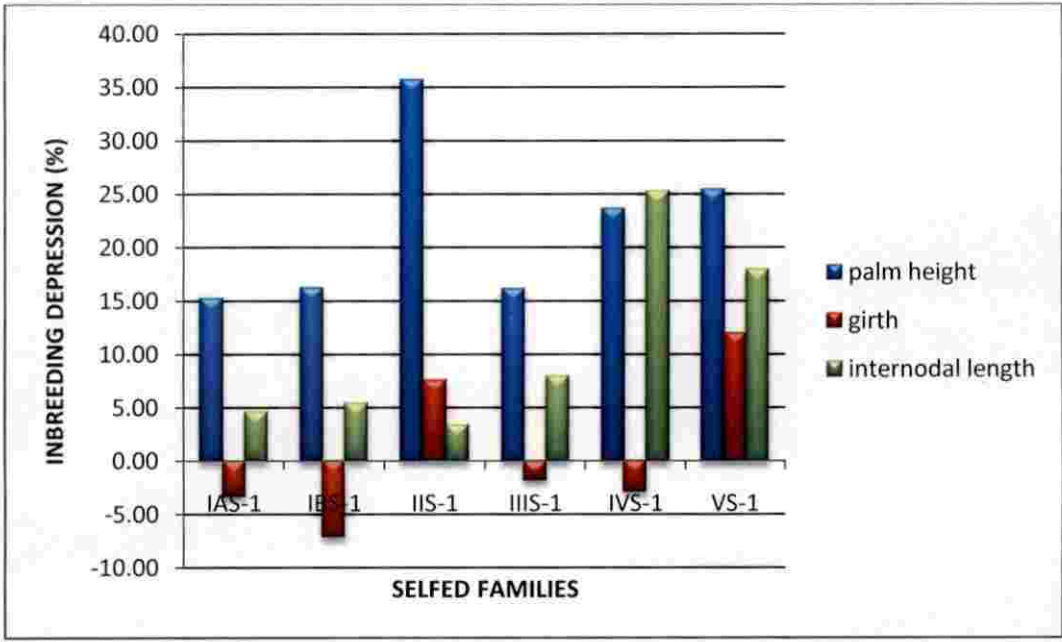


Fig 27: Inbreeding depression of palm height, girth and internodal length of selfed families of WCT coconut

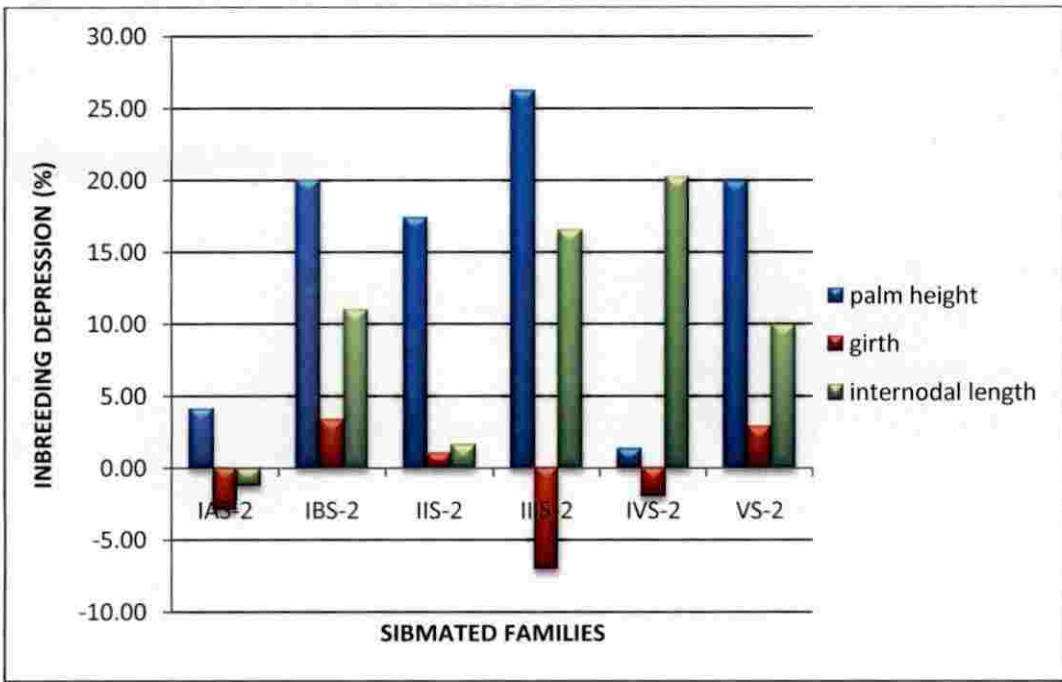


Fig 28: Inbreeding depression of palm height, girth and internodal length of sibmated families of WCT coconut

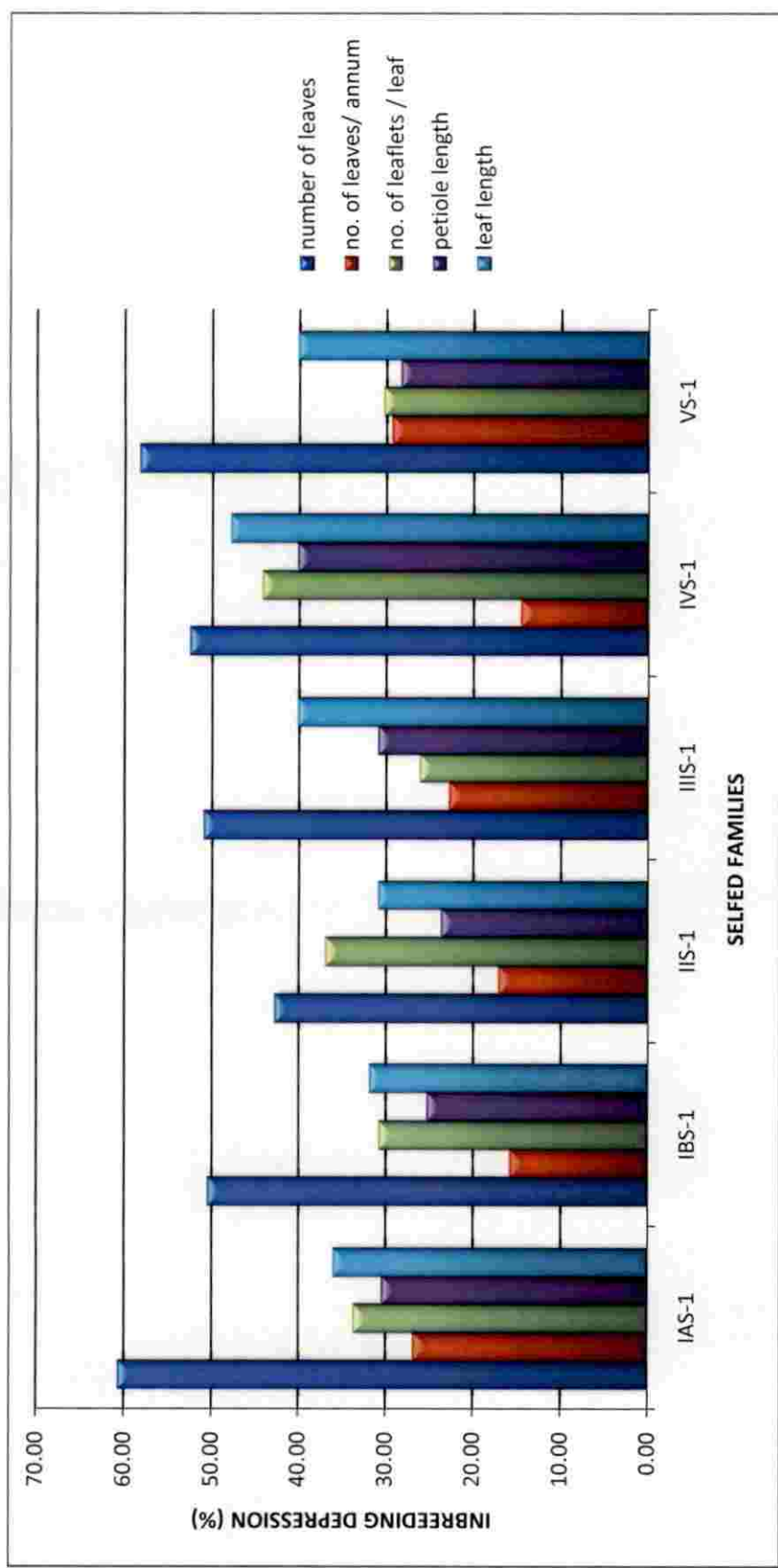


Fig 29: Inbreeding depression of number of leaves, number of leaves per annum, number of leaflets per leaf, petiole length and leaf length of selfed families of WCT coconut

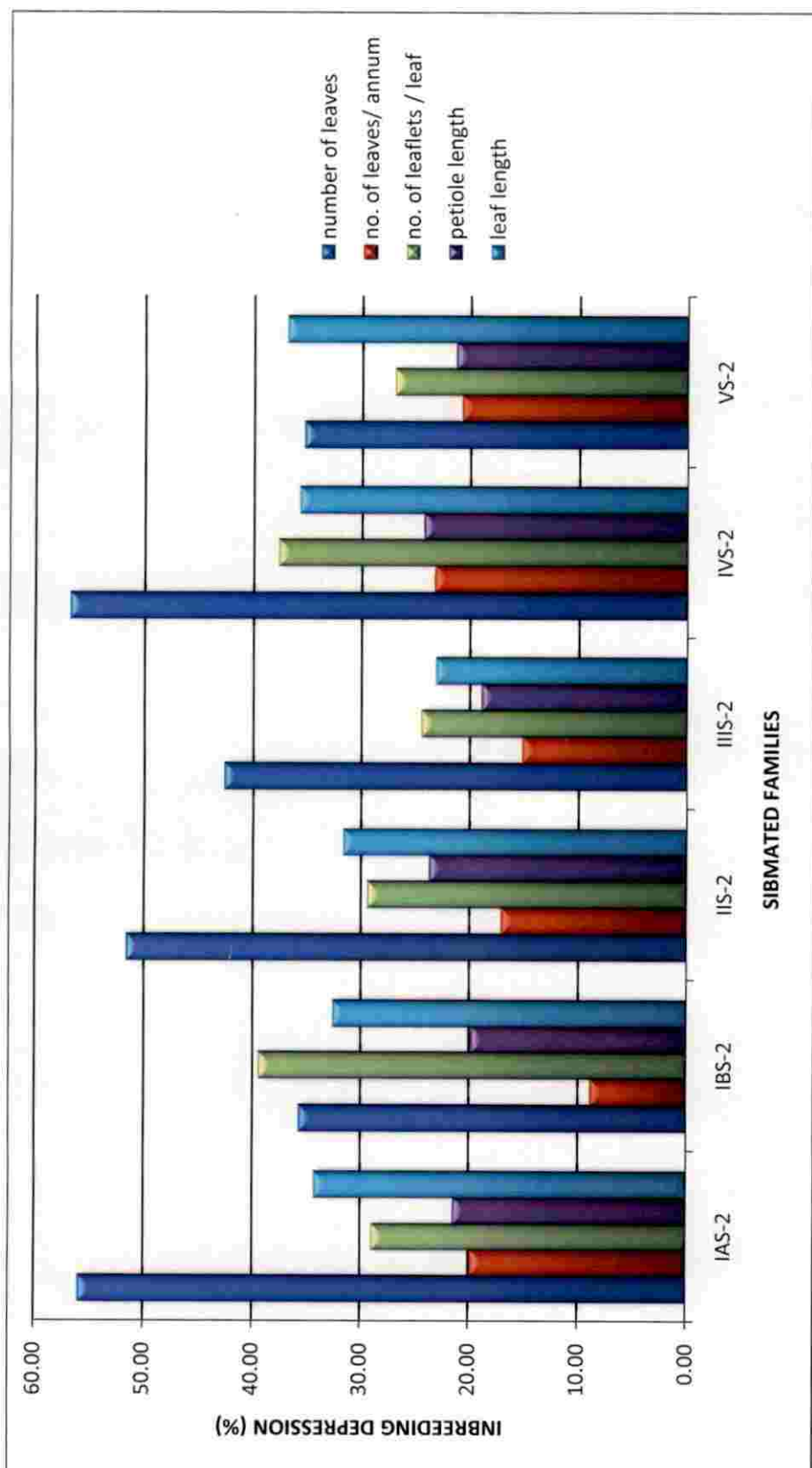


Fig 30: Inbreeding depression of number of leaves, number of leaves per annum, number of leaflets per leaf, petiole length and leaf length of sibmated families of WCT coconut

