MORPHO-MOLECULAR CHARACTERISATION AND EVALUATION OF TxD, DxT AND DxD HYBRIDS OF COCONUT CULTIVAR AYIRAMKACHI (*Cocos nucifera* L.)

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by

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THESIS

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PADANNAKKAD, KASARAGOD 671314

KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled **"Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*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 of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Padannakkad
Date: 13/04/2021

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CERTIFICATE

Certified that this thesis, entitled **"Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*Cocos nucifera* L.)"** is a record of research work done independently by Ms. Haritha M. R. (2018-11-103) 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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We, the undersigned members of the advisory committee of Ms. Haritha M. R. (2018-11-103) a candidate for the degree of Master of Science in Agriculture with major in Plant Biotechnology, agree that the thesis entitled **"Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*Cocos nucifera* L.)"** may be submitted by Ms. Haritha M. R. (2018-11-103), in partial fulfillment of the requirement for the degree.

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Haritha M R

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percentage
˚Bx	Degree Brix
˚C	Degree celsius
µg	Micro gram
µl	Micro litre
µM	Micro molar
ANOVA	Analysis of Variance
CDB	Coconut Development Board
cm	Centimetre
CTAB	Cetyl Trimethyl Ammonium Bromide
ect.	So on
EDTA	Ethylenediamine Tetra Acetic acid
et al.	Co-workers/Co-authors
Fig.	Figure
g	Gram
GD	Genetic distance or genetic diversity
KAU	Kerala Agricultural University
Kb	Kilobase
M	Metre
M	Molar
ml	Millilitre
mm	Millimeter
ng/ µl	Nanogram per microliter
nm	Nanometre
PCR	Polymerase chain reaction
PIC	Polymorphism Information Content
pM	Pico molar
PVP	Polyvinyl polypyrrolidone
RAPD	Random Amplified Polymorphic DNA
SSR	Simple Sequence Repeat
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
Tm	Melting temperature
TSS	Total Soluble Solids
UPGMA	Unweighted Pair Group Method with Arithemetic mean
V	Voltage
viz.	Namely
w/v	Weight/volume

INTRODUCTION

1. INTRODUCTION

Coconut the “Tree of Life” or “Kalpavriksha” or “Tree of Abundance”, is an integral part of our culture for centuries. It can sustain the life of farmers because of its economic importance. Eventhough all the parts of the coconut palm are useful, fruit (nut) is the commercially important one, which provides solid endosperm for culinary purpose, dried copra for oil extraction and liquid endosperm as a natural drink and also used for preparing many value-added products. The non-edible parts are also useful which includes, fiber for making coir, carpets and other commercially important products, shells for certain industrial uses (such as activated charcoal) and as a fuel and timber for various industrial and domestic purposes.

Coconut palm is a tropical, perennial, multipurpose, monoecious plantation crop which is widely cultivated. It is a monotypic species of genus *Cocos*, belonging to monocot family Arecaceae. The scientific name of coconut is *Cocos nucifera* L. The genus name *Cocos* reported having originated from Spanish word *coco* (meaning spectre) and species name *nucifera* a neo-Latin word (meaning bearing nuts) (Niral and Jerad, 2018). Based on growth of stem, age of flowering and mode of pollination the coconut palms can be classified into two major groups - the tall and the dwarf. In India, more than 92 per cent of total coconut production is contributed by Kerala (31.11%), Tamil Nadu (28.33%), Karnataka (27.48%) and Andhra Pradesh (5.70%) (CDB, 2018). Out of which 90 per cent of the commercially grown coconut cultivars are tall and utilized for majority purposes, the remaining 10 per cent constitute dwarf varieties and hybrids.

The two major groups of coconut, *viz.*, Tall and Dwarf have been widely utilized in breeding programmes due to its high intraspecific variability present in the monotypic species. In India, where the hybrid vigour in coconut was exploited for the first time by the cross WCTxCGD made at Coconut Research Station, Nileshwar, Kerala in 1932 (Patel, 1937) and recorded as a landmark in the coconut improvement programme. Since then different combinations of the tall (T) and dwarf (D) cultivars are widely used to exploit heterosis in coconut and most of them were either TxD or DxT, thus most of the commercial hybrids are through inter-varietal crosses. The DxD

hybrids are under-exploited category, but recently attempts are being made to identify superior hybrids from these crosses also.

Any improvement programme in coconut is highly complicated because of its perennial nature, long vegetative phase, high heterozygosity, long generation time and long experimentation period and all these together responsible for slow growth in coconut breeding and also the breeding efforts were mainly confined to conventional techniques such as selection and hybridization. By the developments in biotechnology, through molecular marker technology considerable advancements are possible in the crop improvement programmes. Among the various molecular markers available Simple Sequence Repeat (SSR) markers are widely used for molecular characterization, diversity analysis, identifying parental lines, hybridity testing and marker-assisted selection.

Germplasm collection of various indigenous and exotic cultivars maintained at Regional Agricultural Research Station (RARS), Pilicode, Kasaragod, Kerala has been widely utilized for coconut improvement programmes. One such programme involved crossing different tall and dwarf genotypes (West Coast Tall, Laccadive Ordinary, Philippines Ordinary, Laccadive Micro, Andaman Ordinary and Malayan Yellow Dwarf) with the semitall/dwarf type Ayiramkachi as a common parent with high female flower production but low fruit setting per cent. Three groups of hybrids such as TxD, DxT and DxD were developed through this breeding programme. The present investigation aims at morphological characterisation of hybrids of Ayiramkachi planted at RARS Pilicode during 1994 for important yield attributes and nut quality combined with dwarf stature along with fingerprinting by using microsatellite markers marker (SSR).

In this background the present study was formulated with the following objectives:

1. To evaluate the performance of coconut hybrids produced from the cross of Ayiramkachi with tall and dwarf cultivars
2. Screening of SSR markers for polymorphism between eight parental genotypes and one check cultivar, molecular characterisation and diversity analysis of

parental palms and check, and selection of polymorphic primers for future characterisation of hybrids.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Coconut is a perennial, multipurpose plantation crop widely grown in the tropics. Coconut germplasm collections are maintained in different parts of the world and are widely exploited for desirable traits through selection and hybridization, which resulted in the formation of many varieties. A single classification method cannot accommodate variability among coconut palms throughout the world. There is high intraspecific diversity among palms with respect to specific habit and fruit characters. Presently molecular markers are also available for germplasm characterisation. Simple Sequence Repeat (SSR) markers can be widely utilized because of their abundance, polymorphism and co-dominant nature (Dasanayake *et al*., 2003; Nair *et al*., 2016).

2.1 CLASSIFICATION OF GERMPLASM

Major classification divides the coconut population broadly into- tall (spicata, typica and androgena) and dwarf (nana and javanica), based on breeding behavior and palm stature (Narayana and John, 1949).

Liyanage (1958) adopted a classification system for coconut palms depending on the fruit characters as- typica (tall), nana (dwarf) and aurantiaca (intermediate). Menon and Pandalai (1958) classified the palms based on the growth of stem, age of fruiting, mode of pollination *etc*. into- the dwarf and the tall.

Classification based on pollination behavior put forth by Fremond *et al*. (1966) included two class, the autogamous (mostly dwarfs) and the allogamous (tall). Based on flowering pattern Rognon (1976) classified coconut palms into four types, such as Type I, Type II, Type III and Type IV corresponding to strict allogamous, indirect autogamous, direct autogamous and semi-direct autogamous respectively.

Satyabalan (1997) classified coconut cultivars into different groups such that five groups were included under tall and three groups under dwarfs.

2.1.1 Tall cultivars

Narayana and John (1949) reported that tall palms can reach up to 20-30 m in

height and after five to seven years of planting the palms commence flowering and their economic yield may extend up to 80-100 years.

Ratnambal (2001) reported that tall cultivars have high heterozygosity due to cross pollinating nature and these palms exhibited high variability in morphological and fruit characters.

Tall varieties reported to have a swollen base (bole) and stout trunk, with 25-40 leaves of about six meters in length. The tall palms are mostly allogamous but during summer there is a chance for autogamy in these palms (NIIR Board of Consultants and Engineers, 2006).

2.1.1.1 *West Coast Tall (WCT)*

West Coast Tall is an extensively cultivated tall on western coastal regions of India, and the most popular variety in Kerala with an economic yield for 75 years or more and it can be grown in all soil types and is resistant to water stress. Kannan (1982), who conducted a survey to analyse the performance of coconut cultivars in Kerala, reported better performance of WCT over certain hybrids even under poor management conditions. Balakrishnan *et al.* (1991) also reported that WCT is a stable cultivar.

Ratnambal (2001) reported that the time taken by WCT to set first flower is six to seven years and it is a regular bearer producing 12-13 bunches every year. The reports from NIIR Board of Consultants and Engineers (2006) shows that average yield per palm per year vary from 60-80 nuts but under irrigated conditions it can go up to 100 nuts or more. According to this report, mean copra content per nut and oil content are 165 g and 72% respectively.

2.1.1.2 *Andaman Ordinary (AO)*

This cultivar is widely found in Andaman and Nicobar Islands. They are tall massive palms with vigorous vegetative growth than WCT. Time taken by the palm for first flowering is six to eight years. According to the reports of NIIR Board of Consultants and Engineers (2006) each palm on an average produces 50 nuts in a year.

Nuts are larger in size producing 173 g copra, with oil content of 66%. It is also reported to be tolerant to certain coconut diseases.

2.1.1.3 *Cochin China (CC)*

It is an introduction from Vietnam (Cochin China) and takes six to seven years to reach first flowering. Ratnambal (2001) reported the nuts are greenish-yellow, large and oval in shape and the average yield per palm as 98 nuts every year with a range of 65-150 nuts/palm/year. The average copra and oil content are also recorded to be 220 g and 66% respectively.

2.1.1.4 *Laccadive Micro (LM)*

Laccadive micro is cultivar of Lakshadweep Island and its morphology is similar to WCT. Age of palm at first flowering is 8-9 years. It is a heavy bearer. Nuts are very small, round to oblong with green to various shades of brown. Each palm on an average produces 200 nuts per year. Copra content is 80-100 g and has the highest oil content of 75%. It is good for ball copra making (Ratnambal, 2001).

2.1.1.5 *Laccadive Ordinary (LO)*

Also called as Laccadive Tall, it produces more number of female flowers with high setting per cent (Ohler, 1984), and is a cultivar of Lakshadweep Islands, their growth characters are similar to WCT. Time taken by the palm to set first flowering is 5-6 years. Nuts are oval in shape but smaller than WCT. Colour of the nut varies from green, yellow and shades of brown (Ratnambal, 2001). Average yield, copra content and oil content are 120 nuts/palm/year, 160 g and 72% respectively (NIIR Board of Consultants and Engineers, 2006).

2.1.1.6 *Philippines (PHI)*

It is a Philippine cultivar, reaches 10-12 m in height and takes 6 years for first flowering. It is a heavy yielder with round nuts, each palm may produce 110 nuts per year (range 90-200 nuts). Average copra and oil content are 198 g and 66% respectively. During 1995 CPCRI released this cultivar (Kerachandra) as ‘National variety’ (Ratnambal, 2001).

2.1.2 Dwarf cultivars

These are classified as dwarfs as they may reach upto 8-10 meters in height. Flowering commence after three to four years of planting and the economic yield may extend up to 40-50 years. Early flowering and short stature make them commercially important (Narayana and John, 1949). Dwarfs were supposed to be originated from tall cultivars by inbreeding or by sudden mutations (Swaminathan and Nambiar, 1961). Eventhough dwarfs are reported to be heavy yielder their nuts are smaller compared to tall (Manthriratne, 1972).

Ratnambal (2001) reported that dwarfs are more homozygous because of their self-pollinating nature, thus palms show less variability. Their nuts are smaller with soft copra and oil content is also less. Dwarf cultivars do not possess bole and the trunk is thin (NIIR Board of Consultants and Engineers, 2006).

2.1.2.1 *Ayiramkachi (AYK)*

Varietal classification of coconut palms by Satyabalan (1997) states that Ayiramkachi comes under Group-I of Green Dwarfs variety, having characters such as small fruit size, copra content 32-43% and husked fruits shows a high shell per cent of 26-39%.

Ayiramkachi has no bole and it flowers in about 3.6 years after planting. It is an irregular bearer. The female flowers production of the palm range between 800-1200/palm. But the average production is only 75 nuts/palm/year. The nuts are green and elliptical. Copra content is 98.38 g and is hard, dense and small (NIIR Board of Consultants and Engineers, 2006).

Jayabose *et al*. (2008) studied economically important traits of certain parental palms and their hybrids and reported Ayiramkachi as a high yielding genotype with 129.57 nuts/palm/year and its hybrids also recorded better yield performance.

Ayiramkachi has been identified as a promising general combiner in hybridization programme carried out at RARS, Pilicode involving different tall and dwarf genotypes (WCT, LO, PHI, LM, AO and MYD) (KAU, 2014).

Ayiramkachi was reported to be a dwarf cultivar belongs to the eastern coastal regions of Tamil Nadu. High female flower production with low setting percentage is the important character of this palm. It is an alternate bearer and produces very small sized green coloured fruits which are oblong in shape. The palm yields good quality copra (Sankaran *et al*., 2015).

2.1.2.2 *Malayan Yellow Dwarf (MYD)*

MYD is an introduction from Malaysia for hybrid production and reported to be a good combiner with tall cultivars (Ramachandran *et al*., 1974).

It is a dwarf cultivar with yellow coloured petiole, spathe and nuts (round). It takes about four years for the first flowering. The average yield per palm, copra content and oil percentage are 66 nuts/palm/year, 140 g and 66% respectively (Ratnambal, 2001).

2.2 MORPHOLOGICAL CHARACTERISATION OF CULTIVARS AND HYBRIDS

2.2.1 **Vegetative characters**

Tammes (1955) reported that, in tall cultivars first flowering starts after six to nine year of planting and attains a peak in production between ten to twenty years. Liyanage *et al*. (1986) on further study found that age of palm at first flowering was found least for dwarf cultivar (3 years) followed by hybrids (3.5 years) and highest for tall (4.5 to 5 years) among Sri Lankan cultivars.

Bhaskaran and Leela (1963) reported that the total number of functional leaves present in the crown was higher in hybrid than parents, but rate of leaf production was highest for dwarfs (14.5) followed by TxD (12.9) and tall (10.0).

de Lamothe and Wuidart (1982) identified bole (well developed root bulb) as a distinguishing character of tall palms, which confers resistance against heavy winds and drought. A similar view was put forth by Ekanayake *et al*. (2010) who reported that the tall cultivars can be morphologically distinguished from the dwarf ones with the presence of a well-defined predominant bole at the base and a well spread crown.

Pillai *et al*. (1991) observed that tall and dwarf cultivars vary in their stem girth, number of leaf scars in one meter, length of petiole *etc*. where girth and petiole length was reported higher for tall cultivars and the reverse in case of number of leaf scars in one meter.

Narayanankutty and Gopalakrishnan (1991) studied correlation between vegetative and yield characters of coconut and reported that total number of functional leaves, number of leaflets per leaf, petiole length and girth of palm at the base had positive correlation with yield. Later Namboothiri *et al*. (2007) correlated vegetative and yield characters of F_2 generation of DxT hybrids and reported significant positive correlation of total number of functional leaves on the crown and rate of leaf production with nut yield, but a significant negative correlation was observed for petiole and internode length with yield. Study conducted by Natarajan *et al.* (2010) on vegetative and nut characters also recorded a positive correlation for total number of functional leaves in crown, leaf length and petiole length with nut yield. Path analysis revealed that, yield was greatly affected by total number of leaves directly followed by length of petiole and leaf length. But the study conducted by Subramanian *et al*. (2019) concluded no significant correlation between height of palm, girth of palm, leaf length and petiole length with yield.

Length of petiole and bole size were reported highest for TxT hybrids compared to DxT hybrid in a study conducted by Louis *et al*. (2010). They also observed a significant positive correlation between girth of stem at 20 cm from the base with weight of nut water.

Performance of certain coconut cultivars and hybrids were studied by Ghosh and Bandopadhyay (2015) and reported maximum petiole length in PHI (195 cm) and leaf length in WCTxCOD (564 cm) and least in LO (249 cm).

Mohanalakshmi and Arunkumar (2019) analysed various coconut genotypes for their performance and reported maximum palm height and number of functional leaves for cultivar AO (12.10 m and 35.92) and least for COD (4.23 m and 27.97).

2.2.2 Reproductive characters

Liyanage (1949) reported that approximately 12 inflorescence opens every year and the number of inflorescence produced is reported to be affected by rate of leaf production. According to Menon and Pandalai (1958) every leaf is accompanied by a spadix and under suitable conditions the palm will produce 12 to 15 inflorescence annually.

Liyanage (1954) studied characters of tall cultivars in Ceylon and reported that a new inflorescence opens after the preceding one has lost receptivity of female flower, which also depends on age of the palm and its environmental factors. Abeywardena (1971) reported that the inflorescence primordia is formed 32 months before spathe opening. Once the spathe opens, some of the female flowers will get pollinated within a month and the unpollinated ones will fall off. It was also observed that the chances of immature nutfall is high in early stages of nut development and after four months it will be negligible.

Nambiar and Nambiar (1970) reported that to get improved varieties selection based on reproductive characters such as higher number of female flower production, number of flowers set and per cent of flowers set are effective as they are highly heritable and contribute to yield. Hybrids from cross between tall and dwarf produced more female flowers, indicating the high heritability of character (Arunachalam *et al*., 2014).

In a study conducted by Manna *et al.* (2002) the inflorescence and nut characters of DxT and TxD hybrids were compared with cultivars in West Bengal and observed that the performance of DxT and TxD hybrids were superior with respect to other cultivars.

Thomas and Josephrajkumar (2013) reported that the inflorescence or spadix have a central axis with about 30 lateral branches of 35-55 cm length. Each branch bear 200-300 staminate flowers and one or more pistillate flowers, thus about 20-40 female flowers will be present in each inflorescence. Ratnambal *et al.* (2003) studied floral characters of various tall accessions and reported that ratio between male and

female flowers in tall cultivars is in the ratio 1: 0.004.

Samarasinghe *et al*. (2018) observed that the average number of female flowers produced by the DxT hybrids per inflorescence were significantly higher compared to tall cultivars even under water stress condition.

2.2.3 Nut and yield characters

Child and Nathanael (1950) conducted studies on changes in sugar content in coconut water and reported that seven months old nut contain maximum sugar concentration of five per cent, but thereafter as the nut fully matures (12 to 13 months old) total sugar concentration is reduced to two per cent. Jackson *et al.* (2004) observed change in chemical properties (such as TSS, titratable acidity, sugars, ash, lipid content and turbidity) of coconut water during fruit maturation. Chattopadhyay *et al*. (2013) also reported highest TSS (6.00˚Bx) for nut water at eight months old nuts and lowest (3.18˚Bx) for five months old nuts irrespective of varieties analysed.

Bhaskaran and Leela (1963) reported that the yield potential of TxD hybrids are higher than their tall and dwarf parents. Satyabalan *et al*. (1970) also observed superiority in performance of TxD hybrids. But the combining ability of parents is also reported to be important in producing superior hybrids. Ramachandran *et al*. (1974) identified Chowghat Green Dwarf and Malayalan Yellow Dwarf as the best male combiners with tall cultivars.

Type of cultivar and growing conditions of coconut palm were reported to have high influence on nut weight and nut component characters by Harries (1978). Iyer *et al*. (1981) also reported the influence of these factors on yield.

Louis and Ramachandran (1981) studied oil content in coconut cultivars and found that it varies according to cultivars and the tall varieties recorded to have high percentage of oil and in hybrids the oil content was found closer to that of female parent.

Growth and accumulation of dry matter in coconut was studied by Jayasuriya and Perera (1985) and reported that growth of endosperm starts five months after

fertilization and a rapid increase in dry weight of endosperm was recorded between six to ten months with an average of 39.9 g per month, highest growth rate of 53.1g per month was observed at nine months old and which stopped after 11 months.

The superiority of tall cultivars over dwarfs were identified by Long (1993) and Siju (2003). They found that the tall cultivars had higher kernel thickness and copra content compared to the dwarf.

Guarte *et al*. (1996) reported that in a drier the optimum drying temperature to produce good quality copra (dried coconut meat of about seven percentage moisture) and oil is 90˚C.

Ganesamurthy *et al*. (2004) conducted heterosis studies on TxD and corresponding DxT coconut hybrids. A significant positive heterosis was observed in CCxAYK hybrid for whole nut weight and a significant negative heterosis was observed for both ECTxAYK and AYKxECT hybrids for kernel and copra weight. The hybrids CCxAYK and ECTxAYK also showed a negative heterosis for nut yield. The study also delineates a negative heterosis for whole nut weight in DxT coconut hybrids, but majority of them also shows a significant positive heterosis for dehusked nut weight, kernel weight and copra content.

Hemavathy and Balaji (2006) grouped coconut cultivars into eight clusters based on vegetative, reproductive and nut characteristics and reported that superior hybrids with high yield can be obtained in the cross of AYK (cluster II) with PHI (cluster VII). They also conducted studies on genetic diversity in coconut and reported that the nut characters where more efficient in assessment of genetic divergence.

Jayabose *et al*. (2008) recorded that the hybrids of MYD (as both male and female) with CGD and ECT was high yielding and also hybrids with MYD as female parent shows better yield performance than other hybrids.

Foale and Harries (2009) observed that the size and shape of the nut varies according to cultivar and environmental conditions. The outer skin of the nut is thin which differs in colour *viz*., orange, green, yellow or bronze but once the nut is fully matured it turns brown.

Rachel *et al*. (2010) reported that the kernel thickness has a positive correlation with oil content.

Selvaraju and Jayalekshmi (2011) observed that the yield has a significant positive correlation with both reproductive and vegetative characters. Geethanjali *et al.* (2014) studied yield and nut characters of various tall and dwarf coconut cultivars. A significant positive correlation was observed for fruit length with most of fruit characters analysed. No correlation was observed for kernel thickness with any other fruit parameters. Nut yield had a significant negative correlation with fruit size, nut weight, kernel weight, water content and copra content per nut.

Ghosh and Bandopadhyay (2015) evaluated the performance of certain varieties and hybrids of coconut and reported that hybrid DxT (9.4) produced maximum bunches per palm while maximum yield was recorded by LM (105.2 nuts/pam/year) with minimum nut weight (1265 g). PHI was found superior for volume of water (305 ml), total soluble solids (6.2˚ Brix), copra yield (9.4 kg/palm/year) and oil yield (6.3 kg/ palm).

Niral and Jerard (2018) reported that the time taken by the nuts to get matured is slightly higher for tall varieties (11-12 months) than the dwarf ones (10-11 months).

Mohanalakshmi and Arunkumar (2019) evaluated the yield and nut quality of certain cultivars and hybrids of coconut. AO recorded maximum number of bunches/year (12.58), kernel weight (134.914 g) and yield (118.55 nuts/palm/year). LO reported to have highest whole nut weight (871 g) and dehusked nut weight (387.61 g).

2.2.4 Pests and diseases

Menon and Pandalai (1958) estimated that in India yield loss of minimum ten per cent is caused by rhinocerous beetle directly by damaging spathe. Catley (1969) reported that an adult rhinocerous beetle feed crown region of the palms by boring through petiole in to unfolded leaves and the feeding of immature inflorescence results in yield reduction. Aida *et al.* (2020) conducted studies on resistance and susceptibility of coconut varieties towards rhinocerous beetle and reported that the

variety of palm is having an effect on beetle population. A higher population was observed in MYD and was attributed to its dwarf stature and yellow coloured petiole and fruits, which attracted the pest.

Nambiar and Iyer (1991) reported that a palm infected with stem bleeding disease will produce cracks on the stem with reddish brown exudation from cracks and under severe conditions there is heavy shedding of buttons and nuts.

Abraham *et al.* (1998) listed out certain symptoms caused by Red Palm Weevil on palms which includes, tunnels on base of petiole and trunk, yellowish brown thick fluid oozing from tunnels, presence of frass and under severe condition the stem will break or crown toppling occurs. Faleiro and Rangnekar (2001) reported that Red Palm Weevil has high ovipositional preference in CGD and least in MYD. To tackle its infestation Faleiro (2006) reported that pheromone based food bait management strategy is more suitable and according to Dembilio and Jacas (2015) early detection with proper sanitation and insecticide treatment is the only method to avoid palm death.

Nair (2000) reported that factors such as colour, size and shape of nut and perianth characters influence degree of eriophyid mite attack on coconut. Heavy mite infestation was found on green oblong WCT nuts than round reddish brown coloured nuts. Under severe infestation the buttons dry and shed off and cause malformation and retarded growth in nuts. The economic loss is caused by reduction of copra and malformed fibre (Nair *et al*., 2005).

Levin and Mammooty (2003) reported minimum mite damage in Strait Settlement (8.30 per cent) followed by CC (9.90 per cent) among exotic cultivars, and Lakshaganga (19.40 per cent) among hybrids, and LM (7.40 per cent) among indigenous varieties. Maximum damage was found on Lono (81.10 per cent) among exotic cultivar and Anandaganga (30.00 per cent) among hybrids, and Ayiramkachi (90.20 per cent) among indigenous varieties.

Yang *et al.* (2018) reported that Ayiramkachi can be easily damaged by rodants and nuts are succeptable to Eryophyid mite.

2.3 MOLECULAR CHARACTERISATION OF CULTIVARS AND HYBRIDS

2.3.1 Isolation of genomic DNA

Couch and Fritz (1990) developed a method to isolate genomic DNA from high polyphenol containing plants. The method follows isolating nuclei before lysis. The cytoplasmic impurities are kept away from nuclei by concentrating it and inhibiting oxidized polyphenolic compound formation and its interaction with genetic material in the subsequent steps.

Aitchitt *et al*. (1993) reported that a high concentration of CTAB extraction buffer (3% w/v CTAB) with single chloroform- isoamyl alcohol extraction with an additional step of DNA precipitation using sodium acetate and ethanol worked as an efficient rapid extraction method for DNA isolation from fresh mature coconut and date palm leaves.

Al-Shayji *et al*. (1994) developed DNA isolation protocol for various palm species which was simple, low cost and yielded DNA with sufficient purity. The extraction buffer included potassium metabisulphite and PVP. Proteins and polysaccharides were precipitated using SDS and potassium acetate and finally the DNA was precipitated by adding isopropanol. The DNA recovery was about 930 µg from 1g coconut leaf tissue.

Upadhyay *et al.* (1999) conducted studies on isolation of DNA with good quality from young coconut leaves. They tried two detergents, CTAB and SDS at different concentration and pH (eight and nine), and reported that extraction using 1% SDS at pH 8.0 yielded high quantity of DNA with good quality.

Ramirez *et al*. (2004) reported that DNA extraction using CTAB protocol developed by Doyle and Doyle (1990) modified by Rohde (1995) yield good quality DNA from fresh coconut leaf.

Angeles *et al*. (2005) conducted a study to determine the best part of the coconut palm for DNA extraction and concluded that the young fresh leaf was found better than endosperm and yielded maximum amounts of DNA.

An efficient method for the isolation of genomic DNA of tropical plants was reported by Huang *et al*. (2013) by using extraction buffer with high salt concentration (2 M NaCl) with reagents such as chloroform, β-mercaptoethanol and phenol. The DNase activity was effectively inhibited by the use of ethylenediamine tetra acetic acid (EDTA), cetyl trimethyl ammonium bromide (CTAB) and lauroyl sarcosine (LSS), a deoxidized environment was produced by polyvinyl polypyrrolidone (PVPP) and the interfering compounds were precipitated using borax.

Aina *et al.* (2015) developed a method to yield 8.7-9.8 µg/ml DNA by chemically homogenizing coconut leaf sample with a lysis buffer (Tris HCl, SDS, Potassium acetate) and a detergent followed by centrifugation for supernatant collection and DNA precipitation using ethanol.

2.3.2 Simple sequence repeat markers (SSR)

SSRs also called as microsatellites are repetitive DNA sequences which represent major portion of eukaryotic genomes and the utility of these sequences as genetic markers were reported by Powell *et al*. (1996a). SSR markers are highly discriminative, informative, PCR based, codominant and multi allelic in nature. Compared to many other DNA aasay techniques they require very small quantities of DNA (Powell *et al*., 1996b; Russell *et al*., 1997).

Perera *et al*. (2000) analysed genetic diversity of 94 ecotypes of coconut with 130 individuals including 75 tall and 55 dwarfs and detected 51 alleles in total having a mean 6.4 per locus. Highest number of alleles were detected in tall (50) with a mean of 6.3 per locus compared to dwarfs (26) with 3.3 per locus. Mean gene diversity of tall (0.59) was recorded significantly higher than dwarfs (0.35). They also reported that 116 out of 130 palms were uniquely differentiated with the eight SSR markers (CAC2, CAC3, CAC4, CAC6, CAC8, CAC10, CAC13 and CAC56) used for study.

Perera *et al*. (2001) used eight SSR markers to analyse genetic diversity of 330 genotypes of coconut. Totally 56 alleles were produced with a mean of seven per locus, a very high gene diversity was also detected. Maximum number of alleles was produced by CAC56 (10) and minimum by CAC13 (3). Unique discrimination was

made by eight SSR markers on each genotype.

Dasanayake *et al*. (2003) used 17 SSR markers for germplasm characterisation of coconut, 75 alleles were detected by the markers having a mean value of 4.4 alleles per locus with a range of two (CAC56) to eight (CAC50). Genetic distance values were high (ranged from 0.13-1.00), indicating polymorphic ability of SSR markers.

Genetic relationship study conducted by Perera *et al*. (2003) among 94 varieties of coconut using 12 SSR markers produced 85 alleles in total with a mean of 7.4 per locus. Maximum gene diversity was observed for marker CAC56 (0.84 ± 0.01).

Manimekalai *et al*. (2006) compared ten each of RAPD, SSR and ISSR markers to identify their effectiveness in analyzing polymorphism among coconut accessions and reported that SSR markers have highest polymorphism (100 per cent), PIC value (0.78) and Marker Index (7.60) compared to ISSR and RAPD and also concluded SSR markers were best having high reproducibility and polymorphic ability for identifying cultivars.

Shalini *et al*. (2007) analysed SSR and RAPD markers of coconut to identify its association with mite resistance. When each of the markers were analysed, nine SSR markers were found to be associated and on further multiple regression analysis six SSR markers ($CnCirA9_{99}$, $CnCirS12_{171}$, $CnCirE2_{151}$, $mCnCir86_{194}$, $CnCirF2_{205}$ and $CnCirG4_{207}$) on a combination showed 100 per cent association with resistance and two among them indicates succeptability to mite attack ($CnCirA9_{99}$ and $CnCirE2_{151}$).

Manimekalai and Nagarajan (2007) used ten SSR markers to analyze coconut germplasm for genetic diversity. Total of 92 alleles were produced and all of them where polymorphic. Average PIC value was 0.79 with highest for CnCirB6 and CnCirB12 (0.89) and lowest in CnCirE12 (0.50). SSR markers were found powerful in estimating genetic diversity.

Genetic diversity analysis in coconut by Rajesh *et al*. (2008) using 14 SSR markers, detected 90 alleles in total with a mean of 6.42 per locus. Maximum number of alleles were produced by CnCirE2 (16) and least by CnCirA9 (3). Mean PIC was 0.61, highest for CnCir E2 (0.89) and lowest for CnCir A3 (0.41). They also reported

high heterozygosity in tall cultivars (0.37-0.58) than dwarfs (0.03-0.05).

Dasanayaka et al. (2009) reported that Gene diversity and Polymorphism Information Content (PIC) was highest for tall (0.55 and 0.50) than dwarf cultivars (0.21 and 0.18).

Perera (2010) reported that molecular characterisation can be used as a reliable method to test the validity of coconut hybrids and confirming identity of cultivars. Phenotypic markers are not fully dependent and sometimes misleading also. He used 18 SSR markers to identify varieties (Sri Lanka Tall, Green Dwarf and Yellow Dwarf) and their hybrids (TxD). CAC20 and CNZ6 were found to be polymorphic for each parent and reported as a reliable markers for distinguishing cultivars and confirming validity of hybrids.

Kamaral *et al*. (2016) studied the genotypes of 102 yellow dwarf Sri Lankan cultivars using 30 SSR markers which include ten from each of CAC, CnCir and CNZ markers, 29 out of them were reported to be polymorphic while CnCir89 was monomorphic.

Genetic relationship between nine tall coconut accessions were analysed by Loiola *et al.* (2016) using 25 SSR markers, out of which 19 were reported to be polymorphic. Total of 125 alleles were produced by the markers with a range of four to ten (mean of 6.57 per locus). Maximum number of alleles were produced by locus CNZ10 (10) and PIC value was found highest for CNZ43 (0.82).

Rasam *et al*. (2016) conducted molecular characterisation using 14 SSR and 18 ISSR markers and reported that the average polymorphism was highest for SSR markers (92.9 per cent) compared to ISSR markers (31.9 per cent) and SSR markers were found superior over ISSR markers.

Diversity and association analysis of 79 coconut genotypes were carried out by Geethanjali *et al*. (2018) using 48 SSR markers and the genotypes were classified into two clusters each having two sub-clusters within them. Cluster I included 46 tall cultivars and Cluster II comprised both tall (23) and dwarf (10) genotypes.

Molecular characterisation of coconut carried out by Mahayu and Taryono (2019) using SSR markers reported that all the ten markers used in the study were 100 per cent polymorphic and also efficient in identifying genetic diversity.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Study on morphological and molecular characterisation of hybrids of Ayiramkachi was carried out at the Department of Plant Biotechnology, College of Agriculture, Padannakkad during 2018-2020. Morphological data of hybrids and parental palms were recorded from Regional Agriculture Research Station (RARS), Pilicode and molecular characterisation was done using SSR markers at the Plant Biotechnology Department. This chapter comprises details of materials and methods utilized for the research programme.

3.1 MATERIALS

3.1.1 Coconut palms

Hybrids produced by crossing Ayiramkachi with tall and dwarf cultivars planted during 1994 located in the X-Block (Fig. 1, Plate 1) of RARS, Pilicode was utilized for the present study. The following are the 23 hybrid combinations of Ayiramkachi and their corresponding number of palms in each cross (Table 1)

Table 1: Details of Ayiramkachi hybrids of coconut in the field of RARS Pilicode

Sl. No.	Cross	Hybrids	No. of palms	Palm identity No. (X-Block)
1	TxD	Philippines x Ayiramkachi	5	15, 29, 30, 56, 57
		Cochin China x Ayiramkachi	3	37, 38, 61
		Laccadive Ordinary x Ayiramkachi	2	49, 50
		West Coast Tall x Ayiramkachi	1	55
		Andaman Ordinary x Ayiramkachi	4	18, 39, 41, 42
		Laccadive Micro x Ayiramkachi	3	20, 36, 46
2	DxT	Ayiramkachi x West Coast Tall	1	4
3	DxD	Malayalan Yellow Dwarf x Ayiramkachi	3	13, 14, 59
		Ayiramkachi x Malayalan Yellow Dwarf	1	43

The parental palms consisted of eight cultivars *viz*., Ayiramkachi (Plate 2), MYD, Andaman Ordinary, Cochin China, Philippines, Laccdive Ordinary, WCT and Laccadive Micro, details for which are given in Table 2.

Table 2: Details of parental cultivars of coconut hybrids in the field of RARS, Pilicode

Sl. No.	Cultivar	Block	Palm identity No.
1	Ayiramkachi (AYK)	N4-Block	6, 7, 29, AYK (number not specified)
2	Andaman Ordinary (AO)	D-Block	91, 99
3	Cochin China (CC)	G-Block	166, 167, 168, 169
4	Laccadive Micro (LM)	G-Block	88, 90, 92
5	Laccadive Ordinary (LO)	D-Block	141, 149
6	Malayalan Yellow Dwarf (MYD)	J-Block; T-Block	57, 58, MYD (number not specified)
7	Philippines (PHI)	G-Block	133, 134, 135, 136
8	West Coast Tall (WCT)	G-Block	11, 14

Kerasree (WCTxMYD) located in the T-Block of RARS, Pilicode was used as check palm for the study.

3.1.2 SSR primers

A total of 34 pairs of SSR primers (Merck India Ltd) (including forward and reverse primers) reported in coconut by various research groups were used in the present study, details of which are given in Table 3.

3.1.3 Chemicals, reagents and equipments

Laboratory chemicals (Molecular biology grade) and equipments available at the Plant Biotechnology Department, College of Agriculture, Padannakkad were utilized for the research work.

Plate 1. Experimental plot of Ayiramkachi hybrids of coconut (X-Block, RARS Pilicode)

Plate 2. The common parent 'Ayiramkachi' used in hybridization programme

Table 3: Details of coconut specific SSR markers used for molecular characterisation of parental and hybrid combinations using Ayiramkachi

Sl No.	Primer name	F/R	Primer sequence (5′ → 3′)	GC content (%)
1	CAC 02	F	AGCTTTTTCATTGCTGGAAT	35
		R	CCCCTCCAATACATTTTTCC	45
2	CAC 03	F	GGCTCTCCAGCAGAGGCTTAC	61.9
		R	GGGACACCAGAAAAAGCC	55.6
3	CAC 04	F	CCCCTATGCATCAAAACAAG	45
		R	CTCAGTGTCCGTCTTTGTCC	55
4	CAC 06	F	TGTACATGTTTTTTGCCCAA	35
		R	CGATGTAGCTACCTTCCCC	57.9
5	CAC 08	F	ATCACCCCAATACAAGGACA	45
		R	AATTCTATGGTCCACCCACA	45
6	CAC 10	F	GGAACCTCTTTTGGGTCATT	45
		R	GATGGAAGGTGGTAATGCTG	50
7	CAC 11	F	GATCTTCGGCGTTCCTCA	55.6
		R	TCTCCTCAACAATCTGAAGC	45
8	CnCirA9	F	AATGTTTGTGTCTTTGTGCGTGTGT	40
		R	TCCTTATTTTTCTTCCCCTTCCTCA	40
9	CnCirB12	F	GCTCTTCAGTCTTTCTCAA	42.1
		R	CTGTATGCCAATTTTTCTA	31.6
10	CnCirC12	F	ATACCACAGGCTAACAT	41.2
		R	AACCAGAGACATTTGAA	35.3
11	CnCirE2	F	TCGCTGATGAATGCTTGCT	47.4
		R	GGGGCTGAGGGATAAACC	61.1
12	CNZ 04	F	TATATGGGATGCTTTAGTGGA	38.1
		R	CAAATCGACAGACATCCTAAA	38.1
13	CNZ 05	F	CTTATCCAAATCGTCACAGAG	42.9
		R	AGGAGAAGCCAGGAAAGATTT	42.9
14	CNZ 06	F	ATACTCATCATCATACGACGC	42.9
		R	CTCCCACAAAATCATGTTATT	33.3
15	CAC65	F	GAAAAGGATGTAATAAGCTGG	38
		R	TTTGTCCCCAAATATAGGTAG	38
16	CNZ10	F	CCTATTGCACCTAAGCAATTA	38
		R	AATGATTTTCGAAGAGAGGTC	38

17	CNZ12	F	TAGCTTCCTGAGATAAGATGC	43
		R	GATCATGGAACGAAAACATTA	33
18	CNZ40	F	CTTGATTGCTATCTCAAATGG	38
		R	CTGAGACCAAATACCATGTGT	43
19	CNZ44	F	CATCAGTTCCACTCTCATTTC	43
		R	CAACAAAAGACATAGGTGGTC	43
20	CNZ46	F	TTGGTTAGTATAGCCATGCAT	38
		R	AACCATTTGTAGTATACCCCC	43
21	CnCir01	F	TTGGTCTATTGCATGTTC	39
		R	TGGCATTGAGAGGGT	53
22	CnCirC5	F	ACCACCAAAGCCAGAGC	59
		R	GCAGCCACTACCTAAAAAG	47
23	CnCirHll	F	TCATTCAGAGGACAAAAGTT	35
		R	TAAAAATTCATAAAGGTAAAA	14
24	CnCir51	F	TCTCGTGGATCTCGTC	56
		R	GCTCTTCCAGTTACGTTT	44
25	CnCir A3	F	AATCTAAATCTACGAAAGCA	30
		R	AATAATGTGAAAAAGCAAAG	25
26	CnCir B6	F	GAGTGTGTGAGCCAGCAT	56
		R	ATTGTTCACAGTCCTTCCA	42
27	CnCir C3'	F	AGAAAGCTGAGAGGGAGATT	45
		R	GTGGGGCATGAAAAGTAAC	47
28	CnCir C7	F	ATAGCATATGGTTTTCCT	33
		R	TGCTCCAGCGTTCATCTA	50
29	CnCir E10	F	TGGGTTCCATTTCTTCTCTCATC	43
		R	GCTCTTTAGGGTTCGCTTTCTTAG	46
30	CnCir E12	F	TCACGCAAAAGATAAAACC	37
		R	ATGGAGATGGAAAGAAAGG	42
31	CnCir F2	F	GGTCTCCTCTCCCTCCTTATCTA	52
		R	CGACGACCCAAAACTGAACAC	52
32	CnCir G11	F	AATATCTCCAAAAATCATCGAAAG	29
		R	TCATCCCACACCCTCCTCT	58
33	CnCir H4'	F	TTAGATCTCCTCCCAAAG	44
		R	ATCGAAAGAACAGTCACG	44
34	CnCir H7	F	GAGATGGCATAACACCTA	44
		R	TGCTGAAGCAAAAGAGTA	39

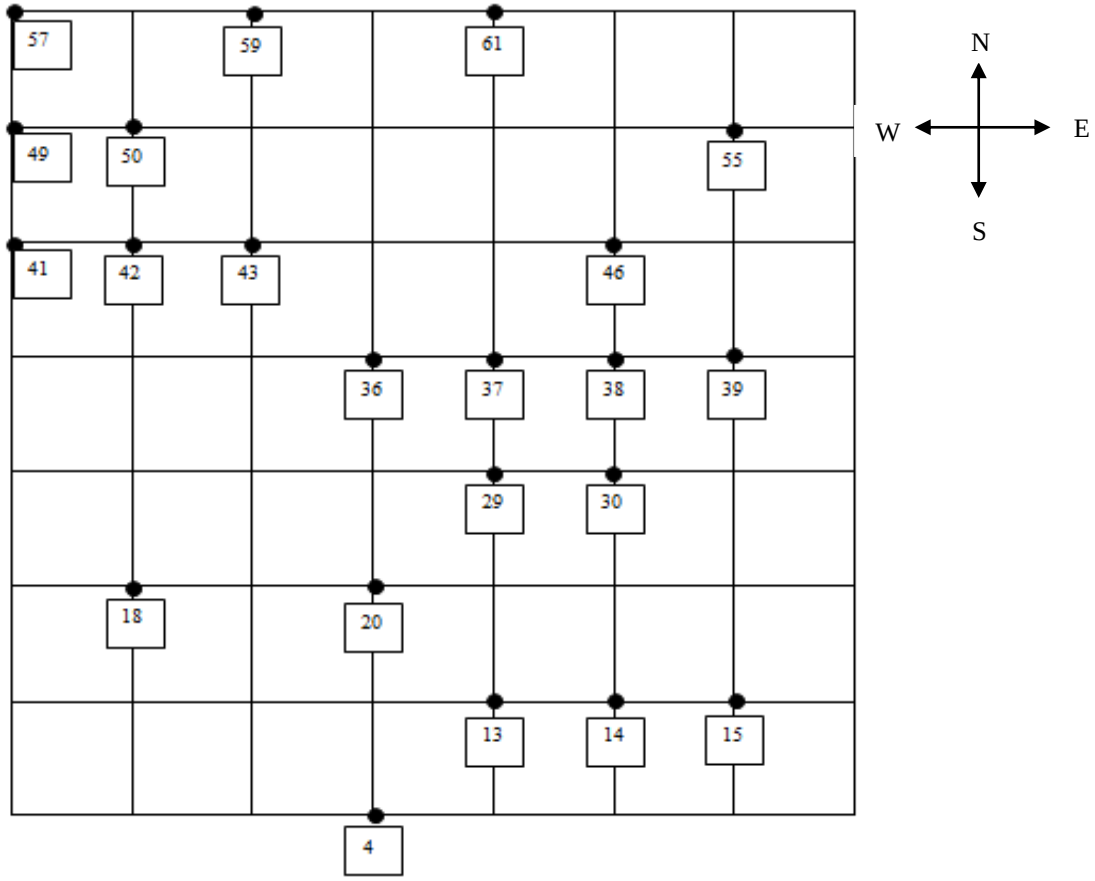


Figure 1. Layout of experimental field (X-Block) RARS, Pilicode

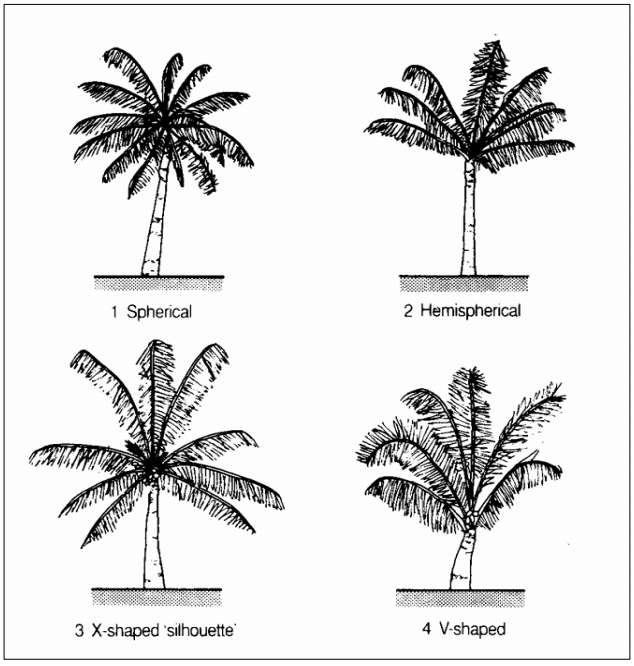


Figure 2. Crown shape of coconut as in IPGRI descriptor

3.2 METHODS

3.2.1 Morphological characterisation

3.2.1.1 *Vegetative characters*

Age of palm at first flowering: Age in years for the emergence of first inflorescence from the date of planting was recorded.

Shape of crown: Crown shape was classified into spherical, hemispherical, X-shaped, V-shaped or others according to descriptors for coconut (Fig. 2).

Height of the palm (m): Height was measured from base of the palm to the point from the crown starts or to the oldest leaf and recorded in meter.

Girth of the palm (cm): Circumference of palm at a height of 1.5 m from the ground was measured with a measuring tape and noted in centimetres.

Internode length (cm): Height of ten leaf scars at 1.5 m from ground was measured and mean height of one leaf scar was recorded in centimetre.

Number of green leaves: Total number of fully opened functional green leaves in the crown at the time of observation were counted.

Rate of leaf production: Number of leaves produced per palm per year.

Petiole colour: Petiole colour was recorded as green, yellow, brown, red or others according to descriptors for coconut.

Petiole length (cm): Length was measured in centimetre from the base of petiole to first nearest leaflet using a measuring tape.

Leaf length (cm): Leaf length was measured in centimetre from base of the petiole to most distal leaflet using a measuring tape.

3.2.1.2 *Reproductive characters*

Total number of inflorescence in the crown at the time of observation: Total number of unopened and opened inflorescence, inflorescence undergoing pollination and also

bunches with nuts at the time of observation were recorded.

Number of unopened inflorescence: Total number of unopened inflorescence present in a palm at the time of observation was recorded.

Number of opened inflorescence undergoing pollination: Total number of inflorescence where female flowers are receptive and male flowers are available for pollination were recorded.

Total inflorescence per palm per year: Total number of inflorescence produced by a single palm during a period of one year was noted (2019-2020).

Period between emergence and opening: Number of days taken from emergence to opening of the inflorescence.

Male phase (days): Number of days taken between anthesis of the first male flower to the last one in an inflorescence.

Female phase (days): Number of days taken between the first to the last female flower become receptive.

Period between phases (days): The period between termination of male phase and initiation of female phase in an inflorescence was recorded in days.

Concordance of phase, if any (days): Number of overlapping days between male and female phases were noted.

Number of female flowers per inflorescence: Total number of female flowers were counted from a freshly opened inflorescence.

Number of female flowers one month after pollination: Number of female flowers were counted one month after pollination of the particular inflorescence.

3.2.1.3 *Yield characters*

Number of bunches per palm per year: It was recorded as the total number of bunches harvested from each palm in a year.

Number of nuts per bunch: Total number of nuts in each matured bunch was counted

and mean was taken.

Number of nuts per palm per year: Number of nuts obtained during each harvest in a year from each palms were added up and recorded (2018-2020).

3.2.1.4 *Nut characters*

Nuts were collected from palms during every harvest and characters were recorded as an average of five nuts.

Fruit colour: Fruit colour was recorded based on visual observations.

Size of unhusked nut (cm): The equatorial and pole to pole circumference of nuts were measured in centimetre using measuring tape and mean was taken.

Fruit weight (g): Weight of unhusked nuts were measured in grams using weighing balance and mean was calculated.

Volume of fruit by water displacement method (ml): Volume of water displaced by the fruit by water displacement method was recorded.

Nut weight (g): Weight of husked nut was measured in grams using weighing balance and mean was calculated.

Shell and meat weight (g): It was recorded in gram using a weighing balance after removing liquid endosperm from nut and mean was calculated.

Kernel thickness at maturity (mm): Kernel thickness from opened nut was measured in millimetre using a measuring scale and average was taken.

Quantity of liquid endosperm (ml): Liquid endosperm was collected on a measuring cylinder and mean quantity was calculated in millilitre.

Sugar content (˚ Brix): Sugar content of coconut water was measured by a hand refractometre and expressed in degree brix.

Copra content (g): The opened nuts were dried under the sun till they attains a constant weight, copra content was calculated by weighing the dried kernel (without shell) using a weighing balance expressed in gram and mean was calculated.

3.2.1.5 *Pest and disease incidence if any*

Any incidence of pest or diseases were recorded.

3.2.2 Statistical analysis

The quantitative data was subjected to statistical analysis to get clear and precise results.

3.2.2.1 *Analysis of variance (ANOVA)*

Analysis of variance was done to find out the significant difference between the cultivars for characters under study. Among the morphological characters-vegetative, reproductive and yield characters were analysed by two-way ANOVA technique as per Panse and Sukhatme (1967) and the nut characters by one-way ANOVA. The analysis was performed using software WASP ver.2.0.

3.2.2.2 *Genetic variability parameters*

1. Coefficient of variations

Phenotypic and genotypic coefficient of variation (PCV and GCV) as per cent were calculated as,

$$PCV = \sigma_p/Mean \times 100$$

$$GCV = \sigma_g/Mean \times 100$$

Where σ_p and σ_g are phenotypic and genotypic standard deviation respectively.

The range of variation is classified as follows (Sivasubramanian and Menon, 1973):

Less than 10% - Low
Between 10-20% - Moderate
More than 20% - High

2. Heritability

Heritability was calculated using the formula by Johnson *et al*. (1955) and

expressed in percentage.

$$H^2 (\%) = V_g/V_P \times 100$$

Where V_g and V_p are genotypic and phenotypic variance respectively

Classification of range for Heritability (Johnson *et al*., 1955):

Less than 30 % - Low
Between 30-60% - Medium
More than 60% - High

3. Genetic gain

It is genetic advance expressed as per cent of mean.

GAM% = Genetic advance/ Mean x 100

Classification of range for GAM% (Johnson *et al*., 1955)

Less than 10% - Low
Between 10-20% - Moderate
More than 20% - High

3.2.2.3 *Correlation and path analysis*

Correlation analysis gives an idea of inter-relationship between the variables under study, but the true contribution of these variables towards a particular character can be only identified by path analysis. Both these analysis were performed using software OPSTAT.

Classification of rage for direct and indirect effect as given by Lenka and Mishra (1973) is as follows:

0.00-0.09 - Negligible
0.10-0.19 - Low
0.20-0.29 - Moderate
0.30-1.00 - High
More than 1.00 - Very high

3.2.2.4 *Heterosis estimation*

Heterosis of the nine hybrids of Ayiramkachi over a standard check (Standard heterosis) and better parent (Heterobeltiosis) was estimated (Rai, 1979).

$$\text{Standard heterosis (SH)} = \frac{\overline{F_1} - \overline{\text{Check}}}{\overline{\text{Check}}} \times 100$$

$$\text{Heterobeltiosis (HB)} = \frac{\overline{F_1} - \overline{\text{Better parent}}}{\overline{\text{Better parent}}} \times 100$$

The significance was tested by student t- test

$$t = \frac{\overline{F_1} - (\overline{\text{Check}} \text{ or } \overline{\text{Better parent}})}{\text{Standard error}}$$

3.2.3 Molecular characterisation

Molecular characterisation of eight parental palms and Kerasree (Check) was done using 34 reported SSR markers and the polymorphic markers were identified and suggested for characterisation of hybrids in future study.

3.2.3.1 *Genomic DNA isolation*

DNA isolation protocol using CTAB method developed by Roger and Bendich (1985) modified by Chethana (2016) was followed. DNA was obtained in low quantity with some impurities, so the protocol was slightly modified. The quality and quantity of DNA obtained was verified using agarose gel electrophoresis and spectrophotometer.

3.2.3.1.1 *Protocol 1 – CTAB method (Chethana, 2016)*

Chemicals and reagents:

1. CTAB extraction buffer (pH-8)

- CTAB : 2%
- Tris : 100 mM

- EDTA : 20 mM
- NaCl : 1.4 M
- Sterilized distilled water

2. 20% PVP
3. β-mercaptoethanol
4. Sodium metabisulphate
5. Chloroform and isoamyl alcohol (24:1 ratio)
6. Isopropanol

Procedure:

1. Preheated 14 ml CTAB buffer in hot water bath (60-65˚C)

2. Wipe one gram young tender coconut leaf (without midrib) with 70% ethanol and cutted it in to small pieces using sterile blade then ground it with 40 µl β-mercaptoethanol, 20 µl PVP (20%) and a pinch of sodium metabisulphate with liquid nitrogen in a sterile cooled mortar and pestle

3. The ground material is then transferred to extraction buffer, mixed thoroughly and incubated at 65˚C in water bath for 30 minutes with intermittent mixing

4. Chloroform: isoamyl alcohol (24:1) at 2/3rd volume was added after incubation and mixed by inverting the tube. Refrigerated centrifugation (4˚C) was done for 15 minutes at 12000 rpm

5. Aqueous layer at the top was collected carefully and transferred to a new eppendorf tube.

6. Slowly inverted the tube several times after adding chilled isopropanol (1/6th volume). Refrigerated centrifugation (4˚C) was done for 15 minutes at 12000 rpm.

7. Solution was decanted carefully leaving the DNA pellet, which is then washed for 2 times using 70% ethanol by centrifugation

8. The DNA pellet was dissolved in ultrapure water after air drying

3.2.3.1.2 *Protocol 2 – Modified procedure*

- Preheat 10 ml extraction buffer instead of 14 ml for 1 g sample
- Step 2, 3, 4 and 5 were followed as on protocol-1
- Treated with RNase (2 µl) and incubated for 30 minutes at 37˚C
- Steps 4, 5, 6 and 7 was followed as on protocol-1
- DNA pellet was dissolved in TE buffer or ultrapure water

3.2.3.2 *Quantity and quality of isolated DNA*

DNA was quantified using Eppendorf Bio-Photometer (spectrophotometer). The instrument was set at 260 nm (absorption maxima of DNA) before quantification. 260/280 ratio 1.8-2 indicate good quality DNA.

Procedure:

1. 50 µl ultrapure water or TE buffer was taken on a sterile clean cuvette and it was set as blank (reading zero) at 260 nm
2. 1 µl sample DNA and 49 µl ultrapure water or TE buffer was taken on another sterile clean cuvette and it was set as sample. Reading was indicated in ng/µl
3. Concentration of DNA in the sample was found out by multiplying the spectrophotometric reading with dilution factor

   DNA concentration = X ng/µl x 50

3.2.3.3 *Agarose gel electrophoresis*

Upon gel electrophoresis the negatively charged DNA molecules migrate towards anode depending on their size through a matrix of agarose gel. DNA within the gel can be visualized using fluorescent dye- Ethidium bromide under UV light. Approximate size, concentration and quality of DNA can be identified by this technique.

Materials required:

1. DNA sample
2. Chemical reagents: Agarose gel – 0.8%

TAE or TBE buffer – 50X

Gel loading dye – 6X

Ethidium bromide (EtBr) - 5µl/100 ml solution

DNA ladder – 1 kb

3. Equipments : Horizontal electrophoresis apparatus

BIO-RAD Gel Doc - UV transilluminator and gel imaging unit

Procedure:

1. 0.8 g agarose was dissolved in 100 ml 1X TAE/TBE buffer by heating (0.8% gel)
2. When cooled (60˚C) EtBr was added and poured into gel casting tray fitted with comb, after mixing
3. Once it is solidified remove the comb and transferred to electrophoresis apparatus with 1X TAE/TBE buffer
4. 5 µl DNA sample was mixed with 1 µl loading dye, but for DNA ladder 1 µl ladder was mixed with 1 µl dye and 4 µl distilled water on a parafilm and loaded on the wells. Constant voltage was kept (90 V)
5. Power was turned off once the tracking dye crossed 3/4th distance on the gel
6. Gel was taken out carefully and kept on Gel-Doc unit for documentation

3.2.3.4 ***Primer dilution***

A total of 68 SSR primers (34 each forward and reverse) were diluted

Procedure:

1. Primers were centrifuged for 2-3 minutes before opening once it was received
2. Master stock (100 µM) was prepared by adding ultrapure water

   100 µM = X nmoles lyophilized primer + (X x 10 µl ultrapure water)
3. Working stock (10 µM) was prepared by diluting the master stock with ultrapure water at 1: 10 ratio

3.2.3.5 ***Standardisation of PCR reaction for SSR primers***

PCR amplification was performed on Himedia thermal cycler. Thirty four SSR primers were screened for polymorphism in coconut genotypes. Reaction mixture

was set up for all the 34 SSR primers as reported by Renju (2012). The annealing temperatures were finalysed by establishing a gradient thermal profile. Gradient was setup at temperatures based on the lowest melting temperature (Tm±5˚C) among forward and reverse primers, for all the 34 primers.