5.2.2. Reproductive characters

The study was conducted for eight reproductive characters viz. number of female flowers per inflorescence, period of male phase, period of female phase, setting percentage, peduncle length, number of spadices, number of yielding bunches, number of nuts per bunches and nut yield. Almost all the characters exhibited positive and high inbreeding depression when selfed as well as sibmated the six groups of WCT coconut, While negative inbreeding depression was exhibited in the characters like period of female phase and setting percentage (Fig). The inbreeding depression was also reported by Pandin (2009) in number of bunches per tree, number of female flowers, and length of inflorescence stalk in S_2 stage of Mapanget Tall Coconut. He further continued selfing for S_3 and S_4 generations and found that the above generative characters occurred inbreeding depression even in fourth generation with percentage.

The reproductive characters are generally correlated with nut yield in coconut. The female flower production decreased in S_2 generation due to inbreeding within the families of open pollinated WCT. Even though male phase didn't show much of inbreeding in S_2 generation, the female phase exhibited negative inbreeding depression in both selfed and sibmated palms of second generation which indicating no change in length of female phase which was 3 to 4 days in general. The setting percentage was also didn't bring depression in second generation. It was same as that of open pollinated WCT, except in a green coloured group IAS_2-1 , orange coloured IBS_2-1 and other yield groups VS_2-1 , IIS_2-1 and IVS_2-2 (Fig). Number of fruit bearing bunches expressed high inbreeding depression throwing light on the action of increasing infertility in WCT coconut. However, number of nuts per bunches didn't show high inbreeding depression except IAS_2-1 and IVS_2-1 in second generation. It denotes the family specific to green coloured and high yielding WCT coconut group. However, nut yield showed high inbreeding depression in both selfed and sibmated families. The nut yield from IAS_2-1 and IBS_2-1 of sibmates were lesser than selfed palms.

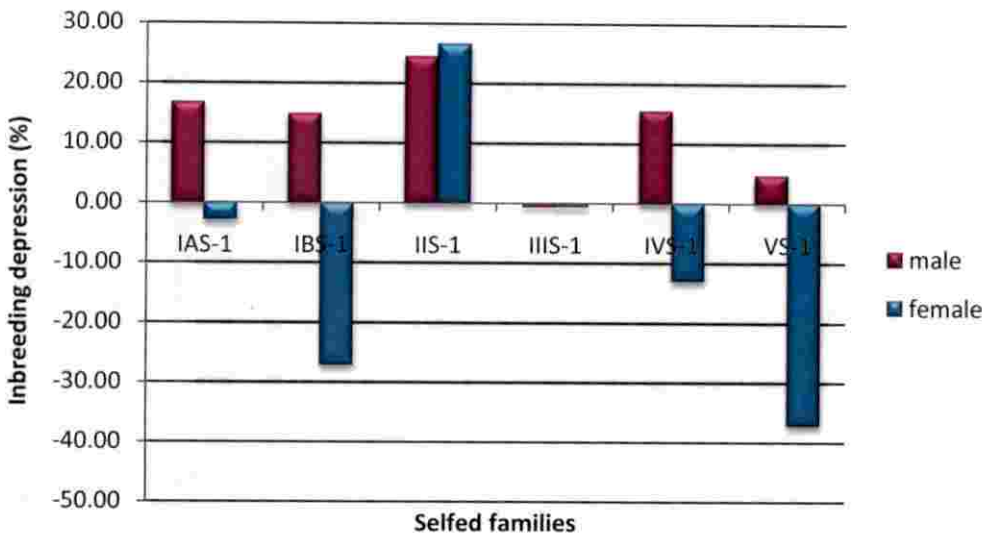


Fig 31: Inbreeding depression of Period of flowering phase in days of selfed families of second generation WCT coconut

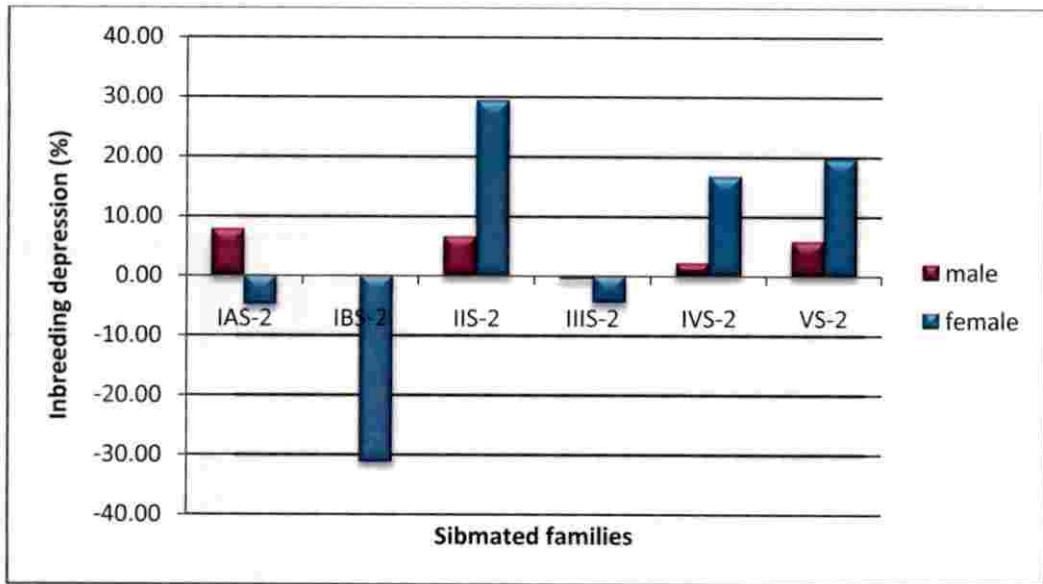


Fig 32: Inbreeding depression of Period of flowering phase in days of sibmated families of second generation WCT coconut

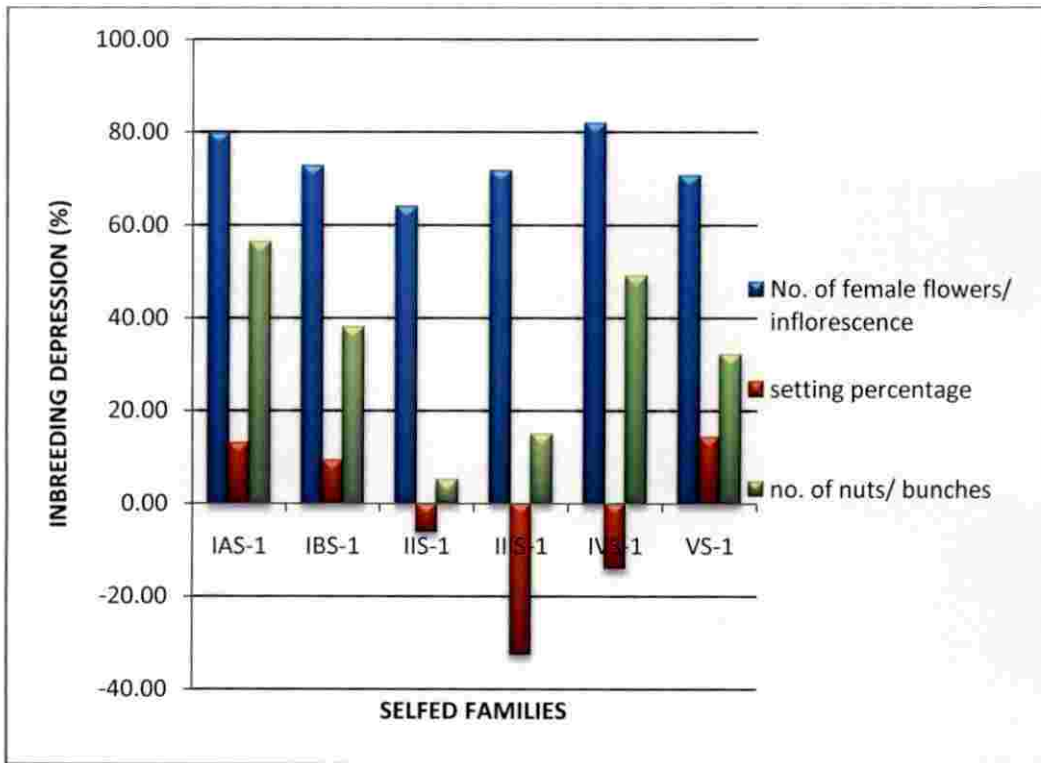


Fig 33: Inbreeding depression of number of female flowers per inflorescence, setting percentage and number of nuts per bunches of selfed families of second generation WCT coconut

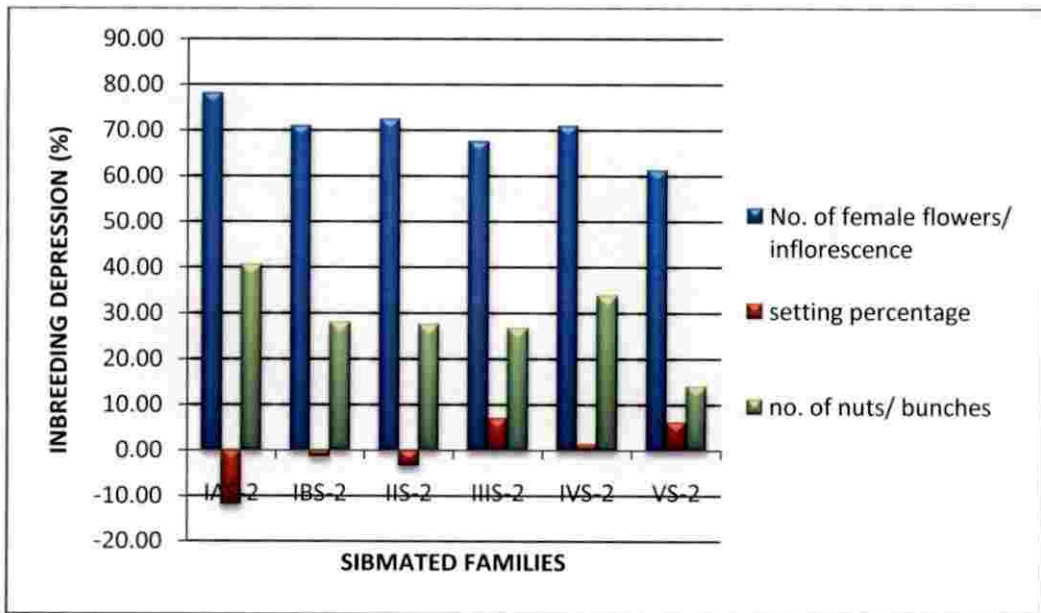


Fig 34: Inbreeding depression of number of female flowers per inflorescence, setting percentage and number of nuts per bunches of sibmated families of second generation WCT coconut

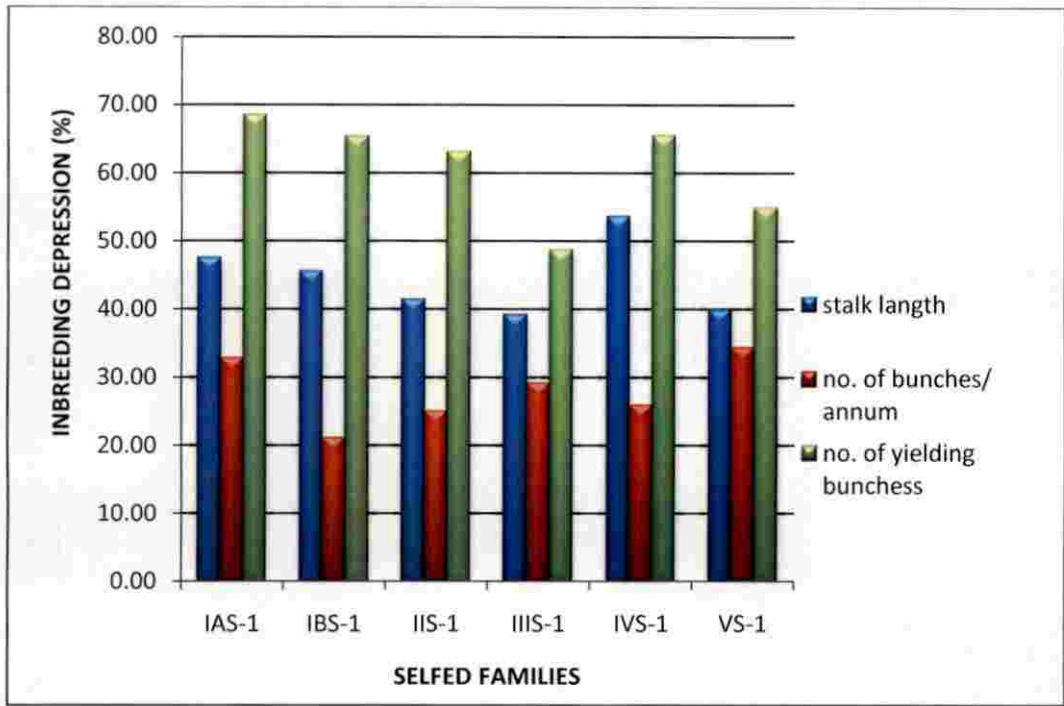


Fig 35: Inbreeding depression of stalk length, number of bunches per annum and number of yielding bunches of selfed families of second generation WCT coconut

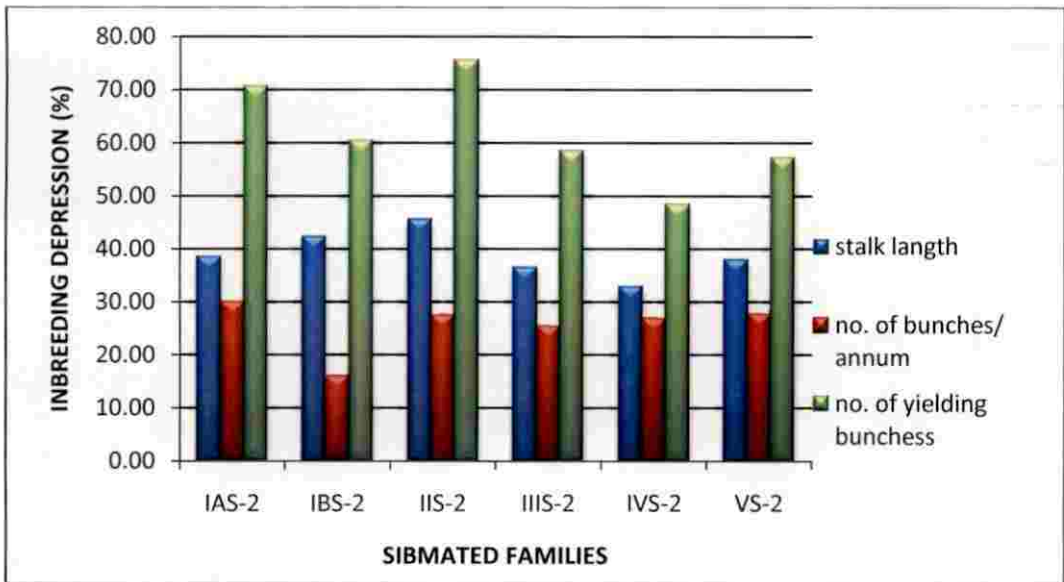


Fig 36: Inbreeding depression of stalk length, number of bunches per annum and number of yielding bunches of sibmated families of second generation WCT coconut

Similar results were reported by Nair and Balakrishnan (1991). Further all other sibmates had given higher yield over second generation selfs.

5.2.3. Nut characters

It is evident that families differ significantly in their behaviour in inbreeding depression of selfs and sibmates. About nine characters were used for studies on inbreeding depression. They were size of nut, weight of unhusked nut and husked nut, diameter of eye, weight of fresh mature kernel, kernel thickness, copra content, oil content and volume of tender nut water. The families differ significantly for all the above said characters. All the nut characters expressed positive and high inbreeding depression for all the characters except diameter of eye which exhibited no inbreeding depression or negative inbreeding depression (Fig). The inbreeding depression of size of nuts was marginal or very less in all the families. The weight of unhusked nut exhibited high inbreeding depression in all the selfed and sibmated families in second generation palms. Kernel thickness also expressed marginal inbreeding depression in all the families of selfed and sibmated S_2 generation of WCT. Similarly the oil content of coconut showed low to moderate inbreeding depression in all the twelve families. The inbreeding depression for tender nut water was high in both selfed and sibmated families of S_2 generation. The high inbreeding depression for fruit component was also reported by Pandin (2009). He reported that the size and weight of fruit approached dwarf coconut after fourth generation of Mapanget Tall Coconut-32.

Inbreeding in coconut was studied by Liyange (1969) on characters like endosperm weight (kernel), embryo weight, leaf production and flowering period and reported that inbreeding depression existed in this characters as compared to open pollinated progenies and the intensity varied among the families. He also found that the endosperm weight and embryo weight were related to the breeding value of grandparents. The result obtained in this study for the number of nuts is in agreement with the above findings with respect to kernel weight and nut weight.

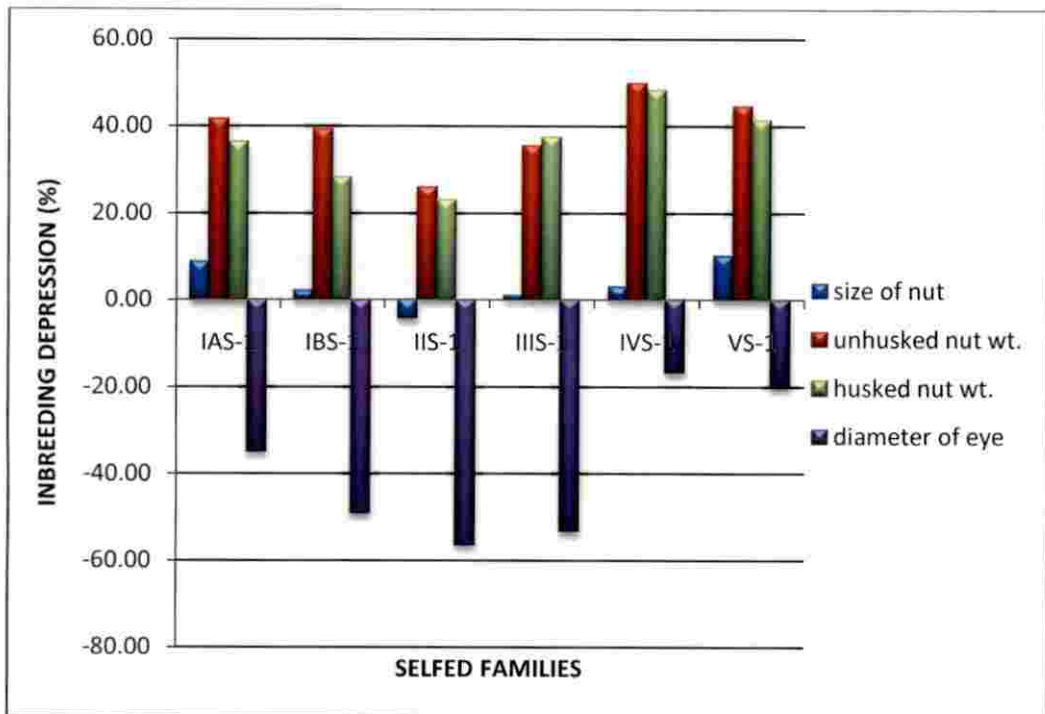


Fig 37: Inbreeding depression of size of nut, unhusked nut weight, husked nut weight and diameter of eye of selfed families of second generation WCT coconut