The PCR products were analysed on 2% agarose gel (Renju, 2012) of 5-10 mm thickness. The standardised PCR condition was setup for characterising eight parental genotypes.

Mastermix (20 µl)	Thermal profile
DNA template – 50 ng/ µl	Initial denaturation: 94˚C- 5 min
10X PCR buffer with $MgCl_2$ – 2 µl	Denaturation: 94˚C- 1 min } 35 cycles
10 mM dNTPs – 1.5 µl	Annealing: X˚C - 1 min } 35 cycles
Taq polymerase (3U) – 0.1 µl	Extension: 72˚C- 2 min } 35 cycles
Forward primer (10 pM) – 1 µl	Final extension: 72˚C- 5 min
Reverse primer (10 pM) – 1 µl	Hold: 4˚C
Sterile distilled water	

(X˚C : Annealing temperature specific to each primer)

3.2.3.6 *Analysis of SSR gel profile data*

The gels were scored for presence (1) or absence (0) of band manually for the nine genotypes (eight parents and one check). Markers which are found polymorphic were suggested for characterisation of hybrids.

Diversity analysis was carried out by Dice dissimilarity matrix using software DARwin ver.6.0.

RESULTS

4. RESULTS

Study on "Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*Cocos nucifera* L.)" was carried out at College of Agriculture, Padannakkad and Regional Agriculture Research Station (RARS), Pilicode during 2018-2020. Twenty three hybrids of Ayiramkachi planted during 1994 were evaluated for its performance with parental palms and a check palm (Kerasree) based on morphological characterisation. The parental palms were subjected to molecular characterisation using SSR markers and identified the markers with polymorphism. Observations on vegetative, reproductive, yield and nut characters were recorded and results after statistical analysis are presented in this chapter.

4.1 MORPHOLOGICAL CHARACTERISATION OF GENOTYPES

4.1.1 Performance of hybrids of Ayiramkachi in comparison with parental palms and check cultivar

Mean performance on morphological characters of six TxD [PHIxAYK (Plate 3), CCxAYK (Plate 4), LMxAYK (Plate 5), LOxAYK (Plate 6), AOxAYK (Plate 7) and WCTxAYK (Plate 8)], one DxT [AYKxWCT (Plate 9)] and two DxD hybrids [MYDxAYK (Plate 10) and AYKxMYD (Plate 11)] of Ayiramkachi were calculated in comparison with parental palms and check cultivar. Mean performance of vegetative characters for the genotypes were studied and presented in Table 4, reproductive and yield characters in Table 5 and nut characters in Table 6.

4.1.1.1 Height of the palm (HT)

A significant variation was observed among the genotypes for palm height (Figure 3, Table 4). For parental palms height ranged from 5.200 m (MYD) to 16.373 m (WCT) and for hybrids 5.257 m (PHIxAYK) to 9.038 m (LOxAYK).

Highest value was recorded by WCT (16.373 m) and was statistically on par with CC (14.449 m) and lowest by MYD (5.200 m) which was on par with MYDxAYK (6.515 m), WCTxAYK (6.350 m), AYKxMYD (5.345 m) and PHIxAYK

(5.257 m). All the hybrids exhibited palm height lower than the check palm Kerasree (KS) (9.915m).

4.1.1.2 Girth of the palm at 20 cm height (GP_20)

Girth of the palm varied significantly among the genotypes (Table 4). Girth varied from 59.625 cm (MYD) to 120.200 cm (PHI) for parents and 80.250 cm (AYKxWCT) to 136.700 cm (AOxAYK) for hybrids. Maximum girth was recorded by AOxAYK (136.700 cm) which was statistically on par with PHI (120.200 cm) and lowest by MYD (59.625 cm) and was on par with LO (79.750 cm).

On comparing with the check (KS), hybrid AOxAYK (136.700 cm) exhibited higher girth value, and hybrids LOxAYK (105.550 cm), CCxAYK (94.483 cm), PHIxAYK (93.690 cm), MYDxAYK (83.700 cm), AYKxMYD (80.500 cm) and AYKxWCT (80.250 cm) recorded a lower value. Hybrids LMxAYK (110.750 cm) and WCTxAYK (110.500 cm) were found statistically on par with KS (113.575 cm).

4.1.1.3 Girth of the palm at 1.5 m height (GP_1.5)

Girth at 1.5 m height differed significantly among genotypes (Table 4). Girth ranged from 57.250 cm (MYD) to 87.983 cm (LM) for parents and 66.150 cm (MYDxAYK) to 85.450 cm (LMxAYK) for hybrids. Maximum girth was recorded by LM (87.983 cm) which was statistically on par with PHI (87.300 cm) and lowest by MYD (57.250 cm).

On comparing with the check (KS), hybrids PHIxAYK (77.520 cm), CCxAYK (75.317 cm), AYKxWCT (73.000 cm), AYKxMYD (72.000 cm) and MYDxAYK (66.150 cm) exhibited a lower girth value, and other hybrids exhibited values on par with the check (KS).

4.1.1.4 Internode length (IL)

Significant difference was observed among the genotypes for internode length (Table 4). For parental palms internode length ranged from 2.925 cm (MYD) to 5.050 cm (WCT) and for hybrids 3.550 cm (AYKxMYD) to 6.825 cm (LOxAYK).

TxD hybrids of Ayiramkachi (AYK) crossed with Philippines (PHI), Cochin China (CC), Laccadive Micro (LM), Laccadive Ordinary (LO), Andaman Ordinary (AO) and West Coast Tall (WCT)

Plate 3a. Palm no. 15

Plate 3b. Palm no. 30

Plate 3. PHIxAYK hybrid palms

Plate 4a. Palm no. 37

Plate 4b. Palm no. 38

Plate 4. CCxAYK hybrid palms

Plate 5a. Palm no. 20

Plate 5b. Palm no. 36

Plate 5. LMxAYK hybrid palms

Plate 6a. Palm no. 50

Plate 6. LOxAYK hybrid palm

Plate 7a. Palm no. 18

Plate 7. AOxAYK hybrid palm

Plate 8. WCTxAYK hybrid palm

DxT hybrid of Ayiramkachi (AYK) crossed with West Coast Tall (WCT)

Plate 9. AYKxWCT hybrid palm

DxD hybrids of Ayiramkachi (AYK) crossed with Malayalan Yellow Dwarf (MYD)

Plate 10a. Palm no. 13

Plate 10b. Palm no. 14

Plate 10. MYDxAYK hybrid palms

Plate 11. AYKxMYD hybrid palm

Bunches produced by the hybrid palms of Ayiramkachi crossed with Tall and Dwarf cultivars

Plate 12. Bunch of PHIxAYK hybrid

Plate 13. Bunch of CCxAYK hybrid

Plate 14. Bunch of LOxAYK hybrid

Plate 15. Bunch of AOxAYK hybrid

Plate 16a. Bunch of LMxAYK hybrid (Palm no. 20)

Plate 16b. Bunch of LMxAYK hybrid (Palm no. 36)

Plate 16. Bunch of LMxAYK hybrid

Plate 17. Bunch of AYKxWCT hybrid

Plate 18. Bunch of MYDxAYK hybrid

Table 4. Mean performance for vegetative characters of coconut hybrids of Ayiramkachi in comparison with parental cultivars and check palm

Genotype	Height of palm (m)	Girth at 20 cm height (cm)	Girth at 1.5 m height (cm)	Internode length (cm)	Number of green leaves	Rate of leaf production	Petiole length (cm)	Leaf length (cm)
PH I x AYK	5.257	93.690	77.520	3.790	22.225	1.660	119.800	457.300
CC x AYK	7.105	94.483	75.317	5.400	28.708	1.667	124.333	504.333
LO x AYK	9.038	105.550	82.475	6.825*	25.813	1.945*	142.250*	529.750
WCT x AYK	6.350	110.500	82.100	6.300	23.400	1.570	138.500*	585.000*
AO x AYK	7.538	136.700*	84.275	6.338*	21.969	1.513	124.500	490.625
LM x AYK	7.585	110.750	85.450	5.275	27.625	1.555	136.500	496.250
AYK x WCT	7.500	80.250	73.000	6.300	19.000	1.200	131.500	543.000*
MYD x AYK	6.515	83.700	66.150	3.800	24.125	1.550	118.667	437.833
AYK x MYD	5.345	80.500	72.000	3.550	18.400	1.250	81.000	331.500
Ayiramkachi	9.064	89.513	73.813	4.250	22.844	1.250	131.875	413.125
Laccadive Micro	9.733	107.550	87.983*	4.467	25.500	1.167	120.833	451.000
Laccadive Ordinary	12.788	79.750	68.250	4.925	28.000	1.375	114.250	453.000
Andaman Ordinary	13.075	98.750	79.250	4.950	26.813	1.125	109.500	432.000
Cochin China	14.449*	105.188	82.688	4.263	36.938*	2.063*	116.750	520.500
Philippines	9.775	120.200*	87.300*	4.938	33.219*	1.250	118.750	516.875
West Coast Tall	16.373*	116.750	83.000	5.050	22.438	1.195	120.000	500.000
Malayalan Yellow Dwarf	5.200	59.625	57.250	2.925	24.813	1.140	92.250	324.750
Kerasree	9.915	113.575	83.000	3.500	28.500	1.438	112.250	475.000
Mean	9.034	99.279	77.823	4.825	25.574	1.439	119.639	470.102
CV	18.320	20.842	7.595	18.897	12.226	15.744	10.899	8.084
CD (0.05)	2.358	30.358	8.574	1.281	4.569	0.332	18.788	54.774

Table 5. Mean performance for reproductive characters of coconut hybrids of Ayiramkachi in comparison with parental cultivars and check palm

Genotype	Total number of inflor. in crown at observation	No. of unopened inflor.	No. of opened inflor. undergoing pollination	No. of inflor. in which pollination over and seed setting started	Total inflor. per palm per year	No. of female flowers per inflor.	No. of female flowers one month after pollination	No. of bunches per palm per year	No. of nuts per bunch	No. of nuts per palm per year
PHI x AYK	10.250	1.667	0.750	0.625	13.333	20.875	13.033	11.000	7.110	81.667
CC x AYK	11.375*	1.458	0.810	0.675	14.333	32.292	20.400	10.667	12.688	150.667
LO x AYK	11.563*	1.625	0.625	0.680	12.500	31.167	18.250	9.500	10.532	116.500
WCT x AYK	8.250	1.125	0.625	0.625	11.000	24.750	17.250	11.000	8.636	95.000
AO x AYK	10.969*	1.823	0.625	0.563	12.750	26.344	16.283	8.500	7.301	61.500
LM x AYK	11.563*	2.375	0.775	0.750	13.000	22.375	14.000	12.000	12.899	155.500
AYK x WCT	10.875*	1.750	0.750	0.625	14.000	30.375	20.000	10.000	9.500	95.000
MYD x AYK	12.000*	2.028	0.825	0.700	13.667	22.292	14.533	9.333	12.225	115.333
AYK x MYD	8.625	1.333	0.500	0.500	8.000	11.875	5.400	8.000	2.625	21.000
Ayiramkachi	11.250*	1.281	0.656	0.679	10.250	66.890*	37.150*	8.250	22.09*	189.000
Laccadive Micro	11.833*	1.524	0.625	0.625	11.667	20.357	14.806	8.000	9.296	75.667
Laccadive Ordinary	11.375*	1.542	0.625	0.563	11.000	27.175	16.750	8.000	8.936	66.500
Andaman Ordinary	11.313*	1.571	0.563	0.625	11.000	25.667	19.042	8.000	8.460	68.000
Cochin China	11.906*	2.031	0.656	0.563	12.750	30.344	21.600	8.500	8.600	71.750
Philippines	11.344*	1.750	0.545	0.545	12.500	18.813	12.863	9.750	7.510	75.250
West Coast Tall	11.938*	1.750	0.698	0.750	11.000	23.063	18.000	9.500	9.600	91.500
Malayalan Yellow Dwarf	6.750	1.500	0.563	0.500	8.500	28.813	15.333	7.000	7.071	49.500
Kerasree	11.781*	2.000	0.719	0.710	13.750	31.563	21.000	10.750	9.809	104.250
Mean	10.831	1.674	0.663	0.628	11.944	25.502	17.539	9.319	9.716	93.532
CV	11.215	21.149	25.065	22.070	17.670	28.886	31.023	7.595	30.344	52.919
CD (0.05)	1.777	-	-	-	-	11.931	8.150	-	6.673	-

Highest internode length was recorded by LOxAYK (6.825 cm) which was on par with AOxAYK (6.338 cm) and lowest by MYD (2.925 cm) and was on par with AYKxMYD (3.550 cm) and KS (3.500 cm).

On comparing with the check (KS), hybrids LOxAYK (6.825 cm), AOxAYK (6.338 cm), WCTxAYK (6.300 cm), AYKxWCT (6.300 cm), CCxAYK (5.400 cm) and LMxAYK (5.275 cm) exhibited higher internode length, and other hybrids recorded a value on par with check (KS).

4.1.1.5 Number of green leaves (NGL)

The genotypes differed significantly for number of green leaves (Table 4). Number of green leaves ranged from 22.438 (WCT) to 36.938 (CC) for parents and 18.400 (AYKxWCT) to 28.708 (CCxAYK) for hybrids. Maximum number of green leaves was recorded by CC (36.938) which was on par with PHI (33.219) and least by AYKxMYD (18.400) and was on par with AYKxWCT (19.000).

On comparing with the check, hybrids LOxAYK (25.813), MYDxAYK (24.125), WCTxAYK (23.400), PHIxAYK (22.225), AOxAYK (21.969), AYKxWCT (19.000) and AYKxMYD (18.400) recorded lesser number of green leaves and other hybrids were found on par with the check (KS).

4.1.1.6 Rate of leaf production (RLP)

Rate of leaf production varied significantly among the genotypes (Table 4). It varied from 1.125 (AO) to 2.063 (CC) for parents and 1.250 (AYKxMYD) to 1.945 (LOxAYK) for hybrids. Maximum rate of leaf production was recorded by CC (2.063) and was statistically on par with LOxAYK (1.945) and least by AO (1.125), MYD (1.140) and LM (1.167).

On comparing with the check, hybrids LOxAYK (1.945), CCxAYK (1.667), PHIxAYK (1.660), WCTxAYK (1.570), LMxAYK (1.555), MYDxAYK (1.550) exhibited higher leaf rate production, and hybrid AYKxMYD (1.250) a lower rate. AOxAYK (1.513) was found on par with the check (1.438).

4.1.1.7 Petiole length (PL)

Significant difference was observed among the genotypes for petiole length (Table 4). Length of petiole ranged from 92.250 cm (MYD) to 131.875 cm (AYK) for parents and 81.000 cm (AYKxMYD) to 142.250 cm (LOxAYK) for hybrids. Maximum value was recorded for LOxAYK (142.250 cm) which was ststistically on par with WCTxAYK (138.500 cm) and least by AYKxMYD (81.000 cm) on par with MYD (92.250 cm). All the hybrids except AYKxMYD (81.000 cm) exhibited petiole length higher than the check (112.250 cm).

4.1.1.8 Leaf length (LL)

Leaf length varied significantly among the genotypes (Table 4). For parental palms it ranged from 324.750 cm (MYD) to 520.500 cm (CC) and for hybrids AYKxMYD (331.500 cm) to 585.000 (WCTxAYK cm). Highest value was recorded by WCTxAYK (585.000 cm) which was statistically on par with AYKxWCT(543.000 cm) and lowest by MYD (324.750 cm) on par with AYKxMYD (331.500 cm).

On comparing with the check, hybrids WCTxAYK (585.000 cm), AYKxWCT (543.000 cm), LOxAYK (529.750 cm), CCxAYK (504.333 cm), LMxAYK (496.250 cm) and AOxAYK (490.625 cm) exhibited higher leaf length and hybrids PHIxAYK (457.300 cm), MYDxAYK (437.833 cm) and AYKxMYD (331.500 cm) a lower value.

4.1.1.9 Total number of inflorescence in the crown at the time of observation (TI)

Total inflorescence differed significantly among the genotypes (Table 5). It varied from 6.750 (MYD) to 11.938 (WCT) for parents and 8.250 (WCTxAYK) to 12.000 (MYDxAYK) for hybrids. Highest value was recorded by MYDxAYK (12.000) which was on par with all genotypes except AYKxMYD (8.625) and WCTxAYK (8.250), and the lowest value was recorded for MYD (6.750).

On comparing with the check, hybrids except AYKxMYD (8.625) and WCTxAYK (8.250) were found statistically similar to the check (11.781).

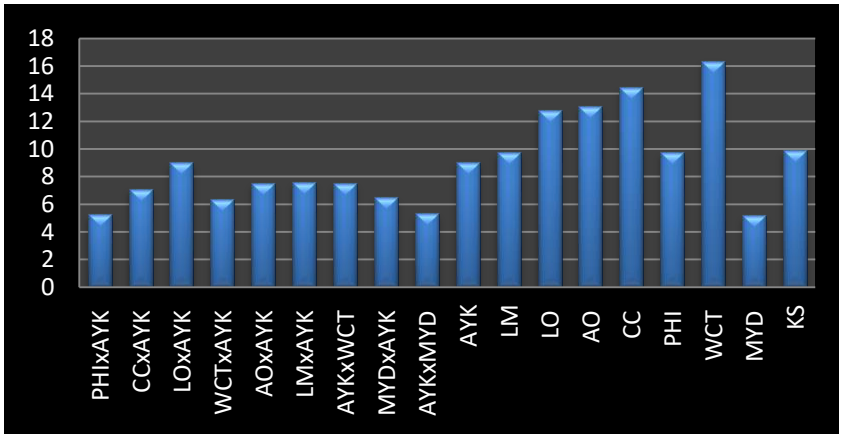


Fig 3. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for palm height

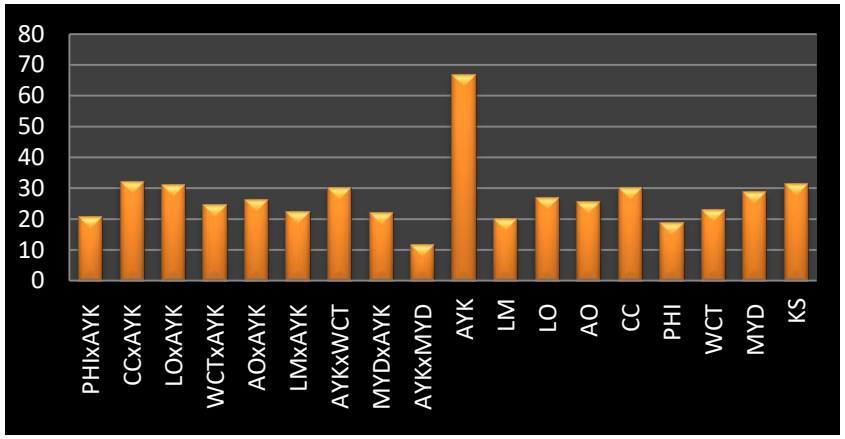


Fig 4. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for number of female flowers per inflorescence

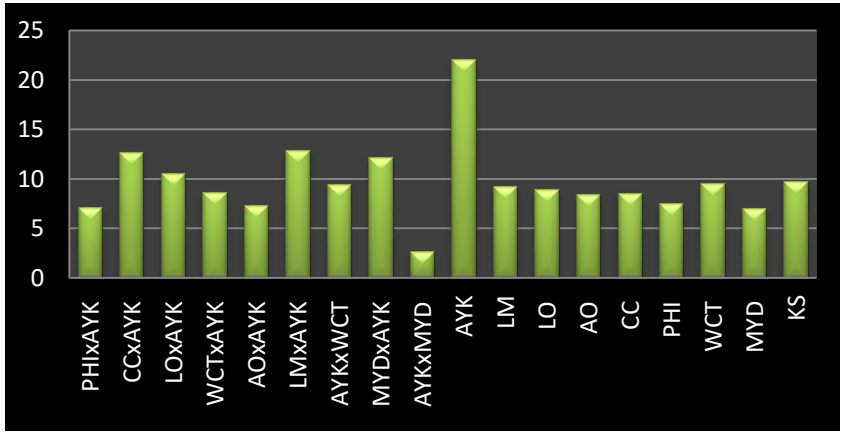


Fig 5. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for number of nuts per bunches

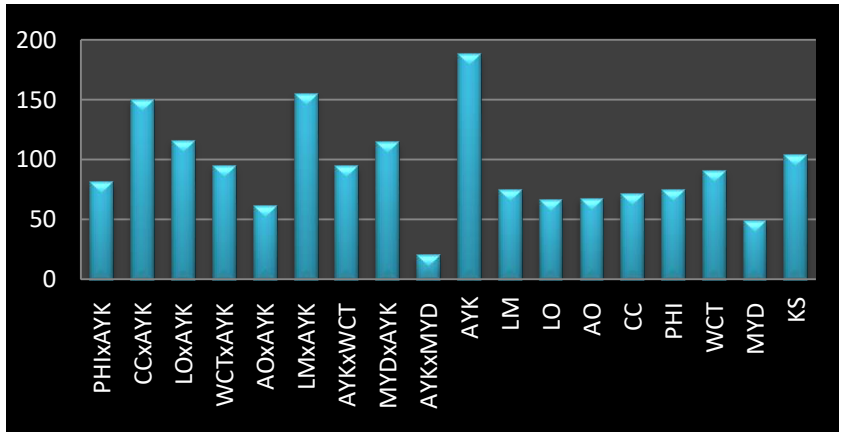


Fig 6. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for number of nuts per palm per year

4.1.1.10 Number of unopened inflorescence (NUI)

No significant difference was observed among the genotypes for number of unopened inflorescence (Table 5). For parents the value ranged from 1.281 (AYK) to 2.031 (CC) and 1.125 (WCTxAYK) to 2.375 (LMxAYK) for hybrids. Mean value of 2.000 was exhibited by the check (KS).

4.1.1.11 Number of opened inflorescence undergoing pollination (IUP)

No significant difference was observed among the genotypes for number of opened inflorescence undergoing pollination (Table 5). The values ranged from 0.545 (PHI) to 0.698 (WCT) for parents and 0.500 (AYKxMYD) to 0.825 (MYDxAYK) for hybrids. Mean value of 0.719 was expressed by the check (KS).

4.1.1.12 Number of inflorescence in which pollination is over and seed setting started (IPOSS)

No significant variation was observed among the genotypes for number of inflorescence in which pollination is over and seed setting started (Table 5). The values ranged from 0.500 (MYD) to 0.750 (WCT) for parents and 0.500 (AYKxMYD) to 0.750 (LMxAYK) for hybrids. Mean value of 0.710 was recorded by the check (KS).

4.1.1.13 Total inflorescence per palm per year (TIPY)

No significant difference was observed among the genotypes for total inflorescence per palm per year (Table 5). The values ranged from 8.500 (MYD) to CC (12.750) for parents and 8.000 (AYKxMYD) to 14.333 (CCxAYK) for hybrids. Mean value of 13.750 was expressed by the check (KS).

4.1.1.14 Number of female flowers per inflorescence (NFF)

Number of female flowers varied significantly among the genotypes (Figure 4, Table 5). It varied from 18.813 (PHI) to 66.890 (AYK) for parents and 11.875 (AYKxMYD) to 32.292 (CCxAYK) for hybrids. Maximum value was recorded by AYK (66.89) and least by AYKxMYD (11.875) on par with PHI (18.813).

On comparing with the check, hybrids LMxAYK (22.375), MYDxAYK (22.292), PHIxAYK (20.875) and AYKxMYD (11.875) exhibited less number of female flowers per inflorescence, but other hybrids recorded a value on par with that of the check (31.563).

4.1.1.15 Number of female flowers one month after pollination (NFFAP)

The genotypes differed significantly for number of female flowers one month after pollination (Table 5). It varied from 12.863 (PHI) to 37.150 (AYK) for parents and 5.400 (AYKxMYD) to 20.400 (CCxAYK) for hybrids. Highest value was recorded by AYK (37.150) and lowest by AYKxMYD (5.400) which was statistically on par with PHI (12.863) and PHIxAYK (13.033).

On comparing with the check, hybrids PHIxAYK (13.033) and AYKxMYD (5.400) recorded lesser number of female flowers one month after pollination but, other hybrids exhibited no significant variation from the check palm (21.000).

4.1.1.16 Number of bunches per palm per year (NBPY)

No significant variation was observed among the genotypes for number of bunches produced by the palm per year (Table 5). For parental palms it varied from 7.000 (MYD) to 9.750 (PHI), and for hybrids 8.000 (AYKxMYD) to 12.000 (LMxAYK). Mean value of 10.750 was recorded by the check (KS).

4.1.1.17 Number of nuts per bunches (NNB)

A significant variation was observed among the genotypes for number of nuts per bunches (Figure 5, Table 5). The corresponding plates showing the bunches of some of the hybrid palms are: PHIxAYK (Plate 12), CCxAYK (Plate 13), LOxAYK (Plate 14), AOxAYK (Plate 15), LMxAYK (Plate 16), AYKxWCT (Plate 17) and MYDxAYK (Plate 18). For parental palms number of nuts per bunches ranged from 7.071 (MYD) to 22.093 (AYK) and for hybrids 2.625 (AYKxMYD) to 12.899 (LMxAYK). Maximum value was recorded for AYK (22.093) and least for AYKxMYD (2.625).

Hybrids except AYKxMYD were found statistically on par with the check (9.809). AYKxMYD (2.625) was found statistically inferior.

4.1.1.18 Number of nuts per palm per year (NNPY)

A very high coefficient of variation (52.919) was recorded for number of nuts produced per palm per year (Table 5). Yield varied from 49.500 (MYD) to 189.000 (AYK) for parental palms and 21.000 (AYKxMYD) to 155.500 (LMxAYK) for hybrids. The check palm (KS) exhibited an average yield of 104.250 (Figure 6).

4.1.1.19 Size of unhusked nut equatorial circumference (SUN_E)

The genotypes differed significantly for size of unhusked nut equatorial circumference (Table 6). For parental palms it ranged from 29.340 cm (AYK) to 52.680 cm (AO) and 37.800 cm (AYKxWCT) to 52.020 cm (PHIxAYK) for hybrids. Genotypes AO (52.680 cm), CC (52.380 cm), PHIxAYK (52.020 cm), LMxAYK (50.500 cm) and MYDxAYK (49.500 cm) was found superior for unhusked nut size and AYK (29.340 cm) was estimated to be the inferior.

On comparing with the check, hybrid PHIxAYK (52.020 cm), LMxAYK (50.500 cm) and MYDxAYK (49.500 cm) recorded highest unhusked nut size, and hybrids CCxAYK (45.400 cm), AOxAYK (42.100 cm), AYKxMYD (41.080 cm) and AYKxWCT (37.800 cm) exhibited least values. Other hybrids recorded size on par with the check (49.000 cm).

4.1.1.20 Size of unhusked nut pole to pole circumference (SUN_P)

Size of unhusked nut pole to pole circumference varied significantly among the genotypes (Table 6). It varied from 42.440 cm (AYK) to 62.560 cm (CC) for parents and 48.740 cm (AYKxMYD) to 58.200 cm (LMxAYK) for hybrids. Highest value was recorded by CC (62.560 cm) which was statistically on par with LMxAYK (58.200 cm) and lowest by AYK (42.440 cm). Genotypes LMxAYK (58.200 cm), LOxAYK (56.600 cm), PHIxAYK (55.760 cm) and MYDxAYK (55.440 cm) were found on par with the check (58.000 cm) and other hybrids recorded a lower pole to pole circumference compared to the check.