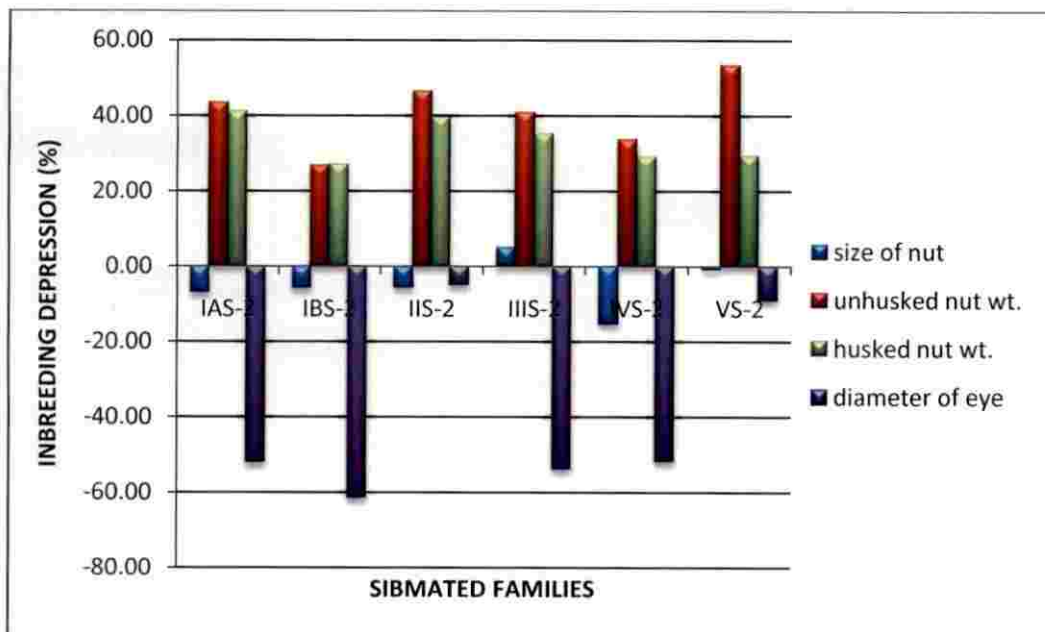


Fig 38: Inbreeding depression of size of nut, unhusked nut weight, husked nut weight and diameter of eye of sibmated families of second generation WCT coconut

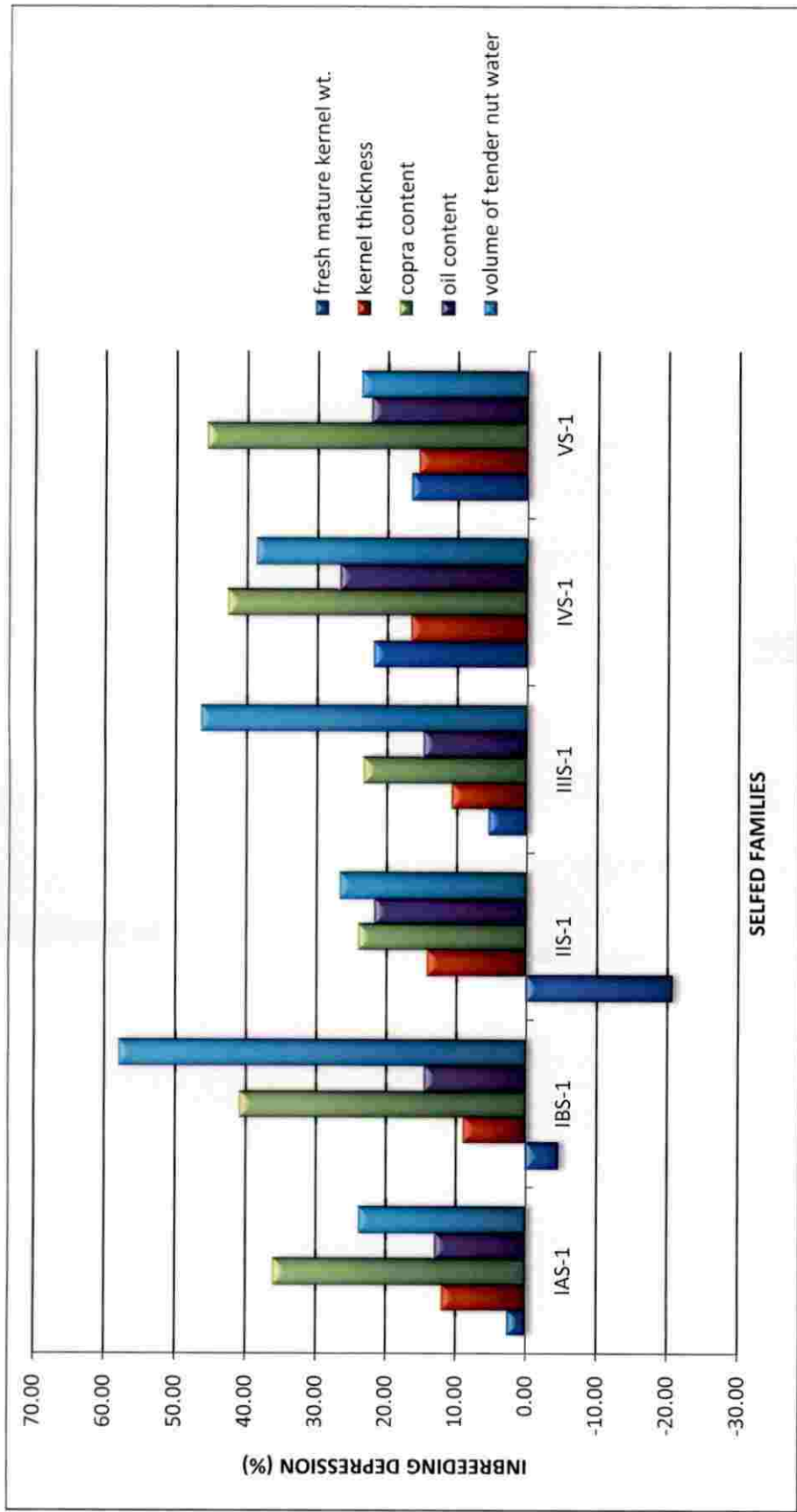


Fig. 39: Inbreeding depression of fresh mature kernel weight, kernel thickness, copra content, oil content and volume of tender nut water of selfed families of WCT coconut

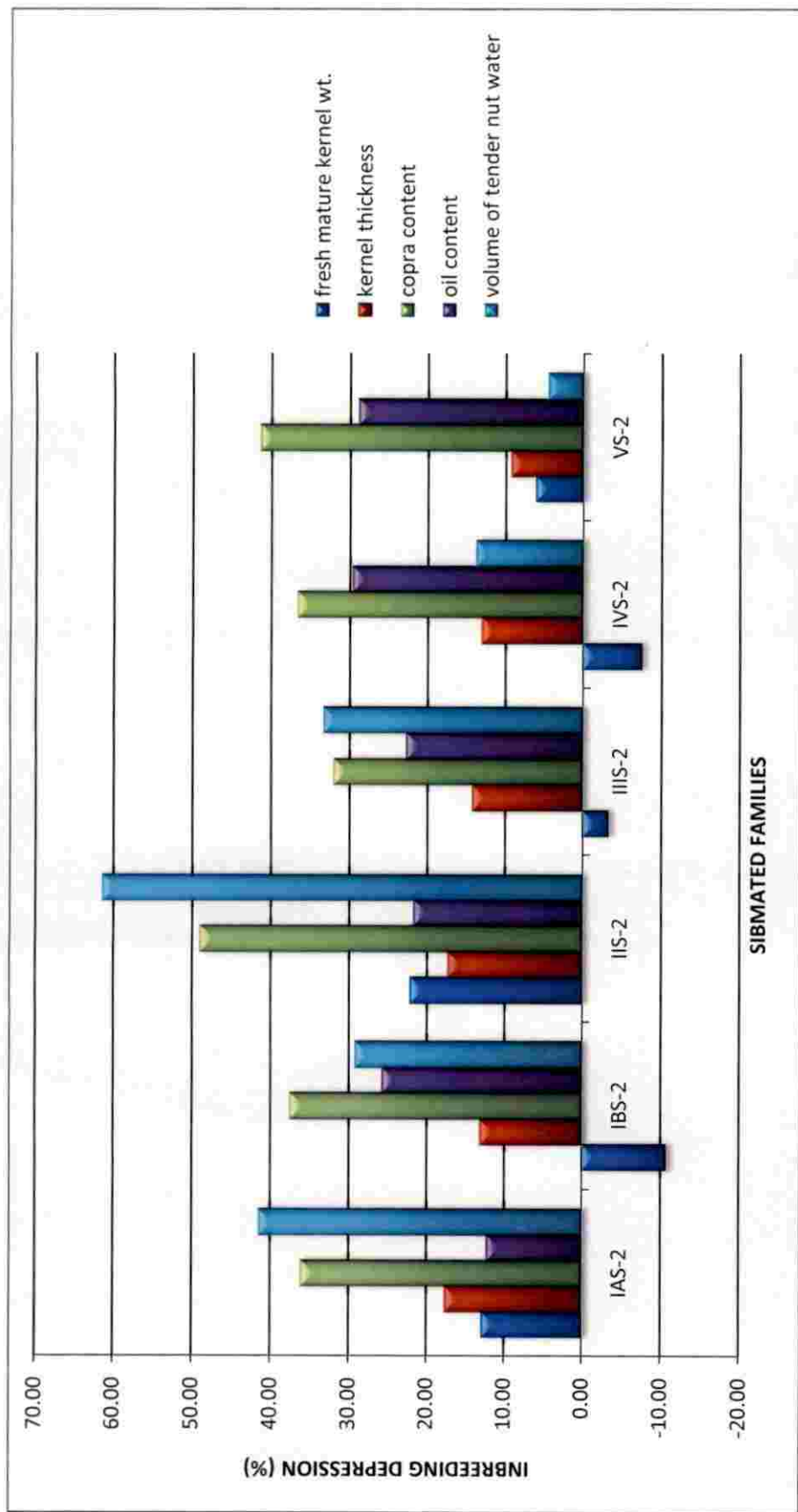


Fig 40: Inbreeding depression of fresh mature kernel weight, kernel thickness, copra content, oil content and volume of tender nut water of sibmated families of WCT coconut

Nambair et al. (1970) opined from his cytological studies on different varieties of coconut that open pollinated varieties showed more chromosomal aberrations and higher percentage of pollen sterility than the self pollinated dwarf varieties. It is evident from the studies that selfing of Tall coconut palms would be expected to reduce vigour and it would be possible to develop inbred lines which could be used for the production of hybrids for higher productivity.

5.3. CHARACTERIZATION OF S₂ PALMS

The first systematic classification of coconut varieties and forms was reported by Narayana and John (1949). This was followed by Liyanage (1958) based on the plant habit and geographical source while Fremond et al. (1966) classified on the basis of floral biology. Even Harris (1978) and Rao and Pillai (1982) attempted the classification based on fruit components, mainly the husk weight and copra weight. Even with all these, it was difficult to identify the cultivars. So, the classification should be done with the parameters which can be assessed accurately.

Basically, the coconut has classified into tall and dwarf, whereas the dwarf without the improvement can call it as semi tall (Pillai et al., 1991). The studied palms of second generation of selfed and sibmated families were characterized using the following important characters viz. palm height, girth of palm, functional leaves, and female flowers per inflorescence, setting percentage, nut yield, copra content and oil content. In selfed S₂ palms based on the above mentioned characters the family has been categorised as tall, semi tall and dwarf. The coloured type IBS₂-1 was characterized to be tall palm within the families of selfed which representing the tall height, stouter girth, medium functional leaves and produced average female flowers with less setting percentage and low yield as well as the nut with the medium copra and high oil content was observed in the family.

Table 23: Characterization of S₂ palms (selfed)

Characters	IAS ₂ -1	IBS ₂ -1	IIS ₂ -1	IIIS ₂ -1	IVS ₂ -1	VS ₂ -1
Palm height	Semi tall	Tall	Dwarf	Semi tall	Semi tall	Semi tall
Girth of palm	Stouter	Stouter	Smaller	Stouter	Stouter	Smaller
Functional leaves	Less	Medium	High	High	High	Less
Female flowers	Less	Average	High	Average	Less	Average
Setting percentage	Less	Less	Medium	High	Medium	Medium
Nut yield	Low	Low	Medium	Medium	Low	Low
Copra content	Medium	Medium	High	High	Medium	Low
Oil content	High	High	High	Low	Low	High

Table 24: Characterization of S₂ palms (sibmated)

Characters	IAS ₂ -2	IBS ₂ -2	IIS ₂ -2	IIIS ₂ -2	IVS ₂ -2	VS ₂ -2
Palm height	Tall	Semi tall	Semi tall	Semi tall	Tall	Semi tall
Girth of palm	Stouter	Stouter	Stouter	Stouter	Stouter	Stouter
Functional leaves	Less	High	Medium	High	Medium	High
Female flowers	Less	Average	Less	Average	Less	High
Setting percentage	Medium	Medium	Medium	Medium	Medium	Medium
Nut yield	Low	Medium	Low	High	Medium	High
Copra content	Medium	Medium	Low	Medium	Medium	Medium
Oil content	High	Low	High	High	Low	Low

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The semi tall characters were noticed in the IIS₂-1 palms which recorded the semi tall stem with stouter girth and produced the average number of functional leaves, the inflorescence with average production of female flowers but the setting percentage was high so that the medium nut yield was noticed with the high copra and low oil content.

The characters of dwarf palms were observed in IIS₂-1 within the selfed families of WCT coconut where the height was short, smaller girth, produce high number of functional leaves with more inflorescence and high number of female flowers with medium setting percentage and nut yield. While the copra content was high with high oil content signifies the dwarf family.

In the sibmated families, the families showing semi tall characteristics were family IIS₂-2 and VS₂-2 while characters of tall palms were noticed in IAS₂-2. The sibmated family of IAS₂-2 was similar to the selfed family IAS₂-1 except for the medium setting percentage and palm height. The semi tall observed in the sibmated families in this study was almost similar to each other in the characters except for the female flowers and oil content. The semi tall type represents with the semi tall height of palm, stouter stem and produced the high number of functional leaves which bear the inflorescence containing average or high female flowers with the medium setting percentage and high nut yield. The semi tall produces the medium copra with high or low oil content.

5.4. SEEDLING VARIABILITY

Being naturally heterozygous, cross pollinated crop with a long gestation period, the progeny of the selected palms may not breed true. Thus it is a challenge to the breeders for developing true to type or inbreds and the farmers to raise the crop commercially economical. There must be some pre requisites to be followed before selection of seedlings and raising of crop. So, ideal seedlings have to be selected based on certain criteria correlated with high yield. Earlier studies had confirmed that early germination of seednuts, rapid growth, and more number of leaves and good vigour seedlings were highly correlated with high and

regular yield (Jack and Sands, 1929; Patel, 1938; Liyanage, 1955, Charles, 1959; Satyabalan et al., 1964; Nampoothiri et al., 1975). The variability in third generation seedlings of different selfed families of selfed and sibmated S₂ palms are discussed in light of the following characters viz. seedling height, collar girth, number of leaves and other characteristics of leaf (leaf length, leaf width, petiole length of 3rd and 5th leaf). The variability existing in each family was classified into high, medium and low mean values groups. The germination percentage recorded on third generation (S₃) seednuts. The results showed that the germination was higher in IBS₃-1 and low in IAS₃-2. Ofcourse, the IBS₃-1 was a orange coloured group. On an average the germination percentage was less in majority of the families in S₃ generation. The open pollinated WCT seedlings has 94.5% germination, all other S₃ families were less than 50% of seed germination. This clearly indicates that the germination of S₃ seednuts was low because of effect of inbreeding by selfing the S₂ parents.

Height of seedling is a primary selection criteria as it show a positive correlation with yield in coconut. The S₃ generation generally produced more of medium tall seedlings (IAS₃-1, IIS₃-1, IIIS₃-1, IVS₃-1, IIS₃-2, IIIS₃-2, VS₃-2) followed by tall (IBS₃-1, IBS₃-2, IVS₃-2, IAS₃-2) and one short (IAS₃-1). The results indicate that the proportion of medium or semi tall was comparatively more in third generation (S₃) of selfed (S₂) than sibmated (S₂). Similarly the percentage of tallness was more (IBS₃-2) in third generation (S₃) of sibmated palms (S₂) than selfed (IBS₃-1). This informed that the heterotic behaviour expressed by sibmated family was continued in third generation on selfing also. Especially IVS₃ family didn't have dwarf seedlings, only tall were present. However the IBS₃, the coloured type naturally expressed tallness in both on selfing (S₃) of sibmated and selfed (S₂). This is in analogy with the findings of Nair and Balakrishnan (1991) in WCT coconut on selfing and sibmating in S₂ generation.

With regard to collar girth yet another important character for selection criteria of vigorous seedlings contributing for nut yield (Pankajakshan and

George, 1961; Ramadasan *et al.*, 1980). The number of S₃ seedlings expressing stouter collar girth was few, that too only in third generation progenies of sibmated palms (S₂). This further confirms the heterotic behaviour of sibmating WCT coconut in development of inbreds. The percentage of occurrence of seedlings with stouter collar girth (narrow) was more in S₃ generation (IAS₃-1, IBS₃-1, IIS₃-1 in selfed and IAS₃-2, IIS₃-2, IIS₃-2, VS₃-2 in sibmated). The medium stout collar girth were found more in S₃ seedlings of selfed (S₂) palms (IIS₃-1, IVS₃-1, VS₃-1) followed by sibmated (S₂) types (IBS₃-2 and IVS₃-2). The WCT generally had narrower collar girth while dwarf had stouter ones.

Sukumaran and Iyer (1982) observed the differences in the total leaf production of WCT coconut seedlings. Similarly, the families noticed with wide variation for the production of leaf in seedlings of S₃ generation. In both selfed and sibmated (S₂) families highly produced were moderate range (5 to 7) leaves. All the seedlings (S₃) of selfed (S₂) produced leaves moderately, while four families only in four sibmated types (IAS₃-2, IBS₃-2, IIS₃-2 and VS₃-2). The sibmated types (S₂) were found with very less number of leaves (2 to 4) i.e., in IIS₃-2 and IVS₃-2. This significantly indicates that the seedlings are exhibiting the inbreeding depression because of selfing. Liyanage (1969) also reported similar results that the inbred plants carried less number of leaves than open pollinated plants. With the 16 families of WCT coconut seedlings also showed the significant difference in collar girth and leaf production (Satyabalan, 1974).

The leaf characters recorded were the leaf length, breadth and petiole length from third and fifth leaf. The third leaf produced was more of medium long (90 to 150cm) from the seedlings of selfed (IAS₃-1, IBS₃-1, IIS₃-1 and IVS₃-1) and sibmated (IIS₃-2, IIS₃-2, IVS₃-2 and VS₃-2) families. The observation on breadth of third leaf also showed that the leaves of S₃ generation were moderately broader in all the five families (IBS₃-2, IIS₃-2, IIS₃-2, IVS₃-2 and VS₃-2) of sibmated types (S₂) and four families of selfed types (IAS₃-1, IIS₃-1, IIS₃-1 and IVS₃-1). The leaves of coloured type selfed in S₂, IAS₃-2 produced narrow leaves in third generation seedlings. However, the VS₃-1 had broader third leaf in S₃

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generation. Mathew and Gopimony (1991) reported that the seedling height, girth of collar and number of leaves had a correlation with leaf area. As in leaf length and breadth, the petiole length of leaf also showed a medium length (30 to 60cm) in all the selfed (S_2) types and four families of sibmated type (S_2) in third generation seedlings. Only IBS₃-2 and IAS₃-2 developed long petiole in S_3 generation. This again throw light on the heterotic tendency of sibmated families (S_2) continuing in third generation (S_3).

The observation on fifth leaf parameters also exhibited moderate number of leaf length (5 to 7 numbers). The fifth leaf was absent in IAS₃-2 a less vigorous family showing inbreeding depression. The short and medium long length of fifth leaf was seen in five and six families each respectively. The four families (IAS₃-1, IIS₃-1, IIS₃-1 and VS₃-1) of selfed (S_2) and two sibmated families (S_2) had shorter leaf (IIS₃-2 and IIS₃-2). In selfed type, IBS₃-1 and IVS₃-1 had medium long (75–125cm) leaves and in sibmated type (S_2) found the medium long leaf in three families (IAS₃-2, IBS₃-2 and VS₃-2). All the families had developed mostly medium broader fifth leaves (13-21cm). The petiole length of fifth leaves were mostly short (10 to 35 cm) except IBS₃-1, IAS₃-2 and IBS₃-2.

Mostly highest frequencies of seedling parameters occurred in the category of medium range. It shows the effect of transition of open pollinated WCT from outcrossing to selfing under inbred development efforts in third generation. This also shows the variation quantitatively shifting to the homozygosity due to selfing in WCT palms. Some studies revealed that both collar girth and number of leaves in seedlings show correlation with the yield (Satyabalan and Mathew, 1976; Nampoothiri et al, 1975). This may say that the reducing collar girth may indicate that the some seedlings are proceeding towards the dwarf.

5.5. INBREEDING DEPRESSION OF SEEDLINGS

The mean and inbreeding depression value of vegetative characters on self-pollination of third generation seedlings with twelve families of selfed and

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sibmated WCT coconut (S_2) were presented in Table 19 and 20. The seedlings of third generation revealed inbreeding depression for the characters: germination percentage, seedling height, collar girth, number of leaves, third leaf length, breadth and petiole length, and fifth leaf length, breadth and petiole length.

Germination percentage revealed the positive inbreeding depression in all the selfed (S_2) families of selfed and sibmated (S_2). Higher inbreeding depression was observed from family IAS₃₋₂, IIS₃₋₂, IVS₃₋₂, VS₃₋₁, IVS₃₋₁ and lowest in family IBS₃₋₁ and IAS₃₋₁. The seednuts harvested was produced by the method of pollination which is through selfing; hence the nuts will go through the barriers during sprouting.

Seedling height expressed inbreeding depression in third generation of all the families of twelve groups. In third generation high inbreeding depression was observed in the family VS₃₋₁ followed by IIS₃₋₂, IIS₃₋₂, VS₃₋₂ and IAS₃₋₁. But families IBS₃₋₁ and IBS₃₋₂ showed lesser or no inbreeding depression indicating that the family reached more or less homozygous condition itself for plant height. The inbreeding depression was comparatively high after selfing of second generation sibmated families compared to all the selfed families except IVS₃₋₂ which showed very less inbreeding depression for seedling height. The result indicates that the pathway to develop inbreds by selfing in selfed families is faster than sibmated families. This reveals that heterogeneity is maintained due to sibmating and may require more generations for attaining homogeneity in the selfed progenies of the sibmated families. However the sibmating was found to be right technique in breeding for maintaining vigour in coconut.