Table 6. Mean performance for nut characters of coconut hybrids of Ayiramkachi in comparison with parental cultivars and check palm

Genotype	Size of unhusked nut (equatorial) (cm)	Size of unhusked nut (pole to pole) (cm)	Fruit weight (g)	Fruit volume (ml)	Nut weight (g)	Shell and meat weight (g)	Kernel thickness (mm)	Quantity of liquid endosperm (ml)	Sugar content (°Bx)	Copra content (g)
PHI x AYK	52.020*	55.760	1507.40*	1416.00*	796.60*	575.400*	12.000*	212.800*	5.700	211.020*
CC x AYK	45.400	51.600	750.400	728.000	485.200	365.800	10.400	115.600	6.000	142.876
LO x AYK	46.300	56.600	930.200	903.000	568.800	429.600	11.700*	135.800	7.040	162.868
WCT x AYK	46.600	53.400	840.800	820.000	416.400	363.000	12.000*	49.200	7.500	156.050
AO x AYK	42.100	52.100	904.400	870.000	545.600	402.600	11.800*	138.200	5.120	159.400
LM x AYK	50.500*	58.200*	1178.800	1129.800	624.200	505.600*	12.600*	114.000	8.000	187.250*
AYK x WCT	37.800	49.500	711.400	675.400	399.400	285.000	9.600	109.200	6.080	128.378
MYD x AYK	49.500*	55.440	1013.400	993.000	587.200	421.200	12.000*	163.200	6.220	172.466
AYK x MYD	41.080	48.740	858.200	834.800	532.600	389.200	11.400	142.000	4.560	149.378
Ayiramkachi	29.340	42.440	395.400	377.000	249.600	206.800	11.000	39.000	5.960	88.600
Laccadive Micro	34.420	47.300	468.800	448.000	273.000	231.000	11.200	41.600	5.760	102.000
Laccadive Ordinary	50.700	58.000	1158.000	1112.000	670.200	475.200	11.400	193.200*	6.200	188.200
Andaman Ordinary	52.680*	57.720	1178.000	1132.000	636.600	458.400	11.600*	176.800	5.580	195.000*
Cochin China	52.380*	62.560*	1286.00*	1244.00*	683.00*	477.600	11.600*	205.400*	6.140	205.400*
Philippines	46.080	53.600	994.800	945.000	556.400	388.600	11.700*	166.000	6.060	170.400
West Coast Tall	45.000	54.000	990.800	949.200	530.600	381.000	11.800*	149.400	5.860	169.000
Malayalan Yellow Dwarf	43.080	49.000	881.200	848.000	438.400	308.000	10.200	129.600	6.460	120.600
Kerasree	49.000	58.000*	1149.000	1091.000	647.200	473.800	11.600*	170.200	6.200	193.000*
Mean	45.221	53.553	955.389	917.567	535.611	396.544	11.422	136.178	6.136	161.216
CV	8.324	6.938	22.431	22.339	21.451	17.792	8.189	38.014	20.474	17.280
CD (0.05)	4.750	4.688	270.393	258.624	144.969	89.022	1.180	82.498	-	35.150

4.1.1.21 Fruit weight with husk (FW)

Fruit weight with husk varied significantly among the genotypes (Figure 7, Table 6). For parental palms fruit weight varied from 395.400 g (AYK) to 1286.000 g (CC) and 711.400 g (AYKxWCT) to 1507.400 g (PHIxAYK) for hybrids. Genotype PHIxAYK (1507.400 g) was recorded superior for fruit weight and was statistically on par with CC (1286.000 g) and AYK (395.400 g) was estimated to be the inferior and was on par with LM (468.800 g).

Hybrid PHIxAYK (1507.400 g) exhibited a higher fruit weight compared to check (1149.000 g) and hybrids LMxAYK (1178.800 g), MYDxAYK (1013.400 g), LOxAYK (930.200 g) and AOxAYK (904.400 g) recorded values on par with the check. Other hybrids recorded fruit weight lesser than the check (KS).

4.1.1.22 Volume of fruit (FV)

A significant variation was observed among the genotypes for fruit volume (Table 6). It ranged from 377.000 ml (AYK) to 1244.000 ml (CC) for parents and 675.400 ml (AYKxWCT) to 1416.000 ml (PHIxAYK) for hybrids. Genotype PHIxAYK (1416.000 ml) recorded highest fruit volume and was statistically on par with CC (1244.000 ml) and AYK (377.000 ml) on par with LM (448.000 ml were estimated to be inferior for fruit volume.

Hybrid PHIxAYK (1416.000 ml) exhibited a higher fruit volume compared to check (1091.000 ml) and hybrids LMxAYK (1129.800 ml), MYDxAYK (993.000 ml), LOxAYK (903.000 ml) and AOxAYK (870.000 ml) recorded values on par with the check. Other hybrids recorded volume of fruit lesser than the check (KS).

4.1.1.23 Nut weight without husk (NW)

Nut weight without husk differed significantly among the genotypes (Figure 8, Table 6). For parental palms nut weight ranged from 249.600 g (AYK) to 683.000 g (CC) and 399.400 g (AYKxWCT) to 796.600 g (PHIxAYK) for hybrids. Genotype PHIxAYK (796.600 g) was estimated to be the superior one and was statistically on par with CC (683.000 g), and AYKxMYD (249.600 g) was recorded to be inferior for

nut weight and was on par with LM (273.000 g).

On comparing with the check, hybrid PHIxAYK (796.600 g) exhibited higher nut weight without husk, and hybrids LMxAYK (624.200 g), MYDxAYK (587.200 g), LOxAYK (568.800 g) and AOxAYK (545.600 g) recorded values on par with the check. Other hybrids recorded nut weight lesser than the check (KS).

4.1.1.24 Shell and meat weight without water (SMW)

The genotypes varied significantly for shell and meat weight without water (Table 6). For parental palms it ranged from 206.800 g (AYK) to 477.600 g (CC) and 285.000 g (AYKxWCT) to 575.400 g (PHIxAYK) for hybrids. Maximum shell and meat weight was recorded for PHIxAYK (575.400 g) and was statistically on par with LMxAYK (505.600 g), and least for AYK (206.800 g) on par with LM (231.000 g).

Hybrid PHIxAYK (575.400 g) on par with LMxAYK (505.600 g) exhibited higher shell and meat weight compared to check (473.800 g). Hybrids LOxAYK (429.600 g) and MYDxAYK (421.200 g) recorded values on par with the check and other hybrids were found inferior to check (KS).

4.1.1.25 Kernel thickness at maturity (KT)

Kernel thickness at maturity differed significantly among the genotypes (Figure 9, Table 6). It ranged from 10.200 mm (MYD) to 11.800 mm (WCT) for parents and 9.600 mm (AYKxWCT) to 12.600 mm (LMxAYK) for hybrids. Maximum kernel thickness was recorded for LMxAYK (12.600 mm) and was on par with MYDxAYK (12.000 mm), WCTxAYK (12.000 mm), PHIxAYK (12.000 mm), WCT (11.800 mm), AOxAYK (11.800 mm), LOxAYK (11.700 mm), PHI (11.700 mm), CC (11.600 mm), AO (11.600 mm) and check (11.600 mm). Genotypes AYKxWCT (9.600 mm) on par with MYD (10.200 mm) were found to be inferior for kernel thickness.

Most hybrids recorded kernel thickness on par with the check except AYKxWCT (9.600 mm) and CCxAYK (10.400 mm).

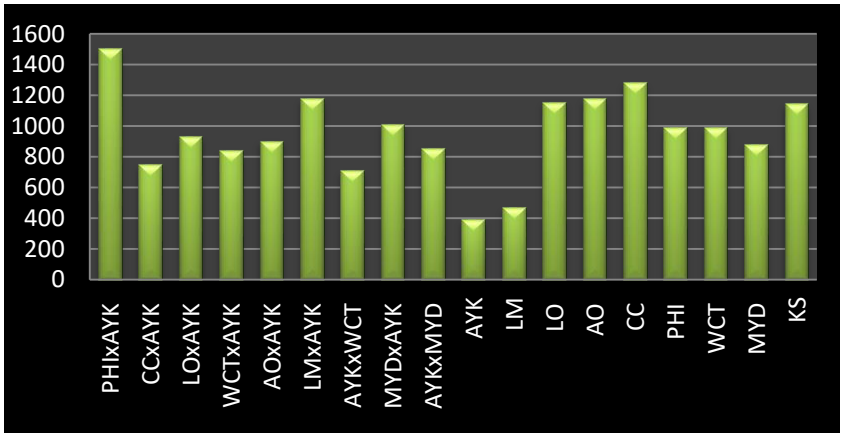


Fig 7. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for fruit weight with husk

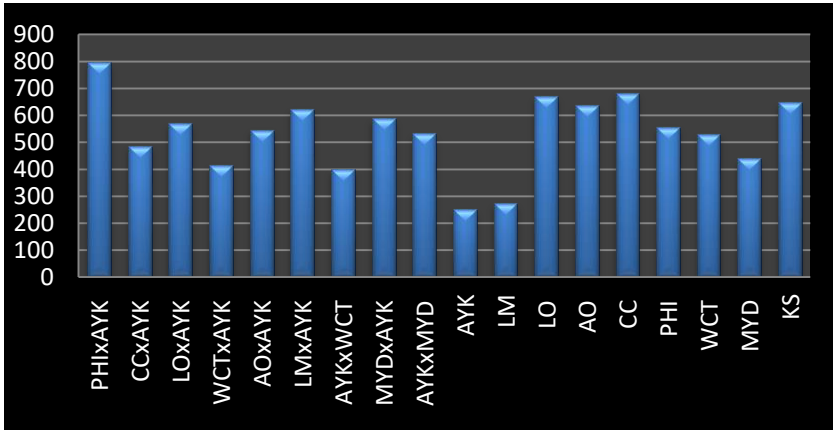


Fig. 8 Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for nut weight without husk

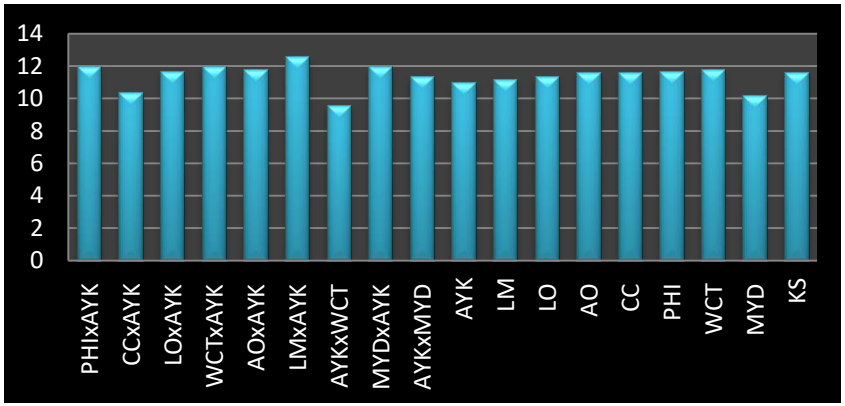


Fig 9. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for kernel thickness at maturity

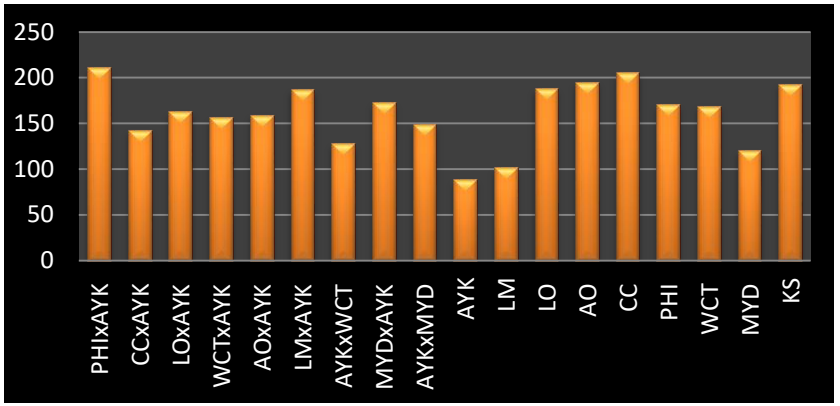


Fig 10. Mean performance of hybrids of Ayiramkachi in comparison with parental and check cultivar for copra content

4.1.1.26 Quantity of liquid endosperm (QLE)

A significant variation was observed among the genotypes for quantity of liquid endosperm (Table 6). For parental palms it varied from 39.000 ml (AYK) to 205.400 ml (CC) and 49.200 ml (WCTxAYK) to 212.800 ml (PHIxAYK). Highest quantity of liquid endosperm was produced by PHIxAYK (212.800 ml) and was statistically on par with CC (205.400 ml) and LO (193.200 ml), and least by AYK (39.000 ml) on par with LM (41.600 ml) and WCTxAYK (49.200 ml).

On comparing with the check, hybrid PHIxAYK (212.800 ml) was found superior for quantity of liquid endosperm and hybrids AYKxWCT (109.200 ml) and WCTxAYK (49.200 ml) were recorded inferior. Other hybrids exhibited values on par with the check (170.200 ml).

4.1.1.27 Sugar content (SC)

No significant variation was observed among the genotypes for sugar content (Table 6). For parental palms it ranged from 5.580 ˚Bx (AO) to 6.460 ˚Bx (MYD) and 4.560 ˚Bx (AYKxMYD) to 8.000 ˚Bx (LMxAYK) for hybrids. Mean value of 6.200 ˚Bx was exhibited by the check (KS).

4.1.1.28 Copra content (CC)

A significant difference was observed among the genotypes for copra content (Figure 10, Table 6). For parental palms it ranged from 88.600 g (AYK) to 205.400 g (CC) and 128.378 g (AYKxWCT) to 211.020 g (PHIxAYK) for hybrids. Genotypes PHIxAYK (211.020 g) was recorded superior for copra content and was statistically on par with CC (205.400 g) and AO (195.000 g). AYK (88.6000 g) on par with LM (102.000 g) was estimated to be the inferior.

On comparing with the check, hybrid PHIxAYK (211.020 g) was found superior for copra content and hybrids LMxAYK (187.250 g), MYDxAYK (172.466 g) and LOxAYK (162.868 g) were found statistically on par with the check (193.000 g). Other hybrids were found inferior for copra content when compared with the check (KS).

4.1.1.29 Qualitative characters

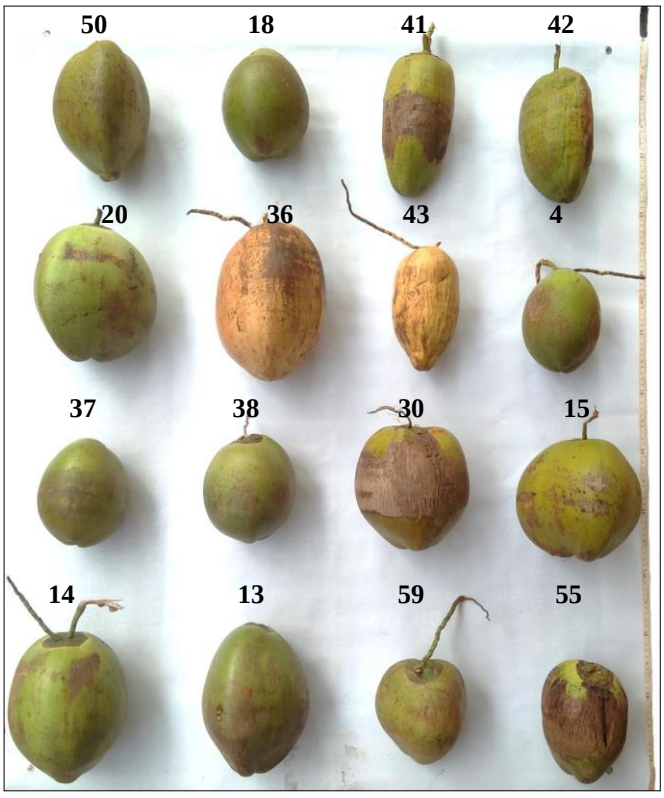
Qualitative characters such as shape of the crown, petiole colour and fruit colour were recorded and presented in Table 7. For all the palms shape of the crown was observed to be spherical except for CC, which was hemispherical in shape.

Petiole colour was reported to be green for all the cultivars under study except for palm no. 36 (LMxMYD), palm no.43 (AYKxMYD) and MYD palms. Similarly nut colour also showed variation among the cultivars studied (Plate 19 and Plate 20). All the palms of hybrid CCxAYK, LOxAYK, WCTxAYK, AOxAYK, AYKxWCT and MYDxAYK; parental palms AYK, LM, LO and WCT produced green coloured nuts, while parental palms AO and CC produced greenish orange coloured nuts. Dwarf cultivar MYD and its hybrid AYKxMYD had yellow coloured nuts. Considering hybrid PHIxAYK palm no.15 and 30 produced greenish orange fruits whereas palm no.29 produced green coloured nuts. Similarly in hybrid LMxAYK, palm no. 20 (Plate 16a) produced green coloured nuts and that of palm no. 36 was orange (Plate 16b). Likewise parental palm PHI also produced green (palm no. 133 and 135) and brownish orange fruits (palm no. 134 and 136).

4.1.1.30 Reproductive characters related to pollination behavior

Some extra reproductive characters related to the pollination behavior of the palms were collected and recorded in Table 8. The dwarfs (AYK and MYD) recorded early flowering (3.67-4 years), while the hybrids took around six years to attain first flowering which is similar to the tall cultivars. Similarly period between inflorescence emergence and opening was also found least for dwarf cultivar MYD (98 days) and highest in tall cultivar LM (129 days).

Concordance between male and female phases were observed only in MYD and provide scope for self-pollination. All the cultivars except MYD had a gap between male and female phases ranging from one day (for hybrids: PHIxAYK, LMxAYK, MYDxAYK and AYKxMYD) to five days (for tall cultivar LM). Thus there is higher chance of cross pollination in these palms.



LO x AYK : 50

AYK x WCT : 4

AO x AYK : 18, 41, 42

CC x AYK : 37, 38

LM x AYK : 20, 36

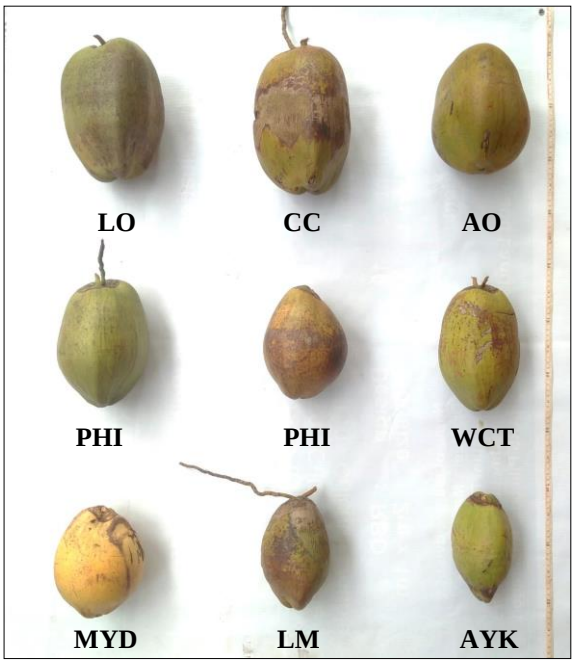
PHI x AYK : 15, 30

AYK x MYD : 43

MYD x AYK : 13, 14, 59

WCT x AYK : 55

Plate 19. Variation in shape, size and colour of nuts from nine different crosses of Ayiramkachi



LO : Laccadive Ordinary

MYD : Malayan Yellow Dwarf

CC : Cochin China

WCT : West Coast Tall

AO : Andaman Ordinary

LM : Laccadive Micro

PHI : Philippines

AYK : Ayiramkachi

Plate 20. Variation in shape, size and colour of nuts from parental palms

Table 7. Details on qualitative characters of the coconut cultivars

Sl no.	Genotype	Palm number	Shape of the crown	Petiole colour	Fruit colour
1	PHI x AYK	15, 30	Spherical	Green	Greenish orange
		29	Spherical	Green	Green
2	CC x AYK	37, 38, 61	Spherical	Green	Green
3	LO x AYK	49, 50	Spherical	Green	Green
4	WCT x AYK	55	Spherical	Green	Green
5	AO x AYK	18, 39, 41, 42	Spherical	Green	Green
6	LM x AYK	20	Spherical	Green	Green
		36	Spherical	Orange green	Orange
7	AYK x WCT	4	Spherical	Green	Green
8	MYD x AYK	13, 14, 59	Spherical	Green	Green
9	AYK x MYD	43	Spherical	Yellow green	Yellow
10	Ayiramkachi	6, 7, 8, 29	Spherical	Green	Green
11	Laccadive Micro	88, 90, 92	Spherical	Green	Green
12	Laccadive Ordinary	141, 149	Spherical	Green	Green
13	Andaman Ordinary	91, 99	Spherical	Green	Greenish orange
14	Cochin China	166, 167, 168, 169	Hemispherical	Green	Greenish orange
15	Philippines	133, 135	Spherical	Green	Green
		134, 136	Spherical	Green	Brownish orange
16	West Coast Tall	11, 14	Spherical	Green	Green
17	Malayalan Yellow Dwarf	57, 58	Spherical	Yellow	Yellow
18	Kerasree	71, 79, 80, 88	Spherical	Green	Green

Table 8. Details of reproductive characters related to pollination behavior in coconut genotypes

Genotype	Age of the palm at first flowering (years)	Period between emergence and opening (days)	Male phase (days)	Female phase (days)	Period between phases (days)	Concordance of phase if any (days)
PHI x AYK	6	112	22	5	1	Nil
CC x AYK		116	21	4	2	Nil
LO x AYK		111	19	5	3	Nil
WCT x AYK		120	21	4	3	Nil
AO x AYK		117	22	3	3	Nil
LM x AYK		110	21	5	1	Nil
AYK x WCT		118	19	3	2	Nil
MYD x AYK		117	19	6	1	Nil
AYK x MYD		115	18	3	1	Nil
Ayiramkachi	3.67	124	19.8	3.8	1.8	Nil
Laccadive Micro	8.5	129	20	5	5	Nil
Laccadive Ordinary	5.5	118	19	4	3	Nil
Andaman Ordinary	7	118	22.3	4.6	2.8	Nil
Cochin China	6.5	123	20.4	4.1	1.8	Nil
Philippines	5	116	18.3	3.6	2.2	Nil
West Coast Tall	6.5	120	19	4	3	Nil
Malayalan Yellow Dwarf	4	98	16.3	6.6	0	6.3
Kerasree	5	116	19	5	2	Nil

Plate 21a. Palm damaged by Red palm weevil attack (holes on stem and destroyed crown)

Plate 21b. Palm attacked by Rhinoceros beetle (Characteristic V-shaped cut on leaves)

Plate 21c. Nuts with coconut mite attack

Plate 21. Pest incidence on coconut palms

4.1.1.31 Pest and disease incidence

Incidence of pest such as rhinoceros beetle (*Oryctes rhinoceros*) (Plate 21b) and eriophyid mite (*Aceria guerreronis*) (Plate 21c) were observed in the field. Palm No. 46 (LMxAYK) was severely infected with red palm weevil attack (*Rhyncophorus ferrugineus*) even before the present study was started and hence no control measures could have been taken. The palm completely collapsed during November 2019 (Plate 21a).

Field sanitation was done and crown cleaning was followed as preventive measures against these pests. Beetles were hooked out from infected palms and neem cake with sand (1:1 ratio) was applied in the innermost 2-3 leaf axils.

4.1.2 Performance of hybrid groups (TxD, DxT and DxD) in comparison with tall (T) and dwarf (D) parental palms

Mean values for observations on 28 morphological characters of six TxD, one DxT and two DxD hybrids of Ayiramkachi and six tall and two dwarf parents were estimated and presented in Table 9. Mean values of the following characters showed significant difference between the groups, tall and dwarf.

4.1.2.1 Height of the palm

Tall cultivars recorded highest value for palm height (12.699 m) and varied significantly from all other palms under study (Table 9). The hybrid palms exhibited height statistically on par with the dwarf cultivars (7.132 m).

4.1.2.2 Girth of palm at 20 cm and 1.5 m height

The palms varied significantly for girth at 20 cm and 1.5 m height (Table 9). Girth at 20 cm was recorded highest for TxD (108.612 cm) and least for dwarf cultivars (74.569 cm). DxT (80.250 cm), DxD (82.100 cm) and tall cultivars (104.698 cm) was found statistically on par with both TxD and dwarf. Girth at 1.5 m was estimated to be higher for talls (81.412 cm) and TxD (81.190 cm) and least for dwarfs (65.532 cm).

Table 9. Mean performance of different groups of coconut hybrids (TxD, DxT and DxD) of Ayiramkachi in comparison with tall (T) and dwarf (D) groups of parental palms

Characters	T x D	D x T	D x D	T	D	Mean	CD (0.05)
Height of palm (m)	7.146	7.500	5.930	12.699*	7.132	6.875	4.251
Girth of palm at 20 cm height (cm)	108.61*	80.250	82.100	104.698*	74.569	90.497	31.326
Girth of palm at 1.5 m height (cm)	81.190*	73.000	69.075	81.412*	65.532	73.706	13.319
Internode length (cm)	5.655*	6.300*	3.675	4.766	3.588	5.130	1.627
Number of green leaves	24.957	19.000	21.263	28.818	23.829	22.043	_
Rate of leaf production	1.652	1.200	1.400	1.363	1.195	1.439	_
Petiole length (cm)	130.98*	131.50*	99.834	116.681	112.063	122.721	26.870
Leaf length (cm)	510.54*	543.00*	384.667	478.896	368.938	482.770	97.361
Total inflorescence present in the crown at the time of observation	10.662	10.875	10.313	11.618	9.000	10.971	_
Number of unopened inflorescence	1.679	1.750	1.681	1.695	1.391	1.774	_
Number of opened inflorescence undergoing pollination	0.702	0.750	0.663	0.619	0.610	0.705	_
Number of inflorescence in which pollination over and seed setting started	0.653	0.625	0.600	0.612	0.590	0.613	_
Total inflorescence per palm per year	12.819*	14.000*	10.834	11.653	9.375	13.106	3.215
Number of female flowers per inflorescence	26.301	30.375	17.084	24.237	47.852*	25.503	18.772
No. of female flowers one month after pollination	16.536	20.000	9.967	17.177	26.242	16.243	_
Number of bunches per palm per year	10.445*	10.000*	8.667	8.625	7.625	9.733	2.293
Number of nuts per palm per year (yield)	110.139	95.000	68.167	74.778	119.250	97.406	_

Number of nuts per bunches	9.861	9.500	7.425	8.734	14.582	9.644	_
Size of unusked nut at equatorial circumference (cm)	47.153	37.800	45.290	46.877	36.210	43.414	_
Size of unhusked nut pole to pole circumference (cm)	54.610	49.500	52.090	55.530	45.720	52.067	_
Fruit weight with husk (g)	1018.66	711.400	935.800	1012.73	638.300	888.622	_
Volume of fruit (ml)	977.800	675.400	913.900	971.700	612.500	855.700	_
Nut weight without husk (g)	572.800	399.400	559.900	558.300	344.000	510.700	_
Shell and meat weight without husk (g)	440.333	285.000	405.200	401.967	257.400	376.844	_
Kernel thickness at maturity (mm)	11.750*	9.600	11.700*	11.550*	10.600	11.017	1.393
Quantity of liquid endosperm (ml)	127.600	109.200	152.600	155.400	84.300	129.800	_
Sugar content (˚Bx)	6.560	6.080	5.390	5.933	6.210	6.010	_
Copra content (g)	169.911	128.378	160.922	171.667	104.600	153.070	_

4.1.2.3 Internode length

Highest internode length was recorded by hybrids DxT (6.300 cm) and TxD (5.655 cm), and least by dwarfs (3.588 cm) and DxD hybrids (3.675 cm) (Table 9).

4.1.2.4 Petiole length

DxT (131.500 cm) and TxD (130.981 cm) hybrids recorded maximum petiole length and least by DxD (99.834 cm). Tall and dwarf cultivars exhibited values statistically on par with all the hybrids (Table 9).

4.1.2.5 Leaf length

The palms differed significantly for leaf length (Table 9). Cross DxT (543.000 cm) and TxD (510.543 cm) were found superior and was statistically on par with cultivars (478.896 cm), and dwarf cultivars (411.250 cm) were estimated to be the inferior and was statistically on par with DxD (384.667 cm).

4.1.2.6 Total inflorescence per palm per year

Hybrids DxT (14.000) and TxD (12.819) were recorded to be superior for total inflorescence produced by palms in a year and dwarf cultivars (9.375) were found inferior. Hybrid DxD (10.834) and tall cultivars (11.653) were statistically on par with both DxT and dwarf cultivars (Table 9).

4.1.2.7 Number of female flowers per inflorescence

Number of female flowers varied significantly among the cultivars studied (Table 9). The dwarf cultivars were recorded to be superior and least by DxD (17.084), tall (24.237) and TxD (26.301). Hybrid DxT (30.375) was found statistically on par with dwarfs, DxD, tall and TxD.