The collar girth is an important parameter for selection of seedlings for high yield and a prepotent palm. It will take only few generations for developing inbreds for this character. Collar girth of the seedlings showed no inbreeding depression compared to the seedling height in all the families. The third generation showed inbreeding depression for the families IIS₃₋₁ and IIS₃₋₂ only. The inbreeding depression of the collar girth showed lower rate than the seedling

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height which signifies that the seedling height showed greater response for the selfing than the collar girth. The seedlings in nursery could nearly resemble to the dwarf coconut seedlings by its appearance where Pandin, 2009 reported the similar results.

The number of leaves of seedlings were analysed for the inbreeding depression in third generations of selfed and sibmated palms. The result revealed a wide range of variation in inbreeding depression. The number of leaves of family IVS₃-2, IIS₃-2, IIIS₃-2 in a sibmated and IIS₃-1 in selfed of S₂ generation palm on selfing for S₃ exhibited high inbreeding depression (above 20), whereas in seedling height also expressed high and for collar girth less inbreeding depression. This characters relates to dwarf stature, hence it may be confirmed that the group IV and II almost reached dwarfness. Similarly almost all families showed inbreeding depression except in VS₃-2 and hence may take more generations to reach inbred line.

The length, breadth and the petiole length of third and fifth leaf were recorded from the seedlings. Almost all families expressed inbreeding depression for the leaf length, breadth and petiole length of third leaf and fifth leaf. The breadth of fifth leaf showed very low inbreeding depression compared to third leaf. For the other two characters viz. leaf length and petiole length inbreeding depression was higher in fifth than third leaf. The fifth leaf was absent in IVS₃-2. All families expressed inbreeding depression for third leaf characters. The S₃ seedlings of the group IB, the orange coloured group expressed low inbreeding depression in selfed third generation of selfed and sibmated palms (S₂) for third leaf characteristics. Hence the group IB, orange coloured group responded faster to inbreeding when selfed and sibmated.

Fifth leaf character was observed for the length, breadth and the petiole length as same as the third leaf. The length of fifth leaf showed higher inbreeding depression in all the families. The observed high inbreeding depressions from families are IIIS₃-2, IIS₃-2, IAS₃-1, IIS₃-1 and IIIS₃-1. For the breadth of third leaf

observed highest inbreeding depressions in the families observed were lesser compared to the other leaf characters where the highest was noticed in VS₃-1, IIS₃-2 and IAS₃-2. The petiole length of the third leaf observed highest inbreeding depression for the families IIS₃-2, VS₃-1, IIS₃-1 and IIS₃-1. Almost all families observed inbreeding depression for the leaf length and petiole length of third leaf while for the leaf breadth has noticed inbreeding depression in seven families only.

The results of the S₃ generation seedling and S₂ generation palms indicated that the inbreeding depression operated in S₂ and S₃ generations were specific to the families and both generations of same family showed similar inbreeding depression generation after generation. Thus it is possible to develop inbred lines in coconut as observed in this study.

5.6. RAPD ANALYSIS

RAPD analysis was done based upon the polymerase chain reaction (PCR) technology. In RAPD, DNA is amplified using random sequences used as primers and the regions of the genome which are complementary are amplified. Scoring is done by comparing the presence or absence of each fragment amplified by the random decamer primer. The analysis will distinguish the similarities or differences between the samples selected, the open pollinated WCT and dwarfs COD and CGD.

From the selected 10 primers, the specific bands for dwarfs were noticed from almost all the primers except for OPAW 09. The bands specific to tall have been noticed from the primer OPBA 03 and OPAU 03. Rajesh *et al.* (2014) also reported that with the primer OPBA 03 could be used to differentiate between tall and dwarfs. The primer OPBA 03 has produced bands specific to tall (950bp) and dwarf (1000bp). Primer OPAU 03 produced two specific bands for tall and two for dwarfs. The two tall specific locus observed were 600bp, 300bp and two dwarf specific were 1300bp 550bp. The specific bands for each genotype were also noticed from different primers such as the WCT specific (OPAU 03),

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CGD (OPAW 15 and OPAW 08), IAS₃-1 (OPAW 12, OPAW 13 and OPAW 08), IBS₃-1 (OPAW 19 and OPAU 03) and IIS₃-1 (OPAW 19).

The analysis by RAPD also signifies that the inbred IIS₃-1 is unique with respect to the absence of most of the bands produced by primers OPBA 03, OPAW 15, OPAW 09 and OPAU 02. Mostly the inbred IIS₃-1 contains only the bands which are monomorphic in all above mentioned primers. These monomorphic bands may be the regions common in all types of coconut palms, irrespective of tall or dwarf. However the primer OPAW 19 could amplify one unique band for this genotype. From the table 19, the characters observed for inbred IIS₃-1 are almost average value between the highest and lowest range.

The coloured types IAS₃-1 and IBS₃-1 were observed as more similar to their parent WCT which were evidently proved by the primer OPBA 03 and OPAW 19. The inbreds IAS₃-1 and IBS₃-1 were also similar to each other from almost 6 primers (OPAW 19, OPAW 15, OPAW 14, OPAW 13, OPAW 12 and OPAU 03) which were actually from the same parent 1/109 except for the coloured difference (Table 1). The family IAS₃-1 has also shared the bands with dwarfs (CGD and COG) and were noticed by the primer OPAW 19, OPAW 08 and OPAU 03. The primer OPAW 09 shared bands between the family IAS₃-1 and CGD and also between family IAS₃-1 with COD. The primer OPAW 08 noticed the specific bands for CGD and also shared with the family IAS₃-1.

Among the primers, highly polymorphic bands between the tall and dwarf were noticed by the OPBA 03 and OPAU 03. The primer OPAW 19 has identified specific for the dwarfs, inbred IBS₃-1 and IIS₃-1 and also noticed the similarities of the family IAS₃-1 with the WCT, CGD, COD, and inbred IBS₃-1.

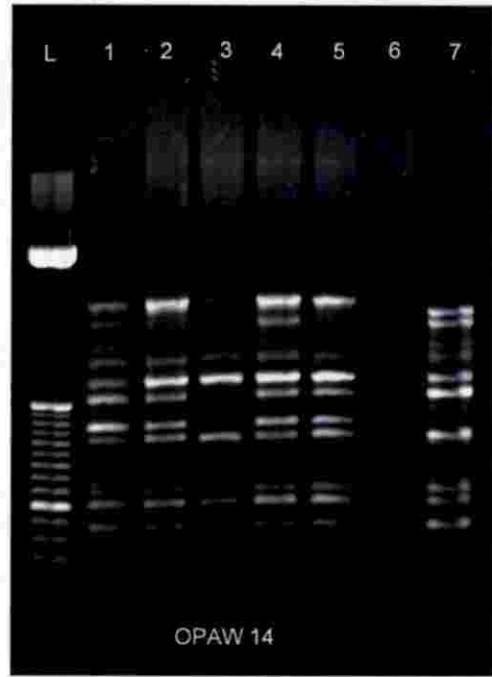
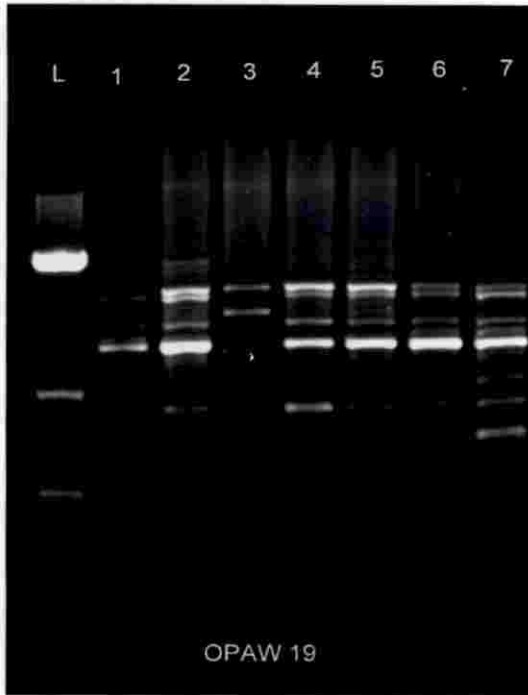
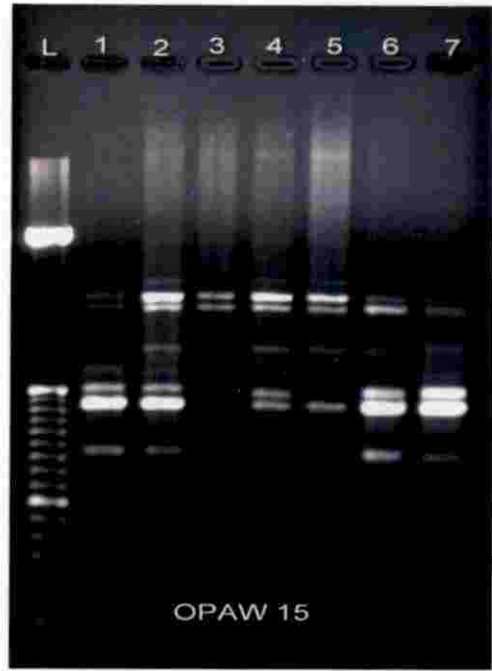
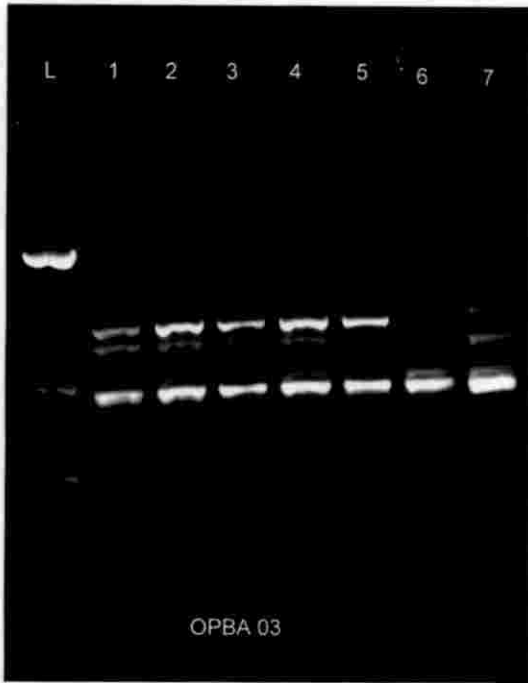


Fig 41: RAPD assay of S₃ WCT families with primers
(a) OPBA 03 (b) OPAW 19 (c) OPAW 15 (d) OPAW 14
L- Ladder; 1- IAS3-1; 2- IBS3-1; 3- IIS3-1; 4- VS3-1; 5- WCT; 6- COD; 7- CGD

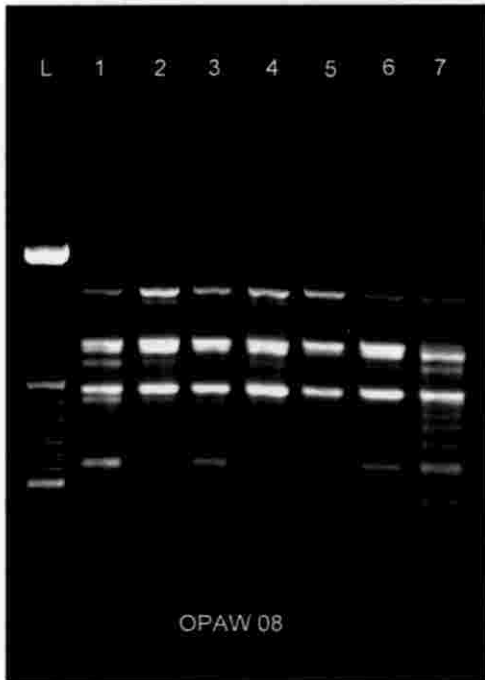
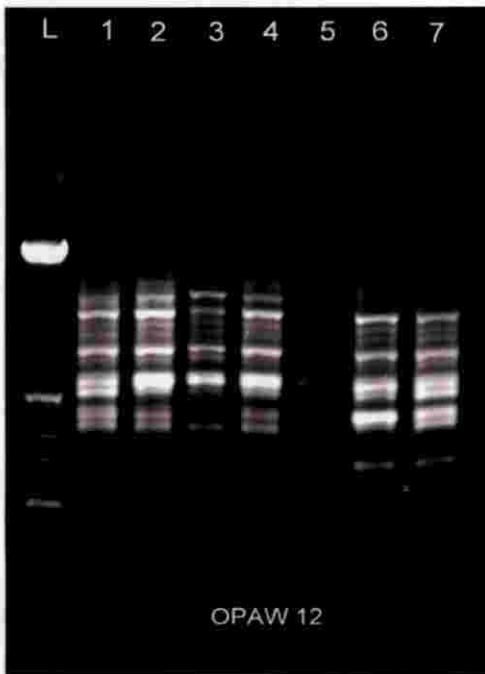
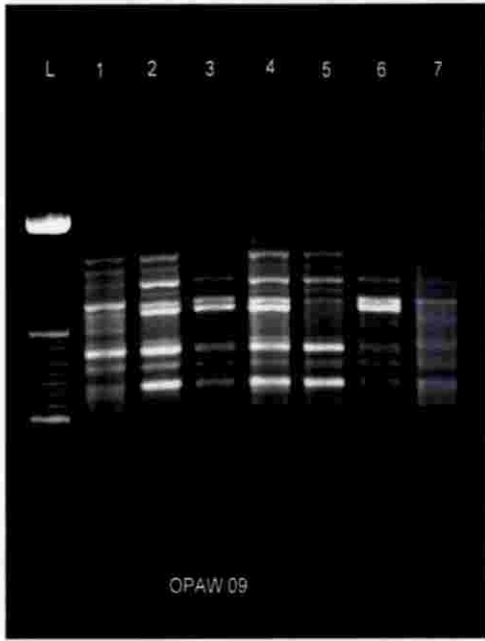
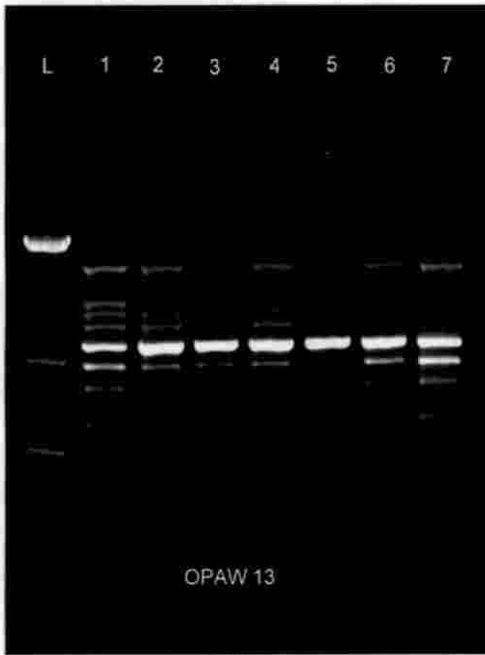


Fig 42: RAPD assay of S_3 WCT families with primers
(a) OPAW 13 (b) OPAW 12 (c) OPAW 09 (d) OPAW 08
L- Ladder; 1- IAS3-1; 2-IBS3-1; 3- IIIS3-1; 4- VS3-1; 5- WCT; 6- COD; 7- CGD

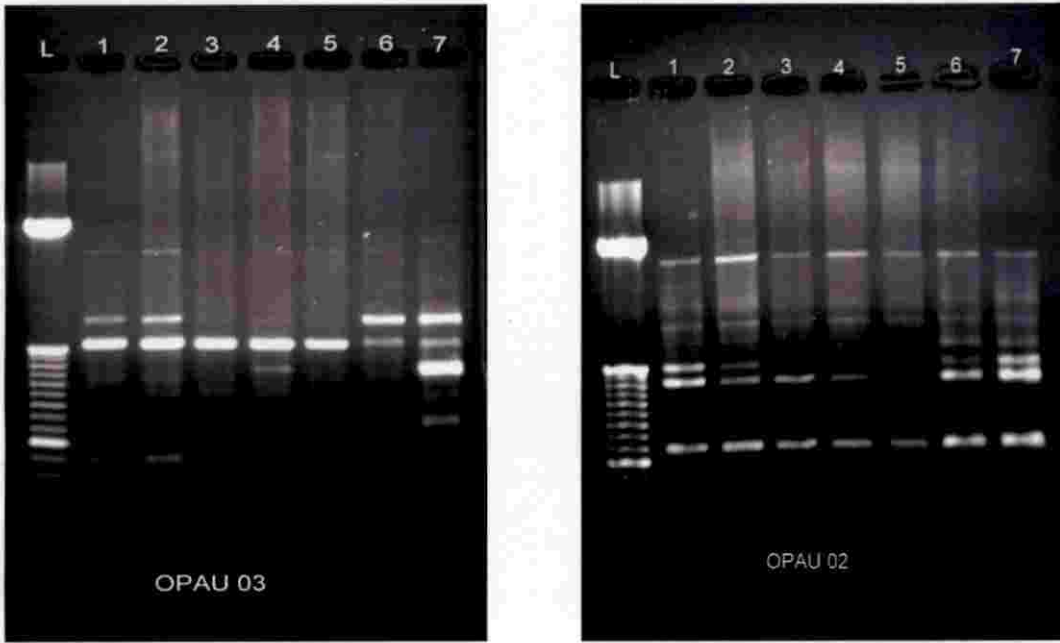


Fig 43: RAPD assay of S_3 WCT families with primers
(a) OPAU 03 (b) OPAU 02
L- Ladder; 1- IAS3-1; 2-IBS3-1; 3- IIIS3-1; 4- VS3-1; 5- WCT; 6- COD; 7- CGD

Summary

6. SUMMARY

In nature, coconut is a cross pollinated crop which influences the high degree of variability and heterogeneous population. The population showing genetically variable is utilized to produce an inbred line for further breeding programme. With the intension of developing inbred line in coconut, the programme was initiated in 1924 by selecting the eighteen WCT palms for selfing to produce the S_1 progenies. The S_1 progenies of six groups of palms were planted in 1927 which were selfed and sibmated to produce the S_2 progenies in 1960 of six groups of WCT and the seedlings planted at RARS, in Pilicode with the replicated trial. This was the present experimental material to characterize and analyze inbreeding depression in S_2 palms and to study the effect of selfing in selfed and sibmated families of West Coast Tall (WCT) coconut palms of Second generation. Of the second generation palms of WCT families were recorded for vegetative, reproductive and nut characters. The performance of 12 families of S_2 palms with WCT (open pollinated), COD and CGD were compared.

Salient features of the study are summarised below:

The analysis of variance revealed significant differences among the twelve families of six groups of WCT for all the characters studied. The tallest palms were observed in IVS_2-2 and IAS_2-2 and on par with the WCT. The shortest palms were recorded from IIS_2-1 and $IIIS_2-2$ but taller than dwarfs. In general the sibmated families were showing more tall stature indicating heterotic behavior. The stem girth was thinner in IAS_2-2 and VS_2-1 . The shorter palms $IIIS_2-2$ and IVS_2-2 showed stouter stem on par with WCT. These indicated the inbreeding leading to semi tall with medium girth at earlier generations. Again the intermodal length was short in $IIIS_2-2$, VS_2-1 and IBS_2-2 indicating expression of dwarfing trait. The internodal length was more in tall palms IAS_2-2 . In general the stem characters indicated that the IAS_2-2 was tall with related trait like stem girth and intermodal length. Similarly the shortest and semi tall stem character were recorded in $IIIS_2-2$ and VS_2-1 .

The IAS₂-1 and IBS₂-1 showed average number of functional leaves as tall while IIS₂-1 had less number of leaves similar to dwarfs. The family IIS₂-2, IVS₂-1 and IBS₂-1 produced more number of leaves. The petiole length was short in IAS₂-1 and IAS₂-2 as well as IIS₂-1, IVS₂-2 and VS₂-1 indicating an advantageous characters high bearing of nuts with less loss due to drooping of leaves. More number of leaflets in IAS₂-2 and IBS₂-2 and IIS₂-1, IIS₂-2 providing more leaf area for photosynthesis inturn high yield, a tall to semi tall characters. IVS₂-1 and IVS₂-2 produced less number of leaflets.

IIS₂-1 and VS₂-1 produced more number of female flowers while IAS₂-1 and IAS₂-2 produced less number of female flowers. In IIS₂-1 and VS₂-1, the period of female phase similar as WCT. The female phase was shorter than dwarfs but more than WCT in IIS₂-2 indicating the entrance of sibmated palms to selfing by prolonging the female phase as in dwarfs. The IIS₂-1 showed long male phase again favoring selfing. The setting percentage was high in IIS₂-1 and IAS₂-2. The peduncle length was shorter in all the sibmated groups. The yielding bunch were reducing in S₂ palms due to low vigour on selfing. The nuts per annum were more in IIS₂-1 and VS₂-1 and less in coloured type.

The largest nuts were produced by IVS₂-2 followed by IAS₂-2 and IBS₂-2 in all sibmats. The nut size was similar to dwarf but lower than WCT indictaing deleterious effect of selfing in nut size. Similarly IBS₂-2 and IIS₂-1 exhibited maximum size of unhusked nut weight. VS₂ exhibited low nut weight, size and husked nut weight. Family IIS₂-1 and IBS₂-1 produced more weighing nuts, where as IVS₂-1 shown low nut weights. The naturally evolved type had high percentage of husk hence less weight than open pollinated forms.

IBS₂-2, IIS₂-2 and IIS₂-1, IVS₂-2 and IIS₂-1 recorded larger eye of the nuts. The meat weight was more in IBS₂-2, IBS₂-1 and IVS₂-2. Thickest kernel noticed in IBS₂-1 and VS₂-2 and less thick in IAS₂-1. More thickness of kernel is tall palm character and thin meat is dwarf palm trait. Highest copra in IIS₂-1 followed by IIS₂-1 and lowest in IIS₂-2. All others families showed on par with

COD in copra content. Similarly the selfing made the palm inferior in oil content also. The IAS₂ yielded more oil on par with dwarf but not equal to WCT. This also indicates the selfing lead inferior progeny for oil content. Tender nut water content was more in IVS₂₋₂ and on par with WCT and CGD but inferior to COD. This also indicates that the nuts have become small by selfing.