4.1.2.8 Number of bunches per palm per year

Hybrids TxD (10.445) and DxT (10.000) were recorded to be superior for number of bunches produced by palms in a year and dwarf cultivars (7.625) were found inferior. Hybrid DxD (8.667) and tall cultivars (8.625) were statistically on par with both TxD and dwarf cultivars (Table 9).

4.1.2.9 Kernel thickness at maturity

The crosses varied significantly for kernel thickness at maturity (Table 9). Maximum kernel thickness was recorded for TxD (11.750 mm) which was statistically similar to DxD (11.700 mm) and tall (11.550 mm) and least by DxT hybrid (9.600 mm).

4.1.3 Palm to palm variations within each cross

Since the parents used for hybrid production in coconut may not be completely homozygous, the hybrid progenies also shows significant palm to palm variation. Hence inorder to identify the best progeny combination, Mean + SD (standard deviation) method was used. For all palm characters except height of palm, higher value than Mean + SD are considered, whereas lower value was taken for height of palm. The palms having maximum desirable characters were considered as superior.

Hybrids combinations which consist of three or more palms per cross were only considered to identify the better palm based on vegetative, reproductive and yield characters.

Table 10. Palm to palm variations within PHIxAYK hybrid combination

Characters	PHI x AYK			Mean	SD	Mean + SD
	15	29	30			
Height of palm (m)	6.00	6.45	6.27	6.24	0.13	6.37
Girth of palm at 20 cm height (cm)	105.15	103.50	82.05	96.90	7.44	104.3
Girth of palm at 1.5 m height (cm)	87.00	80.05	73.00	80.02	4.04	84.06
Internode length (cm)	4.05	4.45	3.05	3.85	0.42	4.27
Number of green leaves	23.13	20.00	25.00	22.71	1.46	24.17
Rate of leaf production	1.57	1.43	2.00	1.67	0.17	1.84
Petiole length (cm)	139.00	105.00	96.50	113.50	12.98	126.4
Leaf length (cm)	548.50	444.00	357.50	450.00	55.22	505.2
Total inflorescence present in the crown at the time of observation	9.25	9.13	12.38	10.25	1.06	11.31
Number of unopened inflorescence	2.38	1.13	1.50	1.67	0.37	2.04
No. of inflorescence undergoing pollination	1.00	0.50	0.75	0.75	0.144	0.89
No. of inflorescence in which pollination over and seed setting started	0.70	0.50	0.68	0.63	0.06	0.69
Total inflorescence per palm per year	14.00	10.00	16.00	13.33	1.76	15.09
No. of female flowers per inflorescence	28.75	16.75	17.13	20.88	3.94	24.82
No. of female flowers one month after pollination	19.80	9.50	9.80	13.03	3.38	16.41
No. of bunches per palm per year	13.00	7.00	13.00	11.00	2.00	13.00
No. of nuts per palm per year (yield)	112.00	40.00	93.00	81.67	21.54	103.2
No. of nuts per bunches	8.62	5.71	7.00	7.11	0.84	7.95

Table 11. Palm to palm variations within CCxAYK hybrid combination

Characters	CC x AYK			Mean	SD	Mean+ SD
	37	38	61			
Height of palm (m)	8.15	7.03	6.14	7.11	0.58	7.69
Girth of palm at 20 cm height (cm)	102.00	85.95	95.50	94.48	4.66	99.14
Girth of palm at 1.5 m height (cm)	78.95	68.00	79.00	75.32	3.66	78.98
Internode length (cm)	6.45	3.65	6.10	5.40	0.88	6.28
Number of green leaves	31.25	32.13	22.75	28.71	2.98	31.69
Rate of leaf production	1.80	1.80	1.40	1.67	0.13	1.80
Petiole length (cm)	102.00	129.00	142.00	124.33	11.78	136.11
Leaf length (cm)	537.50	440.50	535.00	504.33	31.93	536.26
Total inflorescence present in the crown at the time of observation	12.38	11.25	10.50	11.38	0.54	11.92
Number of unopened inflorescence	1.63	1.63	1.13	1.46	0.17	1.63
No. of inflorescence undergoing pollination	1.00	1.00	0.40	0.80	0.20	1.00
No. of inflorescence in which pollination over and seed setting started	0.70	0.73	0.60	0.67	0.04	0.71
Total inflorescence per palm per year	16.00	16.00	11.00	14.33	1.67	16.00
No. of female flowers per inflorescence	39.00	32.75	25.13	32.29	4.01	36.30
No. of female flowers one month after pollination	25.20	23.00	13.00	20.40	3.75	24.15
No. of bunches per palm per year	12.00	13.00	7.00	10.67	1.85	12.52
No. of nuts per palm per year (yield)	185.00	235.00	32.00	150.67	61.06	211.73
No. of nuts per bunches	15.42	18.08	4.57	12.69	4.13	16.82

Table 12. Palm to palm variations within AOxAYK hybrid combination

Characters	AO x AYK				Mean	SD	Mean+ SD
	18	39	41	42			
Height of palm (m)	3.44	9.25	9.44	8.03	7.54	1.40	8.94
Girth of palm at 20 cm height (cm)	90.50	226.10	110.00	120.20	136.70	30.43	167.13
Girth of palm at 1.5 m height (cm)	70.00	96.95	83.25	86.90	84.28	5.57	89.85
Internode length (cm)	3.35	7.25	7.50	7.25	6.34	1.00	7.34
Number of green leaves	28.75	24.00	17.38	17.75	21.97	2.72	24.69
Rate of leaf production	1.71	1.57	1.57	1.20	1.51	0.11	1.62
Petiole length (cm)	117.50	150.50	112.50	117.50	124.50	8.75	133.25
Leaf length (cm)	454.50	556.00	497.50	454.50	490.63	24.03	514.66
Total inflorescence present in the crown at the time of observation	13.25	10.88	8.13	11.63	10.97	1.07	12.04
Number of unopened inflorescence	2.75	1.63	1.17	1.75	1.82	0.33	2.15
No. of inflorescence undergoing pollination	0.70	0.70	0.50	0.60	0.62	0.05	0.67
No. of inflorescence in which pollination over and seed setting started	0.70	0.55	0.50	0.50	0.56	0.05	0.61
Total inflorescence per palm per year	15.00	13.00	11.00	12.00	12.75	0.85	13.60
No. of female flowers per inflorescence	35.00	31.63	17.63	21.13	26.34	4.14	30.49
No. of female flowers one month after pollination	25.80	18.75	10.25	10.33	16.28	3.75	20.03
No. of bunches per palm per year	13.00	8.00	8.00	5.00	8.50	1.66	10.16
No. of nuts per palm per year (yield)	93.00	91.00	38.00	24.00	61.50	17.84	79.34
No. of nuts per bunches	7.15	12.50	4.75	4.80	7.30	1.82	9.12

Table 13. Palm to palm variations within MYDxAYK hybrid combination

Characters	MYD x AYK			Mean	SD	Mean+ SD
	13	14	59			
Height of palm (m)	6.55	7.05	5.95	6.51	0.32	6.83
Girth of palm at 20 cm height (cm)	88.50	92.35	70.25	83.70	6.82	90.52
Girth of palm at 1.5 m height (cm)	61.95	68.50	68.00	66.15	2.10	68.25
Internode length (cm)	3.60	4.00	3.80	3.80	0.12	3.92
Number of green leaves	23.63	25.25	23.50	24.13	0.56	24.69
Rate of leaf production	1.60	1.80	1.25	1.55	0.16	1.71
Petiole length (cm)	115.00	102.50	138.50	118.67	10.55	129.22
Leaf length (cm)	449.00	454.00	410.50	437.83	13.74	451.57
Total inflorescence present in the crown at the time of observation	13.63	12.25	10.13	12.00	1.02	13.02
Number of unopened inflorescence	2.38	2.38	1.33	2.03	0.35	2.38
No. of inflorescence undergoing pollination	0.83	0.85	0.80	0.83	0.01	0.84
No. of inflorescence in which pollination over and seed setting started	0.70	0.80	0.60	0.70	0.06	0.76
Total inflorescence per palm per year	15.00	16.00	10.00	13.67	1.85	15.52
No. of female flowers per inflorescence	21.50	38.38	7.00	22.29	9.07	31.36
No. of female flowers one month after pollination	12.40	26.20	5.00	14.53	6.21	20.75
No. of bunches per palm per year	12.00	9.00	7.00	9.34	1.45	10.79
No. of nuts per palm per year (yield)	98.00	218.00	30.00	115.33	54.96	170.29
No. of nuts per bunches	8.17	24.22	4.29	12.22	6.10	18.32

Among the three palms of the hybrid group PHIxAYK (Table 10), palm No. 15 (Plate 3a) was identified as superior in performance than palm no. 29 and palm No. 30 (Plate 3b). Out of three palms in the group CCxAYK (Table 11), palm No. 38 (Plate 4b) showed superior performance than palm No. 37 (Plate 4a) and palm No.61. AOxAYK group consists of four palms (Table 12), among them palm No. 18 (Plate 7a) was found to be the better performer than palm No. 39, 41 and 42. Among the three palms of the group MYDxAYK (Table 13), palm No. 14 (Plate 10b) was estimated superior than palm No. 13 (Plate 10a) and palm No.59.

4.1.4 Correlation study and Path analysis

Estimation of genotypic correlation gives an idea about inter relationship and degree or extend of association between various parameters studied. It also enables effective selection of parameters contributing to character in concern, mainly the yield and copra content. Genotypic correlation study was conducted using 27 morphological characters including eight vegetative, seven reproductive, three yield and nine nut character and presented in Appendix-I.

True contributions of the characters towards yield cannot be obtained from correlation studies alone. Therefore another study was carried out by path analysis and thus there was an effective partitioning of the characters (or variables) based on a dependent variable in to two: the direct and indirect effects and this will contribute to an efficient selection. For path analysis characters showing positive significant correlation with number of nuts per palm per year (yield) were identified, direct and indirect effects of these characters on yield was estimate and presented in Appendix-II.

Since the results of correlation and path analysis were based only on one year data, the interpretation of the data was not possible. The data analysis can be done with additional data from different years as future programme.

4.1.5 Genetic variability parameters

Genetic parameters such as PCV (Phenotypic coefficient of variation), GCV (Genotypic coefficient of variation), H^2 % (Heritability per cent), GAM % (Genetic

advance value per cent means) were estimated (Table 14).

A high value for all these genetic parameters were observed for Palm height, internode length, number of female flowers, female flowers one month after pollination and shell and meat weight and lowest was recorded for kernel thickness.

4.1.5.1 Phenotypic Coefficient of Variation (PCV)

PCV ranged from 9.710 to 57.529 (Table 14). It was found highest for quantity of liquid endosperm (57.529) followed by yield (53.781), nuts per bunches (48.955), number of female flowers (45.947), number of female flowers one month after pollination (40.704), palm height (38.653), fruit weight (35.047), fruit volume (34.763), nut weight (32.585), shell and meat weight (28.817), internode length (26.499), copra content (26.416), number of inflorescence undergoing pollination (26.299), number of unopened inflorescence (25.060), inflorescence in which pollination over and seed setting started (23.358), girth of palm at 20 cm (22.905), rate of leaf production (22.360), number of bunches per palm per year (21.787) and number of green leaves(20.328).

Moderate PCV was observed for total inflorescence per palm per year (18.907) followed by total inflorescence at observation (16.650), size of unhusked nut (equatorial circumference) (16.051), leaf length (15.308), petiole length (14.652), girth at 1.5 m (11.686) and size of unhusked nut (pole to pole circumference) (11.039).

PCV was recorded lowest for kernel thickness (9.710).

4.1.5.2 Genotypic Coefficient of Variation (GCV)

GCV ranged from 5.216 to 40.607 (Table 14). It was found highest for yield (40.607) followed by number of female flowers (39.222), number of nuts per bunches (37.583), palm height (36.256), female flowers one month after pollination (33.969), quantity of liquid endosperm (31.690), fruit weight (26.929), fruit volume (26.557), nut weight (24.529), shell and meat weight (22.668) and internode length (22.306).

Moderate GCV was observed for copra content (19.98), rate of leaf production

(18.195), number of green leaves (17.402), girth of palm at 20 cm (17.384), Number of unopened inflorescence (16.139), leaf length (14.045), total inflorescence per palm per year (13.942), size of unhusked nut (equatorial circumference) (13.723), total inflorescence at observation (12.636), number of bunches per palm per year (12.426), petiole length (12.171), inflorescence in which pollination over and seed setting started (10.396) and girth of palm at 1.5 m (10.239).

GCV was recorded lowest for kernel thickness (5.216), size of unhusked nut (pole to pole circumference) (8.587) and inflorescence undergoing pollination (8.795).

4.1.5.3 Heritability

Heritability per cent ranged from 11.2 to 88 (Table 14). It was found highest for palm height (88) followed by leaf length (84.2), girth of palm at 1.5 m (76.8), number of green leaves (73.3), Size of unhusked nut (equatorial circumference) (73.1), number of female flowers (72.9), internode length (70.9), female flowers after pollination (69.6), petiole length (69), rate of leaf production (66.2), shell and meat weight (61.9) and size of unhusked nut (pole to pole circumference) (60.5).

Medium range was observed for fruit weight (59) followed by number of nuts per bunches (58.9), fruit volume (58.6), girth of palm at 20 cm (57.6), total inflorescence at observation (57.6), copra content (57.2), yield (57), nut weight (56.7), total inflorescence per palm per year (54.4), number of unopened inflorescence (41.5), number of bunches per palm per year (32.5) and quantity of liquid endosperm (30.3).

Lowest values were recorded for inflorescence undergoing pollination (11.2), inflorescence in which pollination over and seed setting started (19.8) and kernel thickness (28.9).

4.1.5.4 Genetic Advance as Mean per cent

Genetic advance as per cent means ranged from 5.774 to 70.054 (Table 14). It was found highest for palm height (70.054) followed by number of female flowers (68.972), yield (63.159), nuts per bunches (59.438), number of female flowers after pollination (58.397), fruit weight (42.625), fruit volume (41.866), internode length

(38.674), nut weight (38.035), shell and meat weight (36.733), quantity of liquid endosperm (35.960), copra content (31.133), number of green leaves (30.686), rate of leaf production (30.422), girth of palm at 20 cm (27.186), internode length (26.545), size of unhusked nut (equatorial circumference) (24.171), number of unopened inflorescence (21.455), total inflorescence per palm per year (21.179) and petiole length (20.829).

Moderate range was observed for total inflorescence at observation (19.753) followed by girth of palm at 1.5 m (18.481), number of bunches per palm per year (14.585) and size of unhusked nut (pole to pole circumference) (13.759).

Lowest values were recorded for kernel thickness (5.774), inflorescence undergoing pollination (5.935) and inflorescence in which pollination over and seed setting started (9.627).

Table 14. Genetic parameters of morphological characters under study

Characters	Mean	PCV	GCV	H^2%	GAM%
Height of the palm	9.057	38.653	36.256	88	70.054
Girth of the palm at 20cm height	99.279	22.905	17.384	57.6	27.180
Girth of the palm at 1.5m height	77.829	11.686	10.239	76.8	18.481
Internode length	4.818	26.499	22.306	70.9	38.674
Number of green leaves	25.574	20.328	17.402	73.3	30.686
Rate of leaf production	1.439	22.360	18.195	66.2	30.422
Petiole length	119.639	14.652	12.171	69	20.829
Leaf length	470.102	15.308	14.045	84.2	26.545
Total number of inflorescence in the crown at the time of observation	10.831	16.650	12.636	57.6	19.753

Characters	Mean	PCV	GCV	H^2%	GAM%
Number of unopened inflorescence	1.674	25.060	16.139	41.5	21.455
Number of opened inflorescence undergoing pollination	0.663	26.299	8.795	11.2	5.935
Number of inflorescence in which pollination is over and seed setting started	0.623	23.358	10.396	19.8	9.627
Total inflorescence per palm per year	11.944	18.907	13.942	54.4	21.179
Number of female flowers per inflorescence	27.505	45.947	39.222	72.9	68.972
Number of female flowers one month after pollination	17.539	40.704	33.969	69.6	58.397
Number of bunches per palm per year	9.319	21.787	12.420	32.5	14.585
Number of nuts per palm per year; yield	93.532	53.781	40.607	57	63.159
Number of nuts per bunches	9.720	48.955	37.583	58.9	59.438
Size of unhusked nut equatorial circumference	45.221	16.051	13.723	73.1	24.171
Size of unhusked nut pole to pole circumference	53.553	11.039	8.587	60.5	13.759
Fruit weight with husk	955.389	35.047	26.929	59	42.625
Fruit volume	917.567	34.703	26.557	58.6	41.866
Nut weight without husk	535.611	32.585	24.529	56.7	38.035
Shell and meat weight without water	396.544	28.817	22.668	61.9	36.733
Kernel thickness at maturity	11.422	9.710	5.216	28.9	5.774
Quantity of liquid endosperm	136.178	57.529	31.690	30.3	35.960
Copra content	161.216	26.416	19.981	57.2	31.133

4.1.6 Heterosis

The nine hybrids of Ayiramkachi were subjected for heterosis study, to estimate its heterosis for repective characters over a hybrid (Kerasree) and the better parent. Standarad heterosis and heterobeltiosis were estimated for nine characters and are presented in Table 15.

4.1.6.1 Number of nuts per palm per year

Standarad heterosis ranged from 49.16 (LMxAYK) to -79.86 (AYKxMYD), and also LMxAYK and AYKxMYD were the only hybrids that shown a significant positive and negative heterosis respectively. Hybrids such as CCxAYK (44.52), LOxAYK (11.75) and MYDxAYK (10.63) recorded a positive but non-significant heterosis with Kerasree.

Heterobeltiosis varied from -17.73 (LMxAYK) to -88.89 (AYKxMYD). None of the hybrids had shown a significant positive heterosis and five among the nine hybrids produced a significant negative heterosis. Hybrids LMxAYK (-17.73), CCxAYK (-20.28), LOxAYK (-38.36) and MYDxAYK (-38.98) displayed a non-significant heterosis (Table 15).

4.1.6.2 Number of nuts per bunches

Standarad heterosis ranged between 31.49 (LMxAYK) to -73.24 (AYKxMYD) and heterobeltiosis ranged from -41.62 (LMxAYK) to -88.12 (AYKxMYD). None of the hybrids had a significant positive heterosis. AYKxMYD was the only one hybrid that showed a significant negative value for standarad heterosis, but all the nine hybrids exhibited a significant negative heterobeltiosis (Table 15).

4.1.6.3 Size of unhusked nut equatorial circumference

Standarad heterosis varied from 6.16 (PHIxAYK) and -22.86 (AYKxWCT). None of the hybrids had a significant positive heterosis. AYKxWCT (-22.86), AYKxMYD (-16.00) and AOxAYK (-14.08) shown a significant negative heterosis. Hybrids such as PHIxAYK (6.16), LMxAYK (3.06) and MYDxAYK (1.02) exhibited

a nonsignificant but positive heterosis. Heterobeltiosis ranged from 46.72 (LMxAYK) and -20.08 (AOxAYK). Hybrids LMxAYK (46.72), MYDxAYK (14.90) and PHIxAYK (12.89) exhibited a significant positive heterosis and AOxAYK (-20.08), AYKxWCT (-16.00) and CCxAYK (-13.33) showed a negative significant heterosis (Table 15).

4.1.6.4 Size of unhusked nut pole to pole circumference

Standarad heterosis and ranged from 0.35 (LMxAYK) to -15.97 (AYKxMYD). None of the hybrids had a significant positive heterosis. A negative significant heterosis was shown by AYKxMYD (-15.97), AYKxWCT (-14.66), CCxAYK (-11.03) and AOxAYK (-10.17).

Heterosisbeltiosis varied from 23.04 (LMxAYK) to -17.52 (CCxAYK). Hybrids LMxAYK (23.04) and MYDxAYK exhibited a positive significant heterosis and hybrids CCxAYK (-17.52) and AOxAYK (-9.74) a negative significant heterosis (Table 15).

4.1.6.5 Fruit weight with husk

Standarad heterosis showed a range from 31.19 (PHIxAYK) to -38.09 (AYKxWCT). Only PHIxAYK displayed a positive significant heterosis and hybrids AYKxWCT (-38.09), CCxAYK (-34.69), WCTxAYK (-26.82) and AYKxMYD (-25.31) had a negative significant heterosis.

Heterobeltiosis ranged from 151.45 (LMxAYK) to -41.65 (CCxAYK). Significant positive heterosis was shown by LMxAYK (151.45) and PHIxAYK (51.53) and a significant negative heterosis was recorded by CCxAYK (-41.65), AYKxWCT (-28.19) and AOxAYK (-23.23) (Table 15).

4.1.6.6 Nut weight without husk

A range of 23.08 (PHIxAYK) to -38.29 (AYKxWCT) was observed for standarad heterosis. Only PHIxAYK displayed a positive significant heterosis and a negative significant heterosis by AYKxWCT (-38.29), WCTxAYK (-35.66) and

CCxAYK (-25.03). Heterobeltiosis varied from 128.65 (LMxAYK) and -28.96 (CCxAYK). A positive significant heterosis was observed for LMxAYK (128.65), PHIxAYK (43.17) and MYDxAYK (33.94) and significant negative heterosis by CCxAYK (-28.96) (Table 15).

4.1.6.7 Shell and meat weight without water

Standarad heterosis ranged from 21.44 (PHIxAYK) to -39.85 (AYKxWCT). Only PHIxAYK displayed a positive significant heterosis and hybrids AYKxWCT (-39.85), WCTxAYK (-23.39) and CCxAYK (-22.79) had a negative significant heterosis.

A range of 118.87 (LMxAYK) to -25.19 (AYKxWCT) was observed for heterobeltiosis. Hybrids LMxAYK (118.87), PHIxAYK (48.07) and MYDxAYK (36.75) exhibited a positive significant heterosis. A negative significant heterosis was shown by AYKxWCT (-25.19) and CCxAYK (-23.41) (Table 15).

4.1.6.8 Kerenel thickness at maturity

Standarad heterosis and heterobeltiosis ranged from 8.62 (LMxAYK) to -17.24 (AYKxWCT) and -18.64 (LMxAYK) to 12.50 (LMxAYK) respectively. A significant negative heterosis was produced by AYKxWCT and CCxAYK. Hybrid LMxAYK exhibited a positive significant value for heterobeltiosis (Table 15).

4.1.6.9 Copra content

Standarad heterosis varied from 9.34 (PHIxAYK) to -33.48 (AYKxWCT). None of the hybrids had shown a significant positive heterosis and four among nine exhibited significant negative heterosis *viz.,* AYKxWCT (-33.48), CCxAYK (-25.97), AYKxMYD (-22.60) and WCTxAYK (-19.15).

Heterobeltiosis ranged from 83.58 (LMxAYK) to -30.44 (CCxAYK). A positive significant heterosis was observed for LMxAYK (83.58), MYDxAYK (43.01) and PHIxAYK (23.84). A negative significant heterosis was shown by CCxAYK (-30.44), AYKxWCT (-24.04) and AOxAYK (-18.26) (Table 15).

Table 15. Standard heterosis and heterobeltiosis for the nine hybrids of Ayiramkachi

Hybrid palms	Number of nuts per palm per year (yield)		Number of nuts per bunches		Size of unhusked nut at equatorial circumference		Size of unhusked nut at pole to pole circumference	
	SH	HB	SH	HB	SH	HB	SH	HB
PHI x AYK	-21.66	-56.79**	-27.52	-67.82**	6.16	12.89*	-3.86	4.03
CC x AYK	44.52	-20.28	29.35	-42.57*	-7.35	-13.33**	-11.03**	-17.52**
LO x AYK	11.75	-38.36	7.37	-52.33**	-5.51	-8.68	-2.41	-2.41
WCT x AYK	-8.87	-49.74*	-11.96	-60.91**	-4.89	3.56	-7.93	-1.11
AO x AYK	-41.01	-67.46**	-25.57	-66.95**	-14.08**	-20.08**	-10.17*	-9.74*
LM x AYK	49.16*	-17.73	31.49	-41.62*	3.06	46.72**	0.35	23.04**
AYK x WCT	-8.87	-49.74*	-3.15	-56.99**	-22.86**	-16.00**	-14.66**	-8.33
MYD x AYK	10.63	-38.98	24.63	-44.67*	1.02	14.90**	-4.41	13.14**
AYK x MYD	-79.86**	-88.89**	-73.24**	-88.12**	-16.16**	-4.64	-15.97**	-0.53

SH – Standard heterosis HB – Heterobeltiosis

Table 15. Contd.

Hybrid palms	Fruit weight with husk		Nut weight without husk		Shell and meat weight without water		Kernel thickness at maturity		Copra content	
	SH	HB	SH	HB	SH	HB	SH	HB	SH	HB
PHI x AYK	31.19*	51.53**	23.08*	43.17**	21.44*	48.07**	3.45	2.56	9.34	23.84*
CC x AYK	-34.69**	-41.65**	-25.03*	-28.96**	-22.79*	-23.41**	-10.35*	-10.35*	-25.97**	-30.44**
LO x AYK	-19.04	-19.67	-12.11	-15.13	-9.33	-9.59	0.86	2.63	-15.61	-13.46
WCT x AYK	-26.82*	-15.14	-35.66**	-21.52	-23.39*	-4.72	3.45	1.69	-19.15*	-7.66
AO x AYK	-21.29	-23.23*	-15.69	-14.29	-15.03	-12.17	1.72	1.72	-17.41	-18.26*
LM x AYK	2.59	151.45**	-3.55	128.65**	6.71	118.87**	8.62	12.50*	-2.98	83.58**
AYK x WCT	-38.09**	-28.19*	-38.29**	-24.73	-39.85**	-25.19*	-17.24**	-18.64**	-33.48**	-24.04*
MYD x AYK	-11.80	15.00	-9.27	33.94*	-11.10	36.75*	3.45	9.09	-10.64	43.01**
AYK x MYD	-25.31*	-2.61	-17.71	21.49	-17.86	26.36	-1.72	3.64	-22.60*	13.86

SH – Standard heterosis HB – Heterobeltiosis

4.2 MOLECULAR CHARACTERISATION

Inorder to have a comprehensive characterization, fingerprinting using molecular marker (Simple Sequence Repeats) was also envisaged under the study.

4.2.1 Extraction of genomic DNA

DNA isolation protocol using CTAB method developed by Roger and Bendich (1985), modified by Chethana (2016) was followed in the present study. Genomic DNA extracted was in low quantity with some impurities, so the protocol was further modified with 10 ml extraction buffer instead of 14 ml to make it more concentrated and an additional steps of RNase treatment and chloroform: isoamyl alcohol (24:1) extraction was employed to extract high molecular weight, intact DNA free from RNA and protein contaminants.

Freshly collected tender spindle leaves were used for isolating genomic DNA, discarding the midrib. DNA was isolated from eight parental palms *viz*., Ayiramkachi (AYK), Laccadive Micro (LM), Laccadive Ordinary (LO), Andaman Ordinary (AO), Cochin China (CC), Philippines (PHI), West Coast Tall (WCT) and Malayalan Yellow Dwarf (MYD), and from the check cultivar Kerasree (KS).

4.2.2 Estimating quantity and quality of isolated DNA

Quantitation of the DNA samples were done using UV spectrophotometer and approximate size, concentration and quality was ascertained by electrophoresis using agarose gel. The gel image of eight parental palms and the check cultivar (Plate 22) showed intact bands indicating the presence of good quality DNA with no degradation.

The bands produced by LM and MYD were less bright indicating low quantity of DNA compared to other samples. The purity and quantity of DNA was estimated by the ratio of absorbance of DNA at A260/280 by UV spectrophotometer. Data showed a value ranged from 1.53 to 1.89 and the quantity of DNA samples ranged from 200 ng/µl (LM) to 1250 ng/µl (AO) (Table 16).

Table 16. Quantity and quality of isolated genomic DNA

Genotypes	A260/280	DNA concentration (ng/µl)
West Coast Tall (WCT)	1.68	1200
Ayiramkachi (AYK)	1.84	550
Laccadive Micro(LM)	1.89	200
Malayalan Yellow Dwarf (MYD)	1.73	350
Philippines (PHI)	1.59	650
Cochin China (CC)	1.88	800
Laccadive Ordinary (LO)	1.56	900
Andaman Ordinary (AO)	1.83	1250
Kerasree (KS)	1.53	500

4.2.3 Standardised PCR conditions

The composition of components in the master mix and thermal profile were standardised as detailed below. Annealing temperature for each of the primers was optimised by gradient PCR. All the primers except CnCir A3, CnCir C12, CNZ10 and CAC 06 recorded to have an annealing temperature of 58˚C. Primers CnCir A3 and CnCir C12 annealed at 51˚C and, primers CNZ 10 and CAC 06 at 56˚C.

Mastermix (25 µl)	Thermal profile
DNA template – 50 ng/µl	Initial denaturation: 94˚C - 5 min
10X PCR buffer with $MgCl_2$ – 2 µl	Denaturation: 94˚C - 1 min
10 mM dNTPs – 1.5 µl	Annealing: 58, 56 and 51˚C - 1 min
Taq polymerase (5U) – 0.2 µl	Extension: 72˚C - 2 min
Forward primer (10 pM) – 1.0 µl	Final extension: 72˚C - 5 min
Reverse primer (10 pM) – 1.0 µl	Hold: 4˚C
Ultrapure water	35 cycles

4.2.4 Screening of SSR primers

Based on reported SSR markers in coconut, 34 SSR markers were screened for polymorphism between eight parental genotypes (AYK, PHI, LM, AO, CC, LO, WCT

and MYD) and one check cultivar (KS). Best SSR markers were selected by comparative analysis for future studies for screening hybrids palms.

All the SSR markers used were able to amplify the genomic DNA. Primers *viz*. CAC 03 (Plate 23a), CAC 06 (Plate 23b), CAC 65 (Plate 23c), CNZ 04 (Plate 23d), CNZ 06 (Plate 23e), CNZ 10 (Plate 23f), CNZ 46 (Plate 23g), CnCir 01 (Plate 23h), CnCir 51(Plate 23i), CnCir E10 (Plate 23j), CnCir A3 (Plate 23k), CnCir C12 (Plate 23l)and CnCir HII (Plate 23m) produced single monomorphic amplicon (Plate 23). The amplicon size ranged from 85bp (CAC 65 and CnCir 01) to 110 bp (CAC 06, CNZ 10 and CnCir A3). These primers were not selected as they could not produce distinct amplicons.

Primers such as CnCir F2 (Plate 24a), CnCir H7 (Plate 24b), CnCir G11 (Plate 24c) and CNZ 40 (Plate 24d) produced single polymorphic amplicons (Plate 24). All of these primers produced amplicon with a size of about 100 bp and were 100 per cent polymorphic. So these primers were selected and recommended for future studies.

Primers CAC 04 (Plate 25a), CAC 08 (Plate 25b), CAC 10 (Plate 25c), CNZ 05 (Plate 25d), CNZ 12 (Plate 25e) and CnCir B6 (Plate 25f) produced two amplicons, one monomorphic and other polymorphic (Plate 25), showing 50 per cent polymorphism.

Primers *viz*. CAC 02 (Plate 26a), CAC 11 (Plate 26b), CnCir A9 (Plate 26c), CnCir B12 (Plate 26d), CnCir C7 (Plate 26e), CnCir C5 (Plate 26f), CNZ 44 (Plate 26g), CnCir E2 (Plate 26h), CnCir H4 (Plate 26i), CnCir C3 (Plate 26j) and CnCir E12 (Plate 26k) produced two distinct polymorphic amplicons (Plate 26). These primers were 100 per cent polymorphic and were selected for further screening of hybrids. The primers and corresponding type of amplicons produced were represented in Table 17.

4.2.5 Selection of polymorphic SSR primers

The 34 SSR primers generated a total of 51 amplicons out of which 32 amplicons were found polymorphic. The markers produced an average of 52.94 per cent polymorphism and one to two alleles per locus with a mean of 1.5 alleles per locus. The details of SSR amplification is provided in Table 18.

Table 17. Screening of 34 SSR primers

Sl. No.	Primer	Total number of amplicons	Type of amplicons	
			High intensity bands	Faint bands
1	CAC 02	2	0	2
2	CAC 03	1	1	0
3	CAC 04	2	1	1
4	CAC 06	1	1	0
5	CAC 08	2	1	1
6	CAC 10	2	1	1
7	CAC 11	2	1	1
8	CAC 65	1	1	0
9	CNZ 04	1	1	0
10	CNZ 05	2	2	0
11	CNZ 06	1	1	0
12	CNZ 10	1	1	0
13	CNZ 12	2	2	0
14	CNZ 40	1	0	1
15	CNZ 44	2	2	0
16	CNZ 46	1	1	0
17	CnCir A9	2	1	1
18	CnCir 01	1	0	1
19	CnCir 51	1	0	1
20	CnCir E10	1	1	0
21	CnCir A3	1	1	0
22	CnCir C12	1	1	0
23	CnCir B6	2	1	1
24	CnCir B12	2	1	1
25	CnCir E2	2	1	1
26	CnCir C7	2	0	2
27	CnCir H4	2	1	1
28	CnCir E12	2	1	1
29	CnCir C3	2	1	1
30	CnCir F2	1	1	0
31	CnCir H7	1	1	0
32	CnCir G11	1	1	0
33	CnCir HII	1	1	0
34	CnCir C5	2	2	0

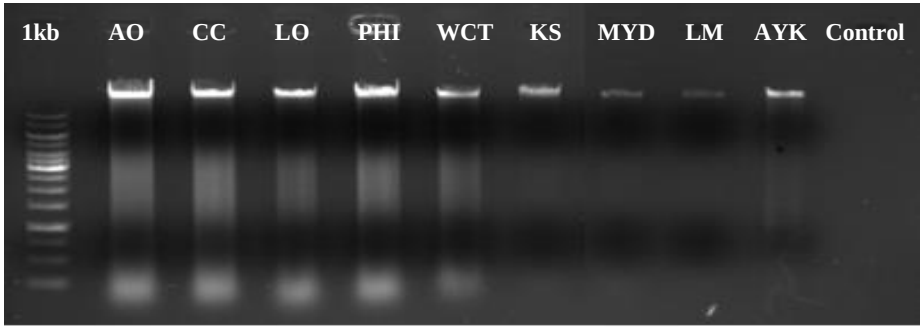


Plate 22. Gel profile of genomic-DNA of parental palms and check

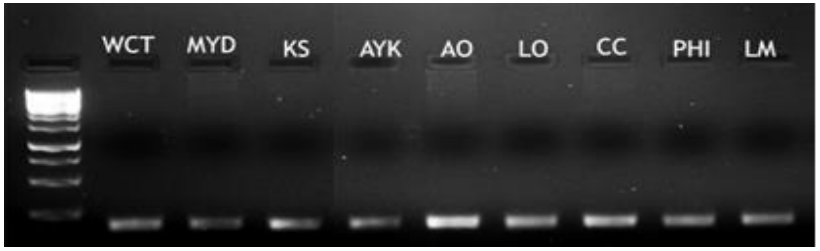


Plate 23a. CAC 03

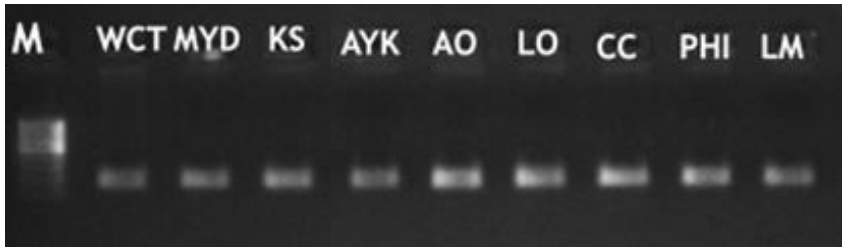


Plate 23b. CAC 06

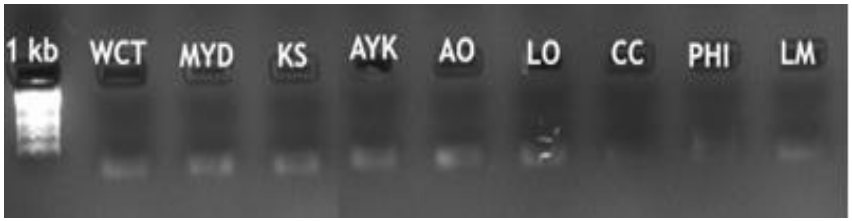


Plate 23c. CAC 65

Plate 23d. CNZ 04

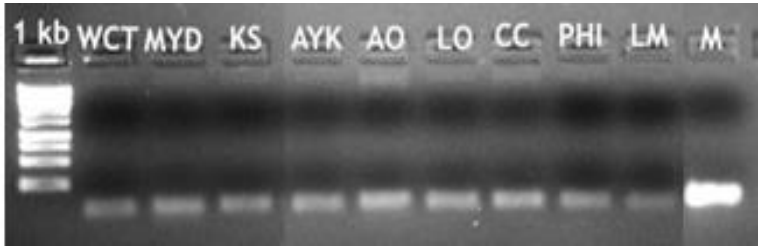


Plate 23e. CNZ 06

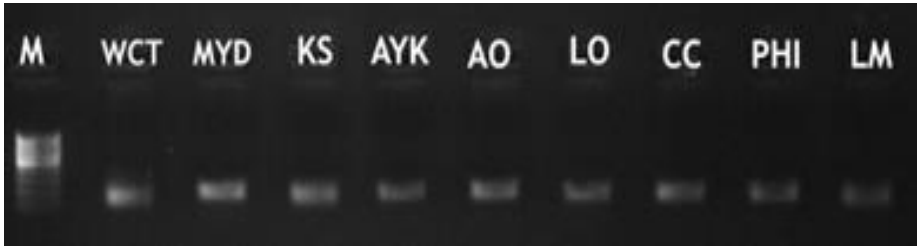


Plate 23f. CNZ 10

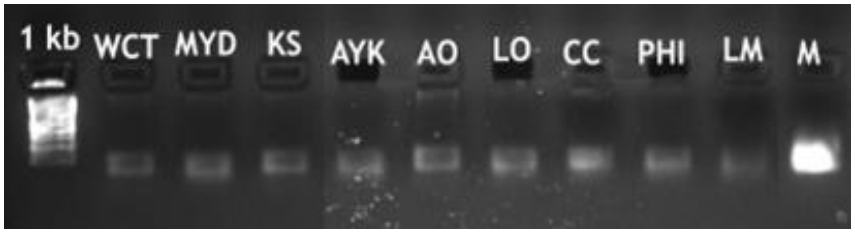


Plate 23g. CNZ 46

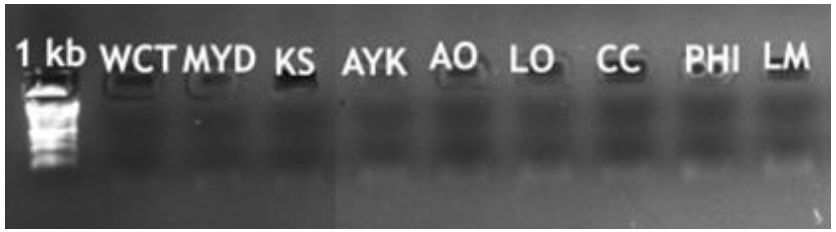


Plate 23h. CnCir 01

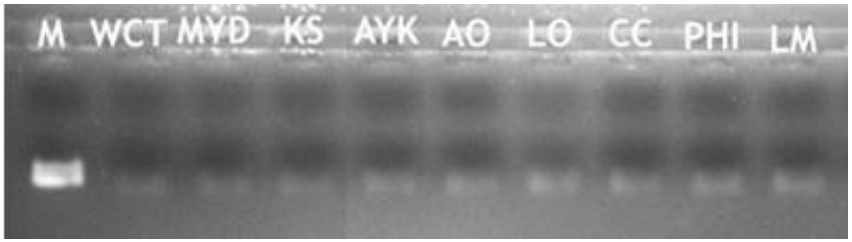


Plate 23i. CnCir 51

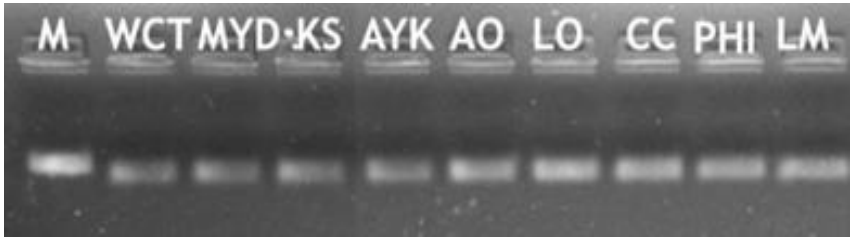


Plate 23j. CnCir E10

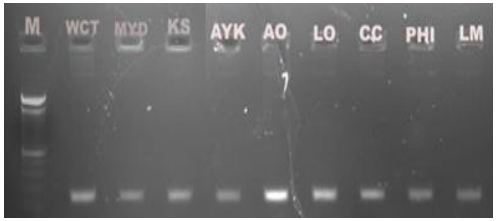


Plate 23k. CnCir A3

Plate 23l. CnCir C12

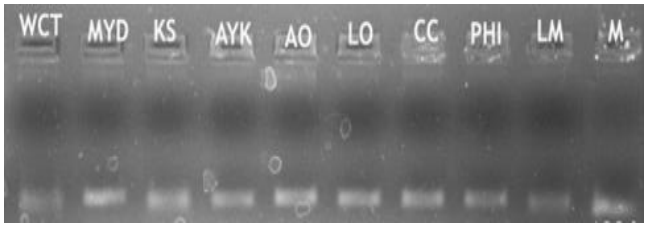


Plate 23m. CnCir HII

Plate 23. Gel profile of primers with single monomorphic amplicon

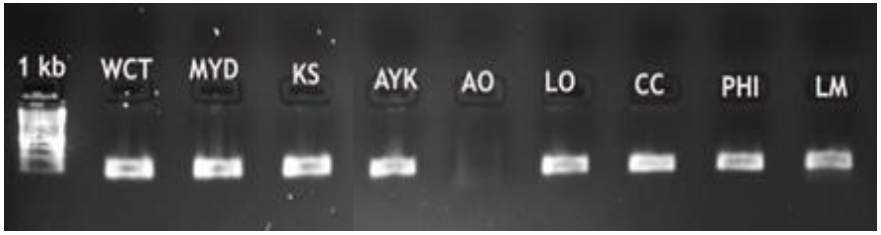


Plate 24a. CnCir F2

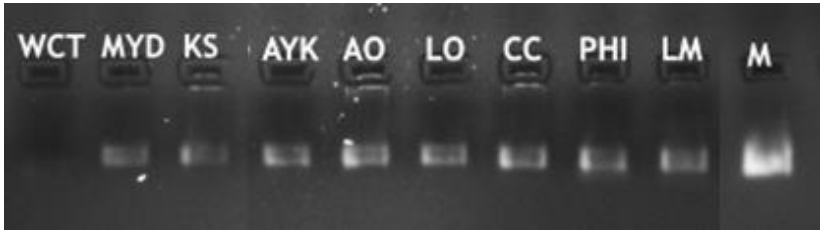


Plate 24b. CnCir H7

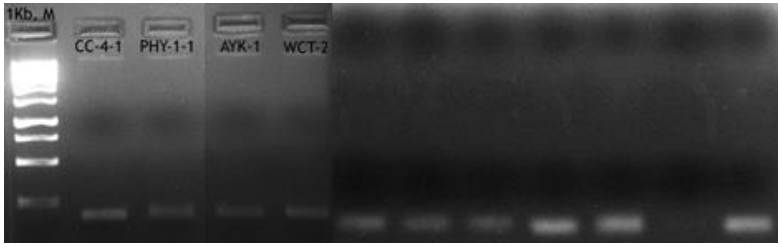


Plate 24c. CnCir GII

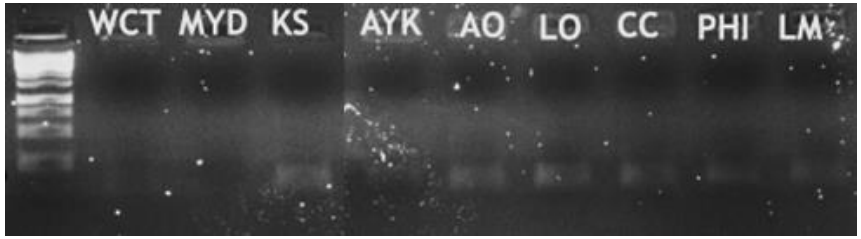


Plate 24d. CNZ40

Plate 24. Gel profile of primers with single polymorphic amplicon

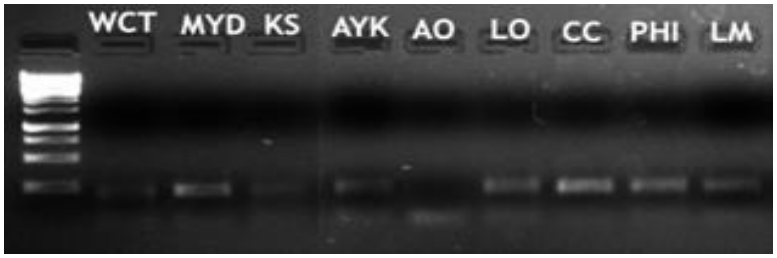


Plate 25a. CAC04

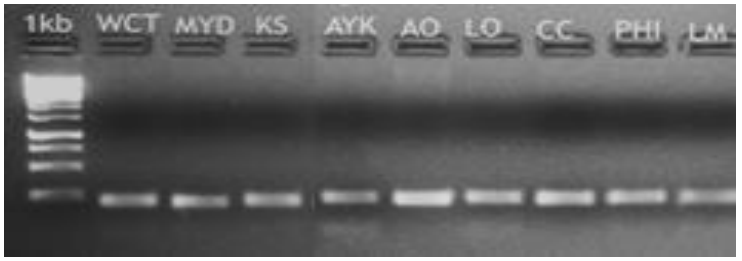


Plate 25b. CAC08

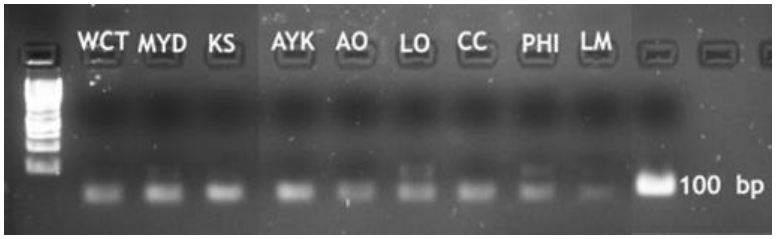


Plate 25c. CAC10

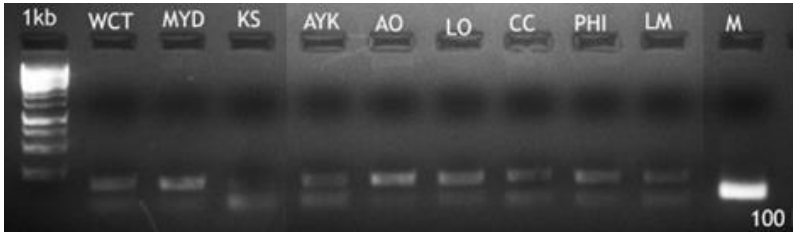


Plate 25d. CNZ05

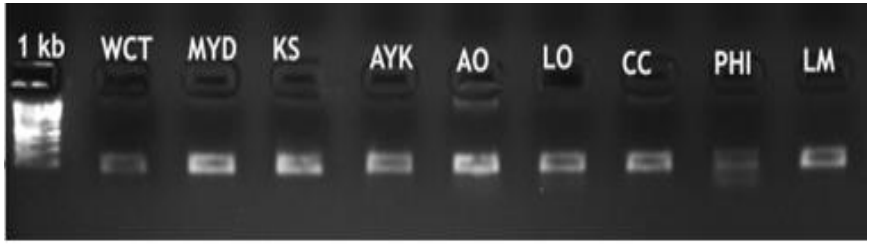


Plate 25e. CNZ12

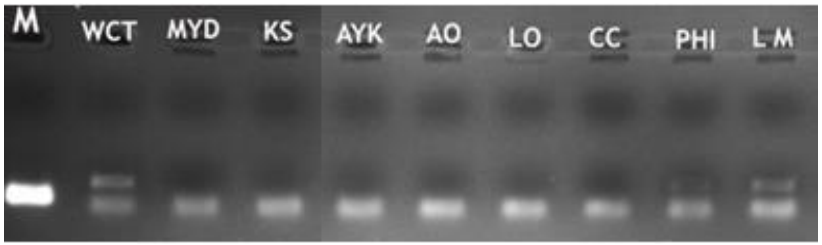


Plate 25f. CnCir B6

Plate 25. Gel profile of primers with two amplicons - a monomorphic and a polymorphic

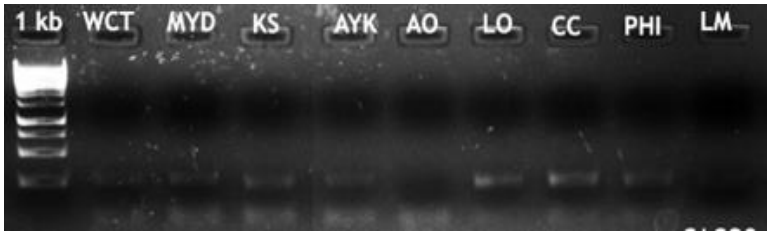


Plate 26a. CAC02

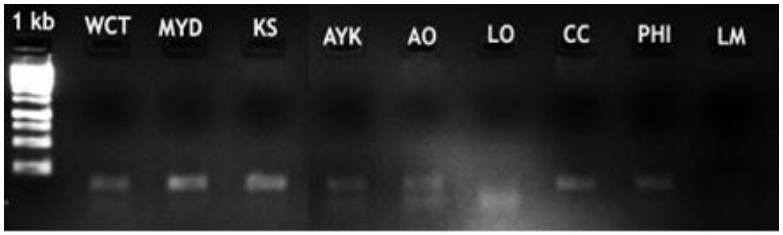


Plate 26b. CAC11

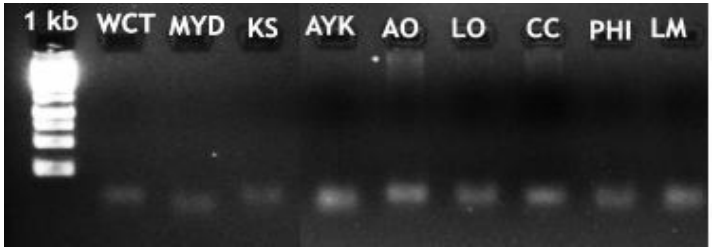


Plate 26c. CnCir A9

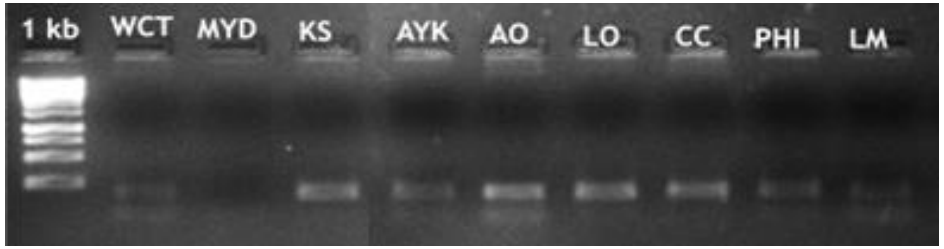


Plate 26d. CnCir B12

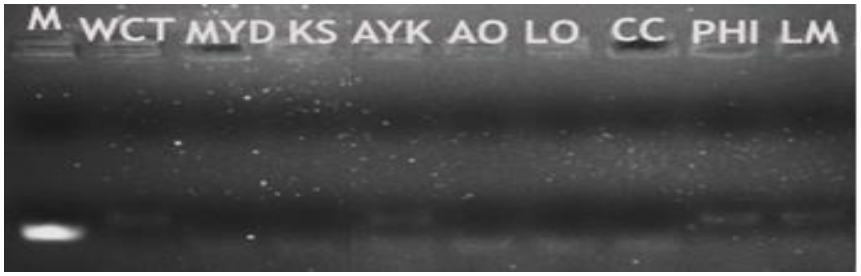


Plate 26e. CnCir C7

Plate 26f. CnCir C5

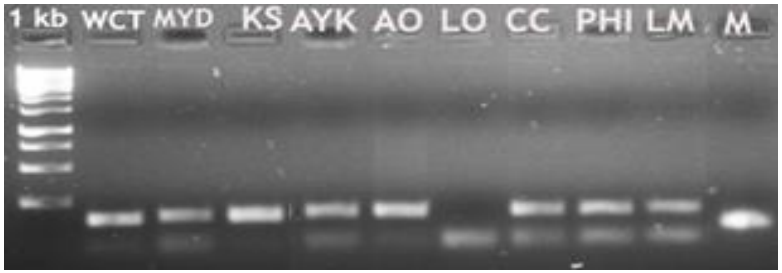


Plate 26g. CNZ 44

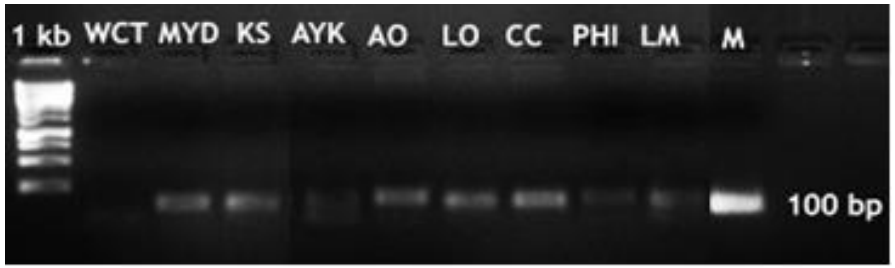


Plate 26h. CnCir E2

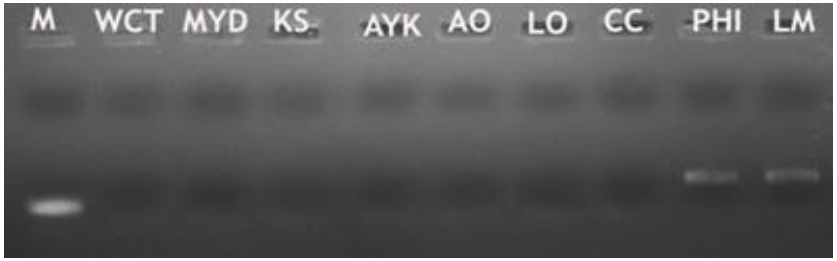


Plate 26i. CnCir H4

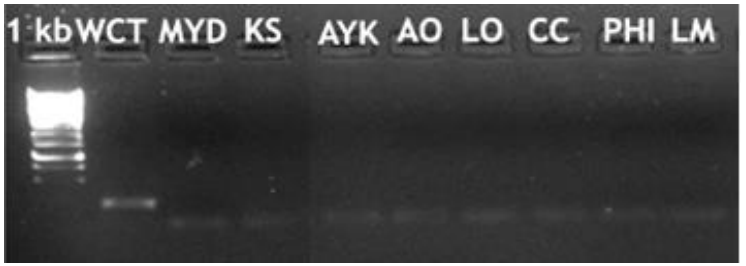


Plate 26j. CnCir C3

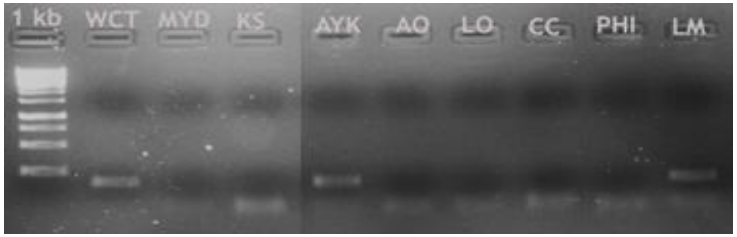


Plate 26k. CnCir E12

Plate 26. Gel profile of primers with two distinct polymorphic amplicons

Table 18. Amplification details of 34 SSR primers

Sl no.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of alleles per locus	Polymor-phism %	Remarks
1	CAC 02	2	2	2	100	Selected
2	CAC 03	1	0	1	0	-
3	CAC 04	2	1	2	50	-
4	CAC 06	1	0	1	0	-
5	CAC 08	2	1	2	50	-
6	CAC 10	2	1	2	50	-
7	CAC 11	2	2	2	100	Selected
8	CAC 65	1	0	1	0	-
9	CNZ 04	1	0	1	0	-
10	CNZ 05	2	1	2	50	-
11	CNZ 06	1	0	1	0	-
12	CNZ 10	1	0	1	0	-
13	CNZ 12	2	1	2	50	-
14	CNZ 40	1	1	1	100	Selected
15	CNZ 44	2	2	2	100	Selected
16	CNZ 46	1	0	1	0	-
17	CnCir A9	2	2	2	100	Selected
18	CnCir 01	1	0	1	0	-
19	CnCir 51	1	0	1	0	-
20	CnCir E10	1	0	1	0	-
21	CnCir A3	1	0	1	0	-
22	CnCir C12	1	0	1	0	-
23	CnCir B6	2	1	2	50	-
24	CnCir B12	2	2	2	100	Selected
25	CnCir E2	2	2	2	100	Selected
26	CnCir C7	2	2	2	100	Selected
27	CnCir H4	2	2	2	100	Selected
28	CnCir E12	2	2	2	100	Selected
29	CnCir C3	2	2	2	100	Selected
30	CnCir F2	1	1	1	100	Selected
31	CnCir H7	1	1	1	100	Selected
32	CnCir G11	1	1	1	100	Selected
33	CnCir HII	1	0	1	0	-
34	CnCir C5	2	2	2	100	Selected
Total		51	32	51	1800	
Average		1.5	0.941176	1.5	52.94118	

Only those primers which were 100 per cent polymorphic are selected and recommended for future screening of hybrids. Thus fifteen SSR markers were selected *viz*. CnCir F2, CnCir H7, CnCir G11, CNZ 40, CAC 02, CAC 11, CNZ 44, CnCir A9, CnCir B12, CnCir E2, CnCir C7, CnCir H4, CnCir E12, CnCir C3 and CnCir C5.