Inbreeding depression was studied for S₂ nut yield and its attributes. The family IIS₂₋₂ and VS₂₋₁ expressed positive inbreeding depression for palm height and intermodal length, but no inbreeding depression for stem girth. Positive inbreeding depression in all the sibmated families was observed and highest in IIS₂₋₂ and lowest in IAS₂₋₂ for these traits. The leaf characteristics also exhibited positive inbreeding depression in S₂. The low value for these characteristics showed that the S₂ families were moving towards the dwarf palm characters by selfing. IAS₂₋₂, IIS₂₋₂ and IVS₂₋₂ showed high inbreeding depression when sibmated.

All eight reproductive characters exhibited high inbreeding depression in both selfed and sibmated. The setting percentage didn't bring inbreeding depression in S₂ palms of both selfed and sibmated. Similarly nuts per bunch also didn't show inbreeding depression. In general, the sibmated families showed less or no inbreeding depression indicating a low heterosis. All the nine characters studied showed inbreeding depression to different extent except diameter of eye. It is evident from the studies that the selfing of WCT palms would reduce vigour and possible to develop inbred lines which could be used for the production of hybrids for higher productivity.

The WCT palms in S₂ generation were characterized primarily based on eight characters separately when selfed and sibmated in S₁ for S₂ generation. In selfed the IBS₂₋₁ showed tall characters with highest stem, stouter stem, medium functional leaves, average female flowers, less setting percentage and low yield, medium nut, high oil percent. Semi tall characters in IIS₂₋₁ which recorded semi tall, stouter stem, average functional leaves, and average inflorescence produced,

average female flowers, medium setting percentage, high copra and low oil content. Dwarf characters observed in IIS₂-1 short, thin stem, high functional leaves, more inflorescence, high female flowers, medium setting percentage, nut yield, copra and oil content high. In sibmated IIS₂-2, VS₂-2 expressed semi tall characters and IAS₂-2 exhibited tall palm characters. The IAS₂ on selfing and sibmating expressed tallness only.

The third generation progenies were developed from second generation palms by selfing the sibmated and selfed families. The germination percentage was higher in IBS₃-1, IAS₃-1, IIS₃-1, IBS₃-2 and VS₃-2. On average, the germination percentage was less in majority of the families. The developed seedlings were analysed for the variability present within families before selection of seedlings for all the seedling characters. The seedlings of S₃ generation generally produced more of medium tall seedlings which was followed by tall (IBS₃-1, IBS₃-2, IVS₃-2, IAS₃-2) and one short (IAS₃-1). Comparatively the selfed S₂ palms produced more medium or semi tall seedlings than the sibmated families. The group IBS₃ naturally expressed tallness in both selfed and sibmated families and the family IVS₃-2 didn't produce any dwarf seedlings.

The other important characters like collar girth and number of leaves were observed the variability. The collar girth expressed less in almost all the families but the medium high girth was high in family IIS₃-1, IVS₃-1, VS₃-1, IBS₃-2 and IVS₃-2. The seedlings expressing stouter collar girth was only in few families that too in sibmated progenies. Almost all the families of selfed produced the leaves from 5 to 7 i.e., in moderate range including four sibmated types (IAS₃-2, IBS₃-2, IIS₃-2 and VS₃-2). The sibmated types were found with very less number of leaves in IIS₃-2 and IVS₃-2. Even tall types were observed from families IIS₃-1, IVS₃-1, IAS₃-2, IBS₃-2 and VS₃-2.

The third leaf length were medium long in all the families whereas the breadth also with moderately broader except in family IBS₃-1 and IAS₃-2. As in leaf length and breadth, petiole length was also medium long except the family IBS₃-2

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and IAS₃-2 which produced the long petiole. However, the fifth leaf was observed as short in five families (IAS₃-1, IIS₃-1, IIIS₃-1, VS₃-1 and IIIS₃-2) and medium long in six families (IBS₃-1, IIIS₃-1, IVS₃-1, IAS₃-2, IBS₃-2 and VS₃-2). All the families developed the medium broader fifth leaves and short petiole length. The families of IBS₃-1, IAS₃-2 and IBS₃-2 expressed with medium long petiole length.

Inbreeding depression for the seedlings in third generation was also observed. The seedling height showed high inbreeding depression in family VS₃-1 followed by IIS₃-2, IIIS₃-2, VS₃-2 and IAS₃-1. But families IBS₃-1 and IBS₃-2 showed lesser or no inbreeding depression indicating that the family reached more or less homozygous condition itself for plant height. Collar girth of the seedlings showed no inbreeding depression compared to the seedling height in all the families. The high inbreeding depression for the families IIS₃-1 and IIS₃-2 whereas low inbreeding depression was observed from family IAS₃-1, VS₃-1, IIIS₃-2 and VS₃-2. The number of leaves exhibited high inbreeding depression (above 20) in family IVS₃-2, IIS₃-2, IIIS₃-2 of sibmated and IIS₃-1 in selfed of S₂ generation palm on selfing for S₃. Similar trend was noticed in seedling height but for collar girth observed inbreeding depression was less.

The molecular study using the promising inbreds was done using 10 primers. The analysis by the primers signifies that the family IIIS₃-1 were unique which was noticed by the 4 primers i.e. OPBA 03, OPAW 19, OPAW 15, OPAW 09 and OPAU 02. The specific bands for dwarfs were noticed from almost all the primers except for the OPAW 09. While the locus specific to tall have noticed from the primer OPBA 03 and OPAU 03. The specific to the samples has also been noticed from some primers which was the WCT specific (OPAU 03), CGD (OPAW 15 and OPAW 08), IAS₃-1 (OPAW 12, OPAW 13 and OPAW 08), IBS₃-1 (OPAW 19 and OPAU 03) and IIIS₃-1 (OPAW 19). The coloured types IAS₃-1 and IBS₃-1 were highest and observed as on par with the WCT which were evidently proved by the primer OPBA 03 and OPAW 19. Among the primers, highly significant bands between the tall and dwarf was noticed by the OPBA 03 and OPAU 03

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Abstract

**ANALYSIS OF INBREEDING DEPRESSION IN WEST COAST TALL
COCONUT (*Cocos nucifera* L.)**

by

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ABSTRACT

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ABSTRACT

In nature, coconut is a cross pollinated crop which influences the high degree of variability and heterogeneous population. With the intension of developing inbred line in coconut, the programme was initiated in 1924 by selecting the eighteen WCT palms and developed S_1 generation. The S_1 plants were selfed and sibmated to produce the S_2 progenies in 1960 and the seedlings planted at CRS (RARS), in Pilicode with the replicated trial. This served the present experimental material to characterize and analyze inbreeding depression in S_2 palms and to study the effect of selfing (S_3) in selfed and sibmated (S_2) families of West Coast Tall (WCT). Vegetative, reproductive and nut characters were recorded in S_2 WCT family.

The analysis of variance revealed significant differences among the twelve families of six groups of WCT for all the characters studied. The tallest palms were observed in IVS_2-2 and IAS_2-2 and on par with the WCT. The shortest palms were recorded from IIS_2-1 and IIS_2-2 but taller than dwarfs. In general the sibmated families were showing more tall stature indicating heterotic behaviour. The IAS_2-1 and IBS_2-1 showed average number of functional leaves as tall while IIS_2-1 had less number of leaves similar to dwarfs. The petiole length was short in IAS_2-1 and IAS_2-2 as well as IIS_2-1 , IVS_2-2 and VS_2-1 indicating an advantageous character of high bearing of nuts with less loss. IIS_2-1 and VS_2-1 produced more number of female flowers while IAS_2-1 and IAS_2-2 produced less number of female flowers. In IIS_2-1 and VS_2-1 , the period of female phase similar to WCT. Thickest kernel was noticed in IBS_2-1 and VS_2-2 and less thick in IAS_2-1 . More thickness of kernel is tall palm character and thin meat is dwarf palm trait.

Inbreeding depression was studied for S_2 nut yield and its attributes. The family IIS_2-2 and VS_2-1 expressed positive inbreeding depression for palm height and internodal length, but no inbreeding depression for stem girth. The leaf characteristics also exhibited positive inbreeding depression in S_2 . The WCT

palms in S₂ generation were characterized primarily based on eight characters separately when selfed and sibmated in S₁ for S₂ generation. In selfed the IBS₂-1 showed tall characters with highest tall, stouter stem, medium functional leaves, and average female flowers, less setting percentage and low yield, medium nut, high oil percent. Semi tall characters in IIS₂-1 which recorded semi tall, stouter stem, average functional leaves, and average inflorescence produced, average female flowers, medium setting percentage, high copra and low oil content. Dwarf characters observed in IIS₂-1 short, thin stem, high functional leaves, more inflorescence, high female flowers, medium setting percentage, nut yield, copra and oil content high.

The S₃ seed nut showed that germination percentage was low in all the families (less than 50%) indicating the operation of deleterious effect of inbreeding in S₃ seed nuts. The semi-tall seedlings were more in selfed and tall in sibmated families of S₃. The percentage of occurrence of stouter collar girth was more in S₃ (IAS₃-1, IBS₃-1, IIS₃-1, IVS₃-2, IIS₃-2, IIS₃-2 and VS₃-2). The leaf production was moderate in all the S₃ seedlings of selfed and four sibmated families (IAS₃-2, IBS₃-2, IIS₃-2 and VS₃-2). Both the third and fifth leaves, the length, breadth and petiole length showed moderate values.

The germination percentage revealed positive inbreeding depression in all the selfed families of 12 families. High inbreeding depression was noticed in IAS₃-2, IIS₃-2, IVS₃-2, VS₃-1 and IVS₃-1 and the lowest in family IBS₃-1 and IAS₃-1. Seedling height expressed high inbreeding depression in VS₃-1. Collar girth didn't show inbreeding depression in S₃. It is an important trait correlated with yield. Both positive and negative inbreeding depression was recorded for total number of leaves produced by the seedlings in S₃ generation.

The molecular study using the promising inbreds was done using 10 primers. The analysis by the primers signifies that the family IIS₃-1 was unique which was noticed by the 5 primers i.e. OPBA 03, OPAW 19, OPAW 15, OPAW 09 and OPAU 02. The specific bands for dwarfs were noticed from almost all the

primers except for the OPAW 09. While the locus specific to tall have noticed from the primer OPBA 03 and OPAU 03. The specificity to the samples has also been noticed from some primers which was the WCT specific (OPAU 03), CGD (OPAW 15 and OPAW 08), IAS₃-1 (OPAW 12, OPAW 13 and OPAW 08), IBS₃-1 (OPAW 19 and OPAU 03) and IIS₃-1 (OPAW 19). The coloured types IAS₃-1 and IBS₃-1 were highest and observed as on par with the WCT which were evidently proved by the primer OPBA 03 and OPAW 19. Among the primers, highly significant bands between the tall and dwarf was noticed by the OPBA 03 and OPAU 03.

It is evident from the studies that the selfing of WCT palms would reduce vigour and possible to develop inbred lines which could be used for the production of hybrids for higher productivity.