CAC 02

This primer produced two polymorphic amplicons which were 80 bp and 100 bp in size, the bands were faint but distinct. The 100 bp band was found polymorphic for AO and the 80 bp band for LM (Plate 26a).

CAC 11

Two distinct polymorphic amplicons were produced by the primer CAC 11, the first band of about 90 bp was clear and polymorphic for LO but the smaller band of 80 bp was faint and polymorphic for KS and CC (Plate 26b).

CNZ 40

Primer CNZ 40 produced a single polymorphic amplicon. The band was faint with a size of 100 bp and found polymorphic for WCT, MYD and AYK (Plate 24d).

CNZ 44

The primer CNZ 44 could generate two clear distinct polymorphic amplicons which were 90 bp and 110 bp in size. The 110 bp band was polymorphic for LO and 80 bp for KS (Plate 26g).

CnCir A9

Two distinct polymorphic amplicons were produced by the primer CnCir A9 were 85 bp and 90 bp in length. The first band of 90 bp was clear and absent in MYD, the second band of 85 bp was faint and and present only in MYD (Plate 26c).

CnCir B12

The primer produced two distinct polymorphic amplicons. The first one was of 100 bp in size was clear and polymorphic for MYD, the second band of 90 bp was

faint and polymorphic for MYD, KS, LO and CC (Plate 26d).

CnCir C3

Primer CnCir C3 produced two distinct polymorphic amplicons of 80 bp and 90 bp in size. the larger band was clear and present only in WCT and the smaller one was faint present in all cultivars except WCT (Plate 26j).

CnCir C5

Two distinct clear polymorphic amplicons were produced by the primer CnCir C5 and these were about 100 bp and 95 bp in size. The larger band was found polymorphic for MYD, AO and LO, and the smaller one for WCT, KS, AYK, CC, PHI and LM (Plate 26f).

CnCir C7

Two distinct faint polymorphic amplicones were produced by CnCir C7 and the bands were about 110 bp and 95 bp in size. The larger band was present only for cultivars WCT, AYK, PHI and LM and the smaller one was absent in WCT, PHI and LM (Plate 26e).

CnCir E2

Primer CnCir E2 generated two distinct polymorphic amplicons. The larger band was clear and about 105 bp in size, it was found polymorphic for WCT. The smaller band was faint and 95 bp in length, it was present only in cultivars WCT, AYK and LM (Plate 26h).

CnCir E12

Two distinct polymorphic amplicons were generated by CnCir E12. The larger band of 95 bp was clear and present only in cultivars WCT, AYK and LM. The smaller one of 85 bp was faint and polymorphic for cultivar WCT (Plate 26k).

CnCir F2

Primer CnCir F2 generated a single clear polymorphic amplicon of size 100 bp and was found polymorphic for AO (Plate 24a).

CnCir G11

A single clear polymorphic amplicon was generated by the primer CnCir G11. The amplicon was about 100 bp in size and found polymorphic for KS (Plate 24c).

CnCir H4

Primer CnCir H4 generated two distinct polymorphic amplicons of size 110 bp and 90 bp. The larger band was clear and present only in PH and LM but the smaller faint band was recorded absent in these cultivars (Plate 26i).

CnCir H7

Single clear polymorphic amplicon of size 100 bp was generated by the primer CnCir H7 and was found polymorphic for WCT (Plate 24b).

4.2.6 Diversity analysis of parental palms and check cultivar using SSR assay data

The eight parental genotypes and the check cultivar was subjected to diversity analysis on the basis of SSR assay data where the data was scored as 1 (presence) or 0 (absence). The genetic distance between each cultivar was analysed using Dice dissimilarity coefficient. Cluster analysis was done using UPGMA method and dendrogram was generated by neighbor joining technique using software DARwin ver.6.0 (Figure 11).

The dendrogram construct based on molecular characterisation classified the nine cultivars in to three major clusters (Table 19). Cluster I included three tall cultivars (Laccadive Micro, Philippines and West Coast Tall) and one dwarf cultivar (Ayiramkachi). Cluster II comprised two tall (Laccadive Ordinary and Andaman Ordinary) and one dwarf cultivar (Malayan Yellow Dwarf). A tall cultivar (Cochin China) and check palm (Kerasree) was grouped under Cluster III. The genetic distance or genetic diversity (GD) among the cultivars ranged between 0.053 (between KS and CC) and 0.240 (between WCT and LO) based on Dice dissimilarity matrix (Table 20).

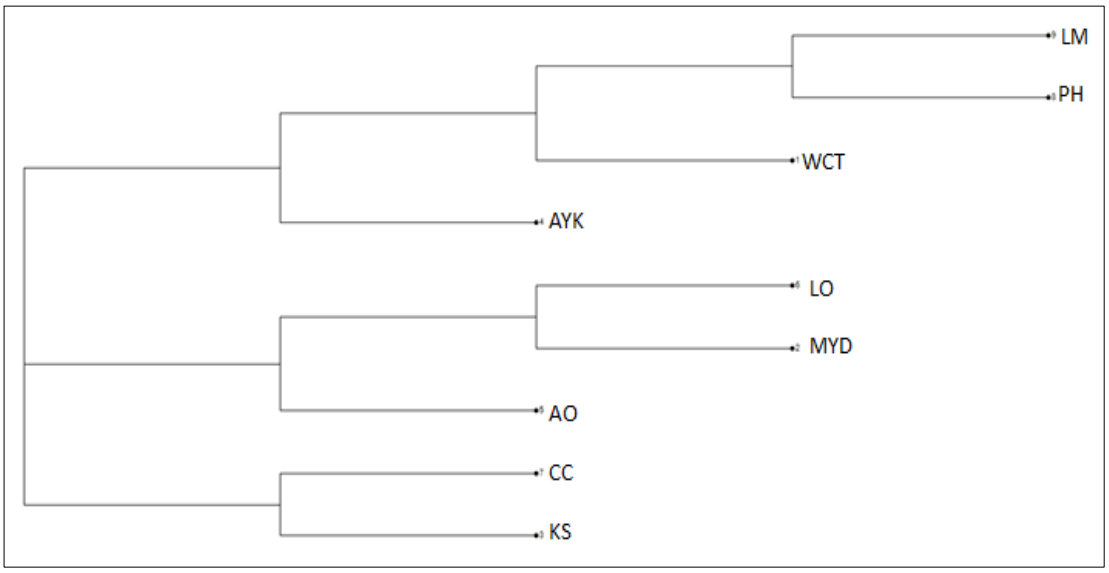


Fig 11. Dendrogram generated for coconut cultivars using DARwin ver.6.0

WCT: West Coast Tall	MYD: Malayan Yellow Dwarf	KS: Kerasree
AYK: Ayiramkachi	AO: Andaman Ordinary	PHI: Philippines
CC: Cochin China	LO: Laccadive Ordinary	

Table 19. Grouping of coconut cultivars based on SSR analysis data using software DARwin ver.6.0

Cluster No.	No. of cultivars	Name of cultivars
Cluster I	4	Laccadive Micro, Philippines, West Coast Tall, Ayiramkachi
Cluster II	3	Laccadive Ordinary, Andaman Ordinary, Malayan Yellow Dwarf
Cluster III	2	Cochin China, Kerasree

Table 20. Dice Dissimilarity matrix of coconut cultivars based on SSR analysis

	WCT	MYD	KS	AYK	AO	LO	CC	PHI
MYD	0.2207							
KS	0.2368	0.1200						
AYK	0.1219	0.1111	0.1250					
AO	0.2207	0.1052	0.1466	0.1358				
LO	0.2405	0.0769	0.1168	0.1084	0.1025			
CC	0.1794	0.0909	0.0526	0.0731	0.0909	0.0886		
PHI	0.1566	0.1463	0.1358	0.1264	0.1463	0.1428	0.1084	
LM	0.1428	0.1807	0.1707	0.0909	0.1807	0.1529	0.1428	0.0561

The dwarf cultivar AYK was found to have more similarity with CC a tall cultivar (GD = 0.0731) and dissimilar to AO (GD = 0.1358). Palm MYD recorded more similarity with LO (GD = 0.0769) and grouped under same cluster and dissimilarity with PHI (GD = 0.1463). On analysing the tall cultivars, LO and WCT was found to be most distinct (GD = 0.2405) thus clustered separately and, PHI and LM the most similar ones (GD = 0.0561) and grouped together in the sub-clustering of the main cluster. Check palm KS is a WCTxMYD hybrid. On analysing the genetic

diversity of KS with its parents, it was found that KS was more similar to MYD (GD=0.1200) than to WCT (0.2368).

Grouping of genotypes in dendrogram was not effective. The tall and dwarf cultivars were grouped under same cluster and the check cultivar Kerasree a hybrid of WCT and MYD was neither grouped with any of the parents and was clustered along with CC (an unrelated genotype). Therefore further study has to be conducted using more number of markers.

DISCUSSION

5. DISCUSSION

Coconut is an important plantation crop in Kerala and is widely cultivated. Most of the cultivated types are selections from tall and there is a need for developing hybrids which can safeguard the farmers need. Thus high yield with desirable qualities like copra and oil content combined with short stature became the prime concern of breeding programmes. Eventhough *Cocos* is a monotypic genus, it has a huge varietal diversity due to cross pollination, which can be utilized for improvement programmes. Thus, in the present study the hybrids of cultivar Ayiramkachi (with high female flower production) belonging to three hybrid groups *viz*. TxD, DxT and DxD developed by crossing with eight diverse tall and dwarf palms were analysed for important yield and nut characters combined with dwarf stature. Simultaneously the parental genotypes were characterised using SSR markers to detect the polymorphic markers suitable for future characterisation of these hybrids also.

The results of morphological evaluation of the hybrids and molecular characterisation of the parents were described in previous chapter, a detailed discussion of which will be presented in this chapter.

5.1 MORPHOLOGICAL CHARACTERISATION OF GENOTYPES

5.1.1 Evaluation of hybrid combinations and hybrid groups (TxD, DxT and DxD)

Aim of the present investigation was to characterise and evaluate the hybrids of Ayiramkachi for important morphological characters, yield attributes and nut quality combined with dwarf stature. Therefore, importance was given to palm height and yield contributing traits like number of female flowers per inflorescence, number of nuts per bunches, fruit weight and copra content in selecting better performing hybrids.

All the hybrids recorded a reduction in palm height compared to check (KS). In general the dwarf stature was found transmitted to all the hybrids of Ayiramkachi. Among them, hybrids MYDxAYK and its reciprocal cross AYKxMYD, WCTxAYK and PHIxAYK exhibited significant height reduction which was comparable to the dwarf palm (MYD). It was also evident from the comparison of overall performance

of TxD, DxT and DxD crosses with tall (T) and dwarf (D) parents, where all the hybrids recorded palm height on par with the dwarf (AYK and MYD). A similar trend was recorded by Raveendran *et al*. (1989) for TxD and DxT hybrids of dwarf cultivars AYK, MGD and MYD with tall cultivar ECT.

Presence of higher girth at 20 cm indicate the presence of bole which is commonly present in tall cultivars and help them to withstand heavy wind (de Lamothe and Wuidart, 1982). Girth at 20 cm was found highest for AOxAYK (which is a TxD hybrid) and least for MYD. When the hybrid groups are compared with tall and dwarf parents, a similar result was obtained. The TxD cross of Ayiramkachi recorded highest girth which was on par with tall, indicating the similarity in behavior of TxD with tall cultivars, and least girth was recorded for the dwarfs. A similar finding was reported by Thampan (1975), where TxD and tall palms recorded maximum girth and least by dwarf palms. Girth at 1.5 m height was recorded highest for LM and PHI (tall cultivars) and least for MYD (dwarf). Menon and Panalai (1958) also reported maximum palm girth for exotic cultivar including PHI.

Highest internode length was recorded for LOxAYK and AOxAYK, which might have been inherited from their tall parents and least by MYD and AYKxMYD. These findings are in agreement with previous reports that the internodal length in hybrid is decided by the parental combination ((Pillai *et al.,* 1991).

Many studies have shown that number of functional leaves, rate of leaf production, petiole length and leaf length are major vegetative characters supporting yield and copra content. Sreelatha (1987) and Pillai *et al*. (1991) reported variation among the cultivars for these characters. Among the hybrids CCxAYK and LMxAYK had highest number of leaves. More the number of leaves per year, higher will be the number of bunches, as in coconut every leaf axil produces one inflorescence and also the rate of photosynthesis will be more resulting higher yield. It was also observed that hybrids CCxAYK and LMxAYK recorded maximum yield among hybrids. Hybrids LOxAYK, CCxAYK, PHIxAYK, WCTxAYK, LMxAYK and MYDxAYK recorded higher rate of leaf production compared to check (KS). But no significant difference was noticed when mean values of leaf number and rate of leaf production in

different groups of hybrids *viz*., TxD, DxT and DxD were compared with that of tall and dwarf.

Direct and reciprocal cross of AYK with WCT recorded maximum leaf length while dwarf cultivar MYD recorded least petiole and leaf length along with its hybrid AYKxMYD (DxD). Generally petiole and leaf length will be higher for tall cultivars compared to dwarf. The comparison of three hybrid groups with tall and dwarf parents also revealed a similar trend. The hybrids involving a tall cultivar as one of its parent (TxD and DxT) recorded highest readings while dwarf (D) or DxD hybrids recorded least petiole and leaf length.

Number of female flowers per inflorescence just after spathe opening and number of female flowers one month after pollination was highest for AYK among the parents, and for CCxAYK among hybrids, while the hybrid AYKxMYD recorded the lowest. Number of nuts per bunch was also found to be highest for AYK. Among hybrids, nuts per bunch was found highest for LMxAYK and CCxAYK which may be due to the high female flower production combined with better setting per cent. All other hybrids except AYKxMYD produced nuts per bunch on par with the check. In AYKxMYD it was significantly low. The mean performance of different groups with respect to these characters were also studied. Generally dwarf cultivars (D) have higher number of female flowers, a similar trend was followed in this study also. However female flowers one month after pollination and number of nuts per bunches does not vary significantly among TxD, DxT, DxD hybrids and tall and dwarf parents.

Nut yield and copra content are considered as economically important yardsticks of coconut. Hence nut characters like size, weight, copra content, shell and meat weight *etc*. are important economic characters in coconut. Selecting palms having higher yield but smaller sized fruits or selecting palms with greater fruit size by compromising yield is not advisable. Geethanjali *et al.* (2014) reported that the cultivars which are yielding more than 100 nuts each year per palm, having medium sized nuts with more than 150 g copra per nut was found suitable for meeting demand of yield and copra. Because of high palm to palm variation, a very high coefficient of variation was observed for the character number of nuts per palm per year (yield), thus

significant difference among the genotypes were not able to be determined. But hybrids LOxAYK, LMxAYK and MYDxAYK yielded more than 100 nuts/palm/year with more than 150 g copra/nut, and thus found superior for these characters.

The hybrid PHIxAYK had four nut characters with highest value among hybrids and significantly high compared to check *viz*., nut weight without husk, shell and meat weight without water, quantity of nut water (liquid endosperm) in the mature nut and kernel thickness. It was also evident from the results of the present study that hybrids LMxAYK, MYDxAYK and LOxAYK recorded better performance or on par with Kerasree (check variety) for most of the nut characters such as size of unhusked nut, weight of nut without husk, shell & meat weight without water and kernel thickness. Kernel thickness was highest in WCTxAYK and AOxAYK but other nut characters were not as superior as the above hybrids.

Except the hybrids of WCT with AYK all other hybrids recorded high quantity of nut water (liquid endosperm) on par with check. However, the potential of these palms to be utilized for tender nut purpose could not be assessed because, nut water content and sugar content were recorded from mature nuts only. Seven months old nuts are preferable for tender nut purpose (Apshara *et al*., 2007).

The comparison of overall performance of the three groups of hybrids *viz*., TxD, DxT and DxD did not revealed any significant difference for yield and nut characters. In general, the vegetative characters of TxD hybrids resembled the respective tall parent while that of the DxD hybrids resembled dwarf parent.

Qualitative characters

Two type of crown shape, spherical and hemi-spherical were observed among the cultivars studied. None of the crowns were X or V-shaped. Dominating nature of spherical shaped crown was reported by Islam *et al*. (2013) and X-shape might have evolved to minimize heavy winds in seashore.

Petiole and nut colour is a widely used morphological marker to distinguish hybrid seedlings and are governed by two genes (*R/r* and *G/g*) (Bourdeix, 1999). The difference in colour of nuts between the reciprocal crosses of AYK and MYD may be

due the following allelic combinations of these genes. The parent AYK was green (possible genotype: *rrGG* or *rrGg*) and MYD was yellow (possible genotype: *rrgg*) and their hybrid AYKxMYD was also yellow (possible genotype: *rrgg*) and MYDxAYK was green (possible genotype: *rrGg*) for petiole and fruit colour.

Similarly for the hybrid LMxAYK, one palm was green (possible genotype: *rrGg* or *rrGG*) and the other was orange (possible genotype: *RRgg* or *Rrgg*) for petiole and fruit colour. As one of the parent AYK was green (possible genotype: *rrGg* or *rrGG*), the other parent (LM) might be brown (possible genotype: *RrGg* or *RRGG*).

A brief description of each hybrid combinations based on the experiment is given below:

LMxAYK

Hybrid LMxAYK (7.5 m) recorded lower palm height compared to the check and produced about 28 green leaves per tree. It is found to be a promising hybrid from TxD category with respect to yield (155.500 nuts/palm/year and 12.899 nuts/bunches) and superior nut characters such as fruit weight, nut weight, shell and meat weight, kernel thickness and copra content (187.250 g). It also recorded highest sugar content of 8˚Bx and thus can be utilized for tender nut purpose also or for extracting neera. One of the palm in this cross produced orange coloured nut while the other was green this may be attributed to genotype of parents for this trait.

When the heterosis was studied, hybrid LMxAYK reported a positive and significant standard heterosis over Kerasree for yield, and positive standard heterosis on par with Kerasree for number of nuts per bunches, size of unhusked nut, fruit weight, shell & meat weight without water and kernel thickness at maturity. It also had a significantly high heterobeltiosis for size of unhusked nut, fruit weight, nut weight, shell & meat weight without water, kernel thickness at maturity and copra content.

LOxAYK

Identified as a promising hybrid from TxD group. It was found superior for

yield (116.50 nuts/palm/year and 10.532 nuts/bunches) and nut characters *viz.* fruit weight, nut weight, shell and meat weight, kernel thickness and copra content (162.868 g).

Heterosis studies shows that LOxAYK recorded a positive standard hetrosis over Kerasree for number of nuts per bunches, yield and kernel thickness at maturity.

MYDxAYK

It is a promising hybrid from DxD group, and has the potential to be released as a commercial DxD hybrid. It was found superior for yield (115.33 nuts/palm/year and 12.225 nuts/bunches) and nut characters *viz*. fruit and nut weight, shell and meat weight, kernel thickness and copra content (172.466 g) combined with dwarf stature (6.515 m) . Jayabose *et al*. (2008) also reported that the hybrids with MYD as maternal parent showed better performance than other hybrids. Which was evident from the heterosis studies also, it recorded a positive standard heterosis over the check (Kerasree) for number of nuts per bunches, yield per palm per year and kernel thickness. A significant positive heterobeltiosis was observed for characters such as size of unhusked nut, nut weight, shell & meat weight without water and copra content.

CCxAYK

It is also found to be a superior hybrid from TxD category with high yield (150.667 nuts/palm/year and 12.688 nuts/bunches) with short stature (7.105 m), but found inferior for nut characters such as size of nut, nut weight, kernel thickness and copra content (142.876 g). This indicated that the high yielding and inferior nut characters of AYK might have been transmitted to hybrid CCxAYK. As one of its parent Cochin China is already reported to have tender nut quality (Selvaraj *et al*., 2019), there is scope for utilizing this hybrid for tender nut purpose, after evaluating nuts at tender nut stage.

On heterosis study CCxAYK hybrid recorded a positive standard heterosis on par with Kerasree for number of nuts per bunches and yield. The observations for the

hybrid CCxAYK was found contradictory to the findings of Ganesamurthy *et al.* (2004), where the standard heterosis of CCxAYK for nut yield (with East Coast Tall as check) was found negative but in the present study (with Kerasree as check) it was highly positive. Similarly they also reported positive significant heterosis for fruit weight, nut weight and copra content, but significant negative values were recorded for these characters studied presently. This might be because of the difference in check variety used and environmental conditions. Heterosis is the effect of allelic or non-allelic interactions under the influence of a specific environment (Kanimozhi *et al*., 2018).

PHIxAYK

Hybrid with promising nut characters such as high fruit and nut weight, kernel thickness, quantity of liquid endosperm and copra content (211.02 g) combined with dwarf stature (5.257 m), but was inferior for yield characters (81.667 nuts/palm/year and low setting per cent (7 nuts/bunches). Hemavathi and Balaji (2006) reported that the hybrids from the cross of AYK and PHI will be superior and high yielding. So the hybrid needs to be further evaluated based on yield data over the years.

When the heterosis was studied PHIxAYK recorded a significantly positive standard heterosis for fruit weight, nut weight and shell and meat weight without water, and positive standard heterosis on par with Kerasree for size of unhusked nut, kernel thickness and copra content. It also had a positive and significant heterobeltiosis for most of the nut characters *viz*., size of unhusked nut, fruit weight, nut weight and shell & meat weight and copra content.

AOxAYK

It was recorded to be promising for nut characters (high fruit weight, nut weight, kernel thickness and copra content (159.400 g), but was found inferior for yield traits (61.500 nuts/palm/year) due to low setting percentage (7 nuts/palm/year). This hybrid recorded a positive standard heterosis on par with the Kerasree for kernel thickness at maturity, and a negative but non-significant standard heterosis and heterobeltiosis was observed for most of the nut characters *viz*., fruit weight, nut

weight, shell and meat weight without water and copra content.

The following combinations were found inferior in their performance for economically important traits but data over a period needs to be analysed to reach any final conclusions.

WCTxAYK

It was a moderate yielding type (95 nuts/palm/year) and was found superior for kernel thickness and copra content (156.050 g). It recorded a positive standard heterosis and heterobeltiosis for kernel thickness at maturity.

AYKxWCT

It was found to be a moderate yielder with 95 nuts/palm/year. However it was found inferior for all the nut characters studied (fruit weight, nut weight, shell and meat weight, kernel thickness, quantity of liquid endosperm and copra content).

AYKxMYD

It was recorded to be inferior for both yield (20 nuts/palm/year) and nut characters (fruit weight, nut weight, shell and meat weight, kernel thickness, quantity of liquid endosperm and copra content). But this hybrid recorded a positive heterobeltiosis for nut weight, shell & meat weight, kernel thickness and copra content. It was found similar to MYD for most of the vegetative and nut characters. So the palm needs to be evaluated further for its suitability as tender nut variety.

The hybrids AYKxWCT and AYKxMYD were recorded to be the inferior ones, but their reciprocal crosses WCTxAYK and MYDxAYK were superior than the later, this might be due to the poor performance of AYK as female parent.

5.1.2 Palm to palm variations within each cross

Variations were found between and also within hybrid combinations as the parents are not homozygous. Hence, a comparison of the different palms available in each hybrid combination was also attempted to identify the best palm in each cross.

Among hybrid PHIxAYK, palm No. 15 was found to be the better performer after analysing vegetative, reproductive and yield characters. It recorded least palm height (6.00 m), highest number of female flowers per inflorescence (28.75) and number of female flowers one month after pollination (19.80), yield (112.00 nuts/palm/year), number of bunches per year (13.00) and also nuts per bunches (8.62).

Analysing the cross CCxAYK palm No. 38 was estimated to be the better palm. It recorded a medium palm height (7.03 m), maximum yield (235.00 nuts/palm/year), number of bunches per year (13.00) and highest number of nuts per bunch (18.08).

Among the hybrids of AOxAYK, palm No. 18 was found to be the better performer. It recorded least palm height (3.44 m), highest number of female flowers per inflorescence (35.00), number of female flowers one month after pollination (25.08), number of bunches per palm per year (13.00) and maximum yield (93.00 nuts/palm/year).

Considering the cross MYDxAYK, palm No. 14 was estimated to be the better performer. Eventhough it exhibited maximum palm height (7.05 m) compared to other two palms in the cross (Palm no. 13 and 59), it recorded highest values for most of the reproductive and yield related characters *viz*., number of female flowers per inflorescence (38.38), number of female flowers one month after pollination (26.20), number of nuts per bunch (24.22) and yield (218.00 nuts/palm/year).

5.1.3 Genetic parameters

The basic information about genetic characteristics of the palms could be understood through phenotypic and genotypic coefficient of variation, depending on which, further improvement methods can be formulated. The present study revealed higher values of PCV over GCV in general, Manju and Gopimony (2006) and Subramanian *et al.* (2019) also reported a similar trend, which indicate that the variations observed was not only because of the genotype alone, but also by the environmental interference.

A high PCV and GCV was recorded for number of female flowers per

inflorescence (Louis, 1981), plam height, internode length, number of female flowers one month after pollination, number of nuts per bunch (Selvaraju, 2008), fruit volume, shell and meat weight, quantity of liquid endosperm, fruit weight and nut weight (Suchithra and Paramaguru, 2018) and number of nuts per palm per year (Subramanian *et al*., 2019). Hence, these characters are more viable, therefore selection for these traits will be efficient. The difference between PCV and GVC ranged from 1.26 (for leaf length) to 25.84 (for quantity of liquid endosperm). As lower the difference less will the environmental effects on that character.

Heritability estimates indicates the extend of inheritance of a particular trait from parent to offspring and also in selecting genotypes on the basis of phenotypic performance. Higher heritability is an indication of higher chance for the character to inherit to future generations, thus they could be improved through selection since there is less environmental interference (Lush, 1940). According to Johnson *et al.* (1955) genetic advance, which is a measure of genetic gain (Jerard, 2002) and heritability should to be considered together for effective selection and high accuracy.

High heritability coupled with high genetic advance as mean per cent was recorded for number of female flowers per inflorescence by Manju (1992) and Renuga (1999), number of green leaves by Natarajan *et al*. (2010), palm height and petiole length by Subramanian *et al*. (2019), internode length, rate of leaf production, leaf length, number of female flowers one month after pollination, size of unhusked nut equatorial circumference, shell and meat weight without water. High heritability with high genetic advance imply the role of additive genes in the inheritance of these characters and thus helps in effective selection.

Characters such yield, number of nuts per bunches, fruit weight and nut weight and copra content recorded medium heritability with high genetic advance, this might be due to the effect of environment in these characters or because of the indirect effect of secondary characters affecting them. A similar result was recorded for number of nuts per bunch and/or yield by Louis (1981) and Manju and Gopimony (2006). Selvaraju and Jayalekshmi (2011) reported high heritability for fruit and nut weight with high genetic advance.

Eventhough the heritability value of yield is of greater importance, its significance when yield alone was considered for selection will be negligible, hence one should take into consideration the yield attributing traits also for effective selection.

5.1.4 Heterosis

Herterosis is a genotypic phenomenon which is the manifestation of hybrid vigor, which in fact is the phenotypic expression (Shull, 1914). Later on certain other components were also added to heterosis by Williams (1959) including *gca* and *sca*, homeostasis and inbreeding depression.

Heterobeltiosis in the hybrids were estimated by comparing its performance over its better parent for nine yield and nut related characters and standard heterosis was also calculated, where the hybrids were evaluated against the standard check Kerasree which is a popular high yielding variety in Kerala. The hybrids possessing favourable standard heterosis can be considered as commercially worthy.

Based on the various vegetative and reproductive characters, among the six different combinations of TxD, four combinations were found promising *viz*., LMxAYK, LOxAYK, PHIxAYK and CCxAYK. Among the two combinations of DxD, which were also reciprocal crosses, Ayiamkachi as pollen parent with MYD as female parent only showed better performance which was on par with the TxD hybrids.

None of the hybrids recorded a positive heterobeltiosis for yield, which was due to the high number of nuts per palm per year but small sized nuts in Ayiramkachi.

According to James *et al*. (2003) there is combined expression of the alleles when different alleles of various genes are associated together, and also the complementation of alleles in different genes were cumulative in phenotype and result in heterosis. On studying both heterobeltiosis and standard heterosis for the nine hybrids of Ayiramkachi, the hybrids LMxAYK and MYDxAYK was found to be the better performers, Rattanapruk *et al.* (1983) also reported the superiority of hybrids having MYD as maternal parent. Therefore these crosses can be utilized for

developing location specific commercially important hybrids.

AYK was reported to be a promising general combiner (KAU, 2014). In the present study hybrids AYKxMYD and AYKxWCT was found to be the poor performers, where as their reciprocal crosses are found to be better performing. A similar trend was also reported by Jayabose *et al*. (2008) who found that the hybrid AYKxSIAM had significantly negative economic heterosis and heterobeltiosis for yield, but SIAMxAYK exhibited a non-significant heterosis. This indicate the poor performance of AYK as maternal parent. Therefore caution should be given in the choice of male and female parents for hybrid development. The negative heterosis might also be due to non-allelic interactions which caused a decrease in heterosis expression (Shinde *et al*., 2018).

5.2 MOLECULAR CHARACTERISATION

5.2.1 Extraction of genomic DNA

Tender leaves of the palm was used for isolating genomic DNA because softer tissues are easy to grind, number of cells per unit area will be more and it may contain less unwanted secondary metabolites and phenolic compounds and hence good quality DNA can be isolated. However as coconut leaves have high phenol content, PVP was added to the grinding mixture to avoid this polyphenol contamination and its oxidation. Angeles *et al*. (2005) also reported that addition of PVP or PVPP helps to reduce polyphenol contamination in the isolated coconut DNA. In this study a high concentration of NaCl (1.4 M instead of 0.5M) was also used to reduce polysaccharide contamination as reported by Fang *et al.* (1992).

5.2.2 Analysing quantity and quality of DNA

Intact and clear bands of the eight parents and the check palm observed in gel electrophoresis confirmed good quality genomic DNA without any degradation or breakage. The brighter and thicker band was produced by AO, whereas the comparatively faint one by LM. This was in line with the spectrophotometric readings which ranged from 200 (LM) to 1250 (AO) ng/µl. A260/A280 of the isolated DNA samples ranged from 1.53-1.89. The few DNA samples with lower ratio were further

subjected to chloroform- isoamyl alcohol precipitation to reduce the contamination by protein.

5.2.3 Standardising PCR conditions

Reaction mixture and thermal profile was standardized based on their efficiency to amplify the DNA. In the present study 50 ng DNA was used as DNA template. This was in line with the findings of Rajesh *et al*. (2013) that SSR assay needs low quantity (35 ng) of DNA. The standardised annealing temperatures were 51˚C for CnCir C12 and CnCir A3, 56˚C for CAC 06 and CNZ 10, and 58˚C for the remaining 32 primers. Rajesh *et al*. (2008) and Renju (2012) identified annealing temperature ranged from 50.2˚C (CnCir C12) to 63.1˚C (CnCir E2) and 52˚C (CnCir A3) to 59˚C (CnCir A9) respectively for primers belonging to CnCir series.

5.2.4 SSR data analysis

A total of 51 amplicons were produced by the 34 SSR primers under study with 32 polymorphic amplicons. Molecular weight of the products ranged from 80-110 bp. Manimekalai *et al*. (2006) and Rasam *et al*. (2016) recorded 100% and 92.90% polymorphism for SSR primers but in the present study it was only 52.94% indicating that selected primers were poor in detecting polymorphism. More number of primers should have been used for the study. Fifteen primers out of 34 primers showed 100% polymorphism among the nine cultivars were suggested for further study.

Among all the 34 SSR primers, CnCir C5 proved the hybridity of Kerasree (WCTxMYD). On analysing the gel profile it was found that both the higher molecular weight band of WCT and lower molecular weight band in MYD were presented in the hybrid palm Kerasree.

5.2.5 Diversity analysis

The dendrogram constructed based on molecular characterisation classified 9 parental cultivars in to 3 major groups. The distance among them ranged between 0.053 (between Kerasree and CC) and 0.240 (between WCT and LO) based on Dice

dissimilarity matrix. However, the grouping of genotypes in the dendrogram was not effective. Non-related cultivars were grouped together in the cluster. The dwarf cultivars AYK and MYD were grouped along with the tall ones and the hybrid Kerasree (WCTxMYD) was recorded to have no relation with any of its parents and was grouped along with CC. When the dissimilarity of KS with WCT and MYD was analysed, Kerasree recorded more similarity with MYD than WCT, but on morphological characterisation Kerasree was found more similar to WCT and was contradictory to above result. This might be because the number and type of markers used in the present study were not sufficient. Study has to be conducted using more SSR primers and other molecular markers also can be included (RAPD, ISSR, SNP's *etc*.) to get a good characterisation in future because Renju (2012) reported that an accurate classification of the cultivars were obtained on analysing a combined data of three markers (SSR, RAPD and ISSR) than analysing individual marker data.

SUMMARY

6. SUMMARY

The study entitled “Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*Cocos nucifera* L.)” was carried out at Department of Plant Biotechnology, College of Agriculture Padannakkad during the year 2018-2020. The plant material included 23 hybrids palms under nine different hybrid combinations under three groups *viz*. TxD [Philippines x Ayiramkachi (PHIxAYK), Cochinchina x Ayiramkachi (CCxAYK), Laccadive Ordinary x Ayiramkachi (LOxAYK), West Coast Tall x Ayiramkachi (WCTxAYK), Andaman Ordinary x Ayiramkachi (AOxAYK) and Laccadive Micro x Ayiramkachi (LMxAYK)]; DxT [Ayiramkachi x West Coast Tall (AYKxWCT)] and DxD [Malayan Yellow Dwarf x Ayiramkachi (MYDxAYK) and its reciprocal (AYKxMYD)]. These hybrid palms were planted during 1994 and was located on the X-Block of RARS, Pilicode.

On evaluating these hybrids along with its parental palms and check (Kerasree) for their morphological characters it was identified that hybrids LMxAYK, LOxAYK and MYDxAYK has the potential to be released commercially for culinary as well as processing purpose and are promising ones with high yield (more than 100 nuts) and superior nut characters. Hybrid MYDxAYK has an additional advantage as it a better performing DxD hyrid. The hybrid CCxAYK was high yielding (150 nuts/pam/year) but inferior in nut characters. As one of the parent in this cross, Cochin China, is suitable for tender nut purpose due to high nut water content, the hybrid nuts also may be further evaluated at tender nut stage to detect its suitability for tender nut purpose. Hybrid PHIxAYK was superior for all the nut characters combined with a shorter stature but the yield was comparatively less (81.67 nuts/ palm/ year). Hybrid AOxAYK recorded good nut characters but was low yielding (61.50 nuts/palm/year). Hybrids of AYK with WCT recorded a moderate yield (95.00 nuts/ palm/ year) with inferior nut characters and hybrid AYKxMYD was inferior for both yield (21 nuts/ palm/ year) and nut characters.

The morphological evaluation alone may not be sufficient to exhibit the

practical utility of the hybrids, as the farmers usually prefer the ruling or existing cultivar in that region. Therefore, it is necessary to prove the superiority of our concerned hybrid over the existing commercial cultivar to attain farmers acceptance. Therefore, standard heterosis was studied and the superiority of hybrids LMxAYK and MYDxAYK was proved. Hybrids AYKxMYD was recorded to be the inferior genotype might be due to the poor performance of AYK as a maternal parent.

Based on the study on genetic parameters further improvement or selection can be formulated. It revealed that characters such as palm height, internode length, number of green leaves, rate of leaf production, petiole length, leaf length, number of female flowers per inflorescence, number of female flowers one month after pollination and shell and meat weight had shown high heritability (H^2) coupled with high genetic advance as per cent means (GAM%). High heritability indicate high chance for these characters to inherit to future generations and less will be the environmental effect. Hence choice of such characters are best suited for selective breeding. Yield and copra content had shown medium heritability with high genetic advance. This might be due to the indirect effect of secondary characters contributing to them.

Inorder to have a comprehensive characterisation, fingerprinting using molecular marker (Simple Sequence Repeats- SSR) was also envisaged under the study. Good quality genomic DNA is the prerequisite for any molecular work. DNA was isolated from all the parental palms and check cultivar Kerasree. The quality and quantity of genomic DNA obtained was verified using agarose gel electrophoresis and spectrophotometer. The quantity of DNA samples ranged from 200 to 1250 ng/µl with good quality.

Molecular characterisation of eight parental genotypes and the check was done using 34 SSR primers. The PCR condition of the reactions were standardised. The markers produced an average of 52.94% polymorphism and 1 to 2 alleles/locus. Fifteen markers out of thirty four showed 100% polymorphism *viz*. CAC02, CAC11, CNZ40, CNZ44, CnCirA9, CnCirB12, CnCirE2, CnCirC7, CnCirH4, CnCirE12, CnCirC3, CnCirF2, CnCirH7, CnCirG11 and CnCirC5 were selected and

recommended for characterising hybrid palms. Among all the 34 SSR primers, CnCir C5 proved the hybridity of Kerasree (WCTxMYD).

The dendrogram construct based on molecular characterisation classified 9 (8 parents and 1 check) cultivars in to 3 major groups. The distance among them ranged between 0.053 (between Kerasree and CC) and 0.240 (between WCT and LO) based on Dice dissimilarity matrix. The clustering was found inefficient as the dwarf cultivars AYK and MYD clustered separately and grouped along with tall cultivars. Check palm Kerasree (KS) is a WCTxMYD, but had not similarity with any of the parent and was clustered along with an unrelated genotype (CC). On analysing the genetic diversity of KS with its parents, it was found that KS was more similar to MYD (GD=0.1200) than to WCT (0.2368). But on morphological evaluation it was found more similar to WCT. Thus the groping of genotypes in the dendrogram was not effective as the number and type of markers used in the present study were not sufficient.

Future scope of the work:

1. LMxAYK and MYDxAYK was found to be the better performers and these crosses can be further studied for releasing as commercially important hybrids

2. The hybrid CCxAYK can be evaluated at tender nut stage for its suitability for tender nut purpose

3. Since the results of correlation and path analysis were based only on one year data, the interpretation of the data was not possible. The data analysis can be as a future programme with additional data from different years.

4. The 15 markers reported in this study were able to detect the polymorphism between the parental palms and hence can be utilized in the future for fingerprinting the hybrid palms

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APPENDIX

APPENDIX-I

GENOTYPIC CORRELATION OF YIELD AND YIELD CONTRIBUTING CHARACTERS OF COCONUT

	HT	GP20	GP1.5	IL	NGL	RLP	PL	LL	TI	NUI	IUP	IPOSS
HT	1											
GP20	0.334**	1										
GP1.5	0.360**	0.924**	1									
IL	0.092NS	0.499**	0.445**	1								
NGL	0.478**	0.292**	0.344**	-0.083NS	1							
RLP	-0.023NS	0.279**	0.265*	0.295**	0.412**	1						
PL	0.079NS	0.472**	0.506**	0.791**	0.076NS	0.454**	1					
LL	0.251*	0.620**	0.633**	0.779**	0.295**	0.500**	0.825**	1				
TI	0.634**	0.546**	0.582**	0.273**	0.385**	0.217*	0.501**	0.462**	1			
NUI	0.247*	0.392**	0.314**	-0.041NS	0.395**	0.314**	0.160NS	0.218*	0.555**	1		
IUP	-0.186NS	-0.061NS	-0.056NS	0.210*	-0.124NS	0.489**	0.882**	0.635**	0.698**	0.779**	1	
IPOSS	0.379**	0.532**	0.503**	0.385**	-0.008NS	0.133NS	0.982**	0.672**	0.756**	0.488**	1.204**	1
TIPY	0.019NS	0.440**	0.413**	0.403**	0.282**	0.479**	0.685**	0.737**	0.690**	0.614**	1.152**	0.650**
NFF	0.108NS	-0.147NS	-0.158NS	0.048NS	0.007NS	0.001NS	0.395**	-0.009NS	0.153NS	-0.305**	0.241*	0.376**
NFFAP	0.325**	0.014NS	0.007NS	0.138NS	0.121NS	0.022NS	0.493**	0.186NS	0.339**	-0.215*	0.257*	0.530**
NBPY	-0.229*	0.498**	0.539**	0.435**	-0.023NS	0.367**	0.719**	0.776**	0.221*	0.400**	0.975**	0.651**
NNPY	-0.041NS	0.115NS	0.161NS	0.259*	0.063NS	0.194NS	0.791**	0.345**	0.459**	0.107NS	0.940**	0.951**
NNB	0.091NS	-0.031NS	0.002NS	0.102NS	0.064NS	0.021NS	0.649**	0.131NS	0.453**	-0.015NS	0.696**	0.834**
SUN_E	0.217*	0.134NS	0.076NS	-0.044NS	0.485**	0.498**	-0.066NS	0.221*	0.114NS	0.501**	0.307**	0.131NS
SUN_P	0.426**	0.294**	0.283**	0.077NS	0.633**	0.633**	0.104NS	0.402**	0.403**	0.718**	0.278**	0.285**
FW	0.188NS	0.139NS	0.081NS	-0.189NS	0.378**	0.456**	-0.150NS	0.101NS	0.081NS	0.573**	0.313**	0.012NS
FV	0.191NS	0.136NS	0.072NS	-0.182NS	0.385**	0.472**	-0.151NS	0.102NS	0.078NS	0.571**	0.303**	0.002NS
NW	0.180NS	0.152NS	0.074NS	-0.139NS	0.372**	0.531**	-0.178NS	0.081NS	0.203NS	0.593**	0.373**	0.012NS
SMW	0.114NS	0.224*	0.167NS	-0.059NS	0.344**	0.591**	-0.060NS	0.160NS	0.199NS	0.602**	0.455**	0.161NS
KT	0.218*	0.811**	0.670**	0.082NS	0.256*	0.472**	0.217*	0.245*	0.413**	0.478**	0.134NS	0.471**
QLE	0.339**	-0.006NS	-0.126NS	-0.317**	0.437**	0.371**	-0.440**	-0.101NS	0.203NS	0.547**	0.149NS	-0.308**
CC	0.319**	0.315**	0.260*	-0.028NS	0.449**	0.532**	-0.051NS	0.275**	0.299**	0.630**	0.372**	0.164NS

GENOTYPIC CORRELATION OF YIELD AND YIELD CONTRIBUTING CHARACTERS OF COCONUT (Contd…)

	NFF	NFFAP	NBPY	NNPY	NNB	SUN_E	SUN_P	FW	FV	NW	SMW	KT	QLE
NFF	1												
NFFAP	0.982**	1											
NBPY	-0.181NS	-0.133NS	1										
NNPY	0.694**	0.686**	0.494**	1									
NNB	0.874**	0.868**	0.162NS	0.928**	1								
SUN_E	-0.519**	-0.423**	0.397**	-0.248*	-0.430**	1							
SUN_P	-0.409**	-0.272**	0.394**	-0.177NS	-0.339**	0.958**	1						
FW	-0.491**	-0.416**	0.390**	-0.341**	-0.495**	0.943**	0.914**	1					
FV	-0.499**	-0.425**	0.378**	-0.347**	-0.501**	0.955**	0.926**	1.000**	1				
NW	-0.485**	-0.429**	0.383**	-0.305**	-0.475**	0.927**	0.915**	0.980**	0.984**	1			
SMW	-0.471**	-0.415**	0.524**	-0.177NS	-0.388**	0.936**	0.911**	0.996**	1.001**	0.995**	1		
KT	-0.384**	-0.334**	0.454**	0.051NS	-0.065NS	0.664**	0.722**	0.747**	0.758**	0.709**	0.782**	1	
QLE	-0.501**	-0.445**	0.037NS	-0.577**	-0.644**	0.870**	0.891**	0.902**	0.903**	0.963**	0.929**	0.527**	1
CC	-0.487**	-0.367**	0.462**	-0.243*	-0.421**	0.976**	1.001**	1.009**	1.015**	0.990**	0.971**	0.778**	0.982**

**Significant at 1% *Significant at 5%

HT- Height of the palm, **GP_20**- Girth of the palm at 20cm height, **GP_1.5**- Girth of the palm at 1.5m height, **IL**- Internode length, **NGL**- Number of green leaves, **RLP**- Rate of leaf production, **PL**- Petiole length, **LL**- Leaf length, **TI**- Total number of inflorescence in the crown at the time of observation, **NUI**- Number of unopened inflorescence, **IUP**- Number of opened inflorescence undergoing pollination, **IPOSS**- Number of inflorescence in which pollination is over and seed setting started, **TIPY**- Total inflorescence per palm per year, **NFF**- Number of female flowers per inflorescence, **NFFAP**- Number of female flowers one month after pollination, **NBPY**- Number of bunches per palm per year, **NNPY**- Number of nuts per palm per year (Yield), **NNB**- Number of nuts per bunches, **SUN_E**- Size of unhusked nut equatorial circumference**, SUN_P**- Size of unhusked nut pole to pole circumference, **FW**- Fruit weight with husk, **FV**- Fruit volume, **NW**- Nut weight without husk, **SMW**- Shell and meat weight without water, **KT**- Kernel thickness at maturity, **QLE**- Quantity of liquid endosperm, **CC**- Copra content

APPENDIX-II

PATH ANALYSIS: DIRECT AND INDIRECT EFFECTS OF DIFFERENT CHARACTERS ON YIELD

	IL	**PL**	**LL**	**TI**	**IUP**	**IPOSS**	**TIPY**	**NFF**	**NFFAP**	**NBPY**	**NNB**	**Genotypic correlation**
IL	**0.1121**	-0.0654	-0.0767	0.0964	-0.0135	-0.1369	-0.0569	0.0199	-0.0529	0.3264	0.1066	0.259
PL	0.0887	**-0.0826**	-0.0812	0.1772	-0.0564	-0.3492	-0.0967	0.1632	-0.1895	0.5395	0.6780	0.791
LL	0.0874	-0.0681	**-0.0984**	0.1634	-0.0406	-0.2389	-0.1040	-0.0039	-0.0714	0.5828	0.1370	0.345
TI	0.0306	-0.0414	-0.0455	**0.3537**	-0.0446	-0.2688	-0.0974	0.0631	-0.1302	0.1662	0.4733	0.459
IUP	0.0236	-0.0729	-0.0625	0.2468	**-0.0639**	-0.4279	-0.1625	0.0994	-0.0989	0.7319	0.7270	0.940
IPOSS	0.0432	-0.0811	-0.0662	0.2675	-0.0769	**-0.3554**	-0.0917	0.1555	-0.2038	0.4886	0.8714	0.951
TIPY	0.0452	-0.0566	-0.0726	0.2441	-0.0736	-0.2310	**-0.1411**	-0.0059	-0.0418	0.5292	0.2249	0.421
NFF	0.0054	-0.0326	0.0009	0.0539	-0.0154	-0.1337	0.0020	**0.4134**	-0.3775	-0.1359	0.9135	0.694
NFFAP	0.0154	-0.0407	-0.0183	0.1199	-0.0165	-0.1885	-0.0153	0.4061	**-0.3842**	-0.0997	0.9074	0.686
NBPY	0.0488	-0.0594	-0.0764	0.0783	-0.0624	-0.2313	-0.0995	-0.0749	0.0510	**0.7505**	0.1688	0.494
NNB	0.0114	-0.0536	-0.0129	0.1602	-0.0445	-0.2964	-0.0304	0.36135	-0.3337	0.1213	**1.0449**	0.928

IL- Internode length, **NGL**- Number of green leaves, **PL**- Petiole length, **LL**- Leaf length, **TI**- Total number of inflorescence in the crown at the time of observation, **IUP**- Number of opened inflorescence undergoing pollination, **IPOSS**- Number of inflorescence in which pollination is over and seed setting started, **TIPY**- Total inflorescence per palm per year, **NFF**- Number of female flowers per inflorescence, **NFFAP**- Number of female flowers one month after pollination, **NBPY**- Number of bunches per palm per year, **NNB**- Number of nuts per bunches.

MORPHO-MOLECULAR CHARACTERISATION AND EVALUATION OF TxD, DxT AND DxD HYBRIDS OF COCONUT CULTIVAR AYIRAMKACHI (*Cocos nucifera* L.)

By

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Abstract of the Thesis

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ABSTRACT

Study on "Morpho-molecular characterisation and evaluation of TxD, DxT and DxD hybrids of coconut cultivar Ayiramkachi (*Cocos nucifera* L.)" was carried out at the Department of Plant Biotechnology, College of Agriculture, Padannakkad during 2018-2020. This investigation aimed at morphological and molecular characterisation of hybrids planted at RARS Pilicode during 1994, in the hybridization programme involving different tall and dwarf genotypes (WCT, Laccadive Ordinary, Philippines, Laccadive Micro, Andaman Ordinary and Malayan Yellow Dwarf) with Ayiramkachi, for important yield attributes and nut quality combined with dwarf stature.

The evaluation based on morphological characters recorded during 2018-2020 revealed that the hybrids LMxAYK, MYDxAYK and LOxAYK were promising ones with high yield (155.50, 115.33 and 116.50 nuts/ palm/year respectively) and superior nut characters such as fruit weight, nut weight, shell and meat weight, kernel thickness and copra content. Hybrid PHIxAYK was superior for all the nut characters combined with a shorter stature but the yield was comparatively less (81.67 nuts/ palm/ year). The hybrid CCxAYK was high yielding (150.67 nuts/ palm/ year) but inferior in nut characters. Hybrid AOxAYK recorded good nut characters but was low yielding (61.50 nuts/palm/year). Hybrids of AYK with WCT recorded a moderate yield (95.00 nuts/ palm/ year) with inferior nut characters, and hybrid AYKxMYD was inferior for both yield (21 nuts/ palm/ year) and nut characters. On analysing the TxD, DxT and DxD groups with their tall (T) and dwarf (D) parents, all the hybrids recorded palm height statistically similar to the dwarf cultivars, which may be an indication of inheritance of shorter stature from the common parent AYK.

Study on genetic parameters revealed that characters such as palm height, internode length, number of green leaves, rate of leaf production, petiole length, leaf length, number of female flowers per inflorescence, number of female flowers one month after pollination and shell and meat weight had shown high heritability (H^2) coupled with high genetic advance as per cent means (GAM%). Hence choice of such characters are best suited for selective breeding. Yield and copra content had shown

medium heritability with high genetic advance. This might be due to the indirect effect of secondary characters contributing to them.

Estimation of heterobeltiosis (superiority over better parent) and standard heterosis (superiority over standard check, Kerasree) for the nine hybrids of Ayiramkachi revealed that the hybrids LMxAYK and MYDxAYK were found to be better performers with respect to yield as well as nut characters, and can be exploited for developing commercially important hybrids suitable for culinary as well as processing purposes (copra and other value added products). The hybrid CCxAYK was high yielding but inferior in nut characters. As one of the parent in this cross, Cochin China, is suitable for tender nut purpose due to high nut water content, the hybrid nuts also may be further evaluated at tender nut stage for its suitability for tender nut purpose.

Thirty four SSR markers were screened for polymorphism among the eight parental cultivars and the check palm (Kerasree). The markers produced an average of 52.94% polymorphism and 1 to 2 alleles/locus. Out of 34 SSR markers screened for polymorphism, 15 markers *viz*., CAC02, CAC11, CNZ40, CNZ44, CnCirA9, CnCirB12, CnCirE2, CnCirC7, CnCirH4, CnCirE12, CnCirC3, CnCirF2, CnCirH7, CnCirG11 and CnCirC5 were able to detect the polymorphism between the parental palms and hence can be utilized in future for fingerprinting the hybrid palms.

സംക്ഷിപ്തം

പടന്നക്കാട് കാർഷിക കോളേജിന്റെ, സസ്യ ജൈവസാങ്കേതിക വിഭാഗത്തിന് കീഴിലായി 2018-2020 കാലയളവിൽ ആയിരംകാച്ചി തെങ്ങിനത്തിന്റെ സങ്കരയിനങ്ങളെക്കുറിച്ച് രൂപശാസ്ത്രപരവും ജനിതകപരവുമായി പഠനം നടത്തുകയുണ്ടായി. 1994 കാലഘട്ടത്തിൽ പിലിക്കോട് പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിൽ നടത്തിയ തെങ്ങിലെ സങ്കരണ പദ്ധതിയുടെ ഭാഗമായി ഉയരം കൂടിയ തെങ്ങിനങ്ങളായ വെസ്റ്റ് കോസ്റ്റ് ടാൾ, ആൻഡമാൻ ഓർഡിനറി, ലക്ഷദ്വീപ് ഓർഡിനറി, ലക്ഷദ്വീപ് മൈക്രോ, ഫിലിപ്പൈൻസ്, കൊച്ചിൻ ചൈന എന്നിവയും കുള്ളൻ തെങ്ങിനങ്ങളായ മലയൻ യെല്ലോ ഡ്വാർഫ് ആയിരംകാച്ചി എന്നിവയും തമ്മിൽ പരാഗണം നടത്തി സങ്കരയിനങ്ങളെ ഉത്പ്പാദിപ്പിച്ചു. ഈ തെങ്ങിനങ്ങളെയാണ് പഠനവിഷയം ആക്കിയത്.

2018-2020തിൽ നടത്തിയ രൂപശാസ്ത്രപരമായ മൂല്യനിർണയത്തിൽ നിന്നും ലക്ഷദ്വീപ് മൈക്രോ x ആയിരംകാച്ചി, മലയൻ യെല്ലോ ഡ്വാർഫ് x ആയിരംകാച്ചി, ലക്ഷദ്വീപ് ഓർഡിനറി x ആയിരംകാച്ചി എന്നീ സങ്കരയിനങ്ങൾ പ്രതിവർഷ നാളികേരത്തിന്റെ എണ്ണത്തിലും (155.50, 115.33, 116.50 എണ്ണം) ഗുണമേന്മയിലും മികവ് പുലർത്തി. ഫിലിപ്പൈൻസ് x ആയിരംകാച്ചി ഇനം നാളികേരത്തിന്റെ ഗുണമേന്മയിൽ മുന്നിട്ടുനിന്നു, എന്നാൽ വിളവ് താരതമ്യേന കുറവായിരുന്നു (പ്രതിവർഷം 81.67 നാളികേരം). കൊച്ചിൻ ചൈന x ആയിരംകാച്ചി എന്ന ഇനം മികച്ച വിളവ് (പ്രതിവർഷം 150.67 നാളികേരം) നൽകിയിരുന്നുവെങ്കിലും നാളികേരത്തിന്റെ ഭാരത്തിലും, കൊപ്രയുടെ അളവും കുറവ് രേഖപ്പെടുത്തി. മറ്റൊരു സങ്കരയിനമായ ആൻഡമാൻ ഓർഡിനറി x ആയിരംകാച്ചി നാളികേര ഗുണമേന്മയിൽ മികവുറ്റതായിരുന്നുവെങ്കിലും വിളവ് കുറവായിരുന്നു (പ്രതിവർഷം 61.50 നാളികേരം). ആയിരംകാച്ചിയും വെസ്റ്റ് കോസ്റ്റ് ടാൾഉം തമ്മിലുള്ള സങ്കരയിനങ്ങൾ ഇടത്തരം വിളവ് നൽകി (പ്രതിവർഷം 95.00

നാളികേരം). ആയിരംകാച്ചി x മലയൻ യെല്ലോ ഡ്വാർഫ് ഇനത്തിന് വിളവിലും (പ്രതിവർഷം 21.00 നാളികേരം) ഗുണമേന്മയിലും മികവ് പുലർത്താൻ കഴിഞ്ഞില്ല.

മേൽപ്പറഞ്ഞ ഒൻപത് സങ്കരയിനങ്ങളെ ടാൾxഡ്വാർഫ്, ഡ്വാർഫ്xടാൾ, ഡ്വാർഫ്xഡ്വാർഫ് എന്നിങ്ങനെ മൂന്നായി തരംതിരിച്ചു പഠനം നടത്തിയപ്പോൾ എല്ലാ ഇനങ്ങളും തെങ്ങിന്റെ ഉയരത്തിൽ കുള്ളൻ തെങ്ങുകളോട് സാമ്യം പുലർത്തി. ഈ സവിശേഷത ആയിരംകാച്ചി എന്ന കുള്ളൻ ഇനത്തിൽ നിന്നും പാരമ്പര്യമായി ലഭിച്ചതാണെന്ന് അനുമാനിക്കാം.

സങ്കരയിനങ്ങളുടെ ഗുണമേന്മയെക്കുറിച്ച് കൂടുതൽ മനസ്സിലാക്കുന്നതിനായി ഹെറ്റെറോസിസ് പഠനം നടത്തുകയുണ്ടായി. കേരശ്രീ എന്ന സങ്കരഇനം തെങ്ങാണ് പഠനത്തിന് അടിസ്ഥാനമായി ഉപയോഗിച്ചത്. ആയിരംകാച്ചിയുടെ സങ്കരഇനങ്ങളായ ലക്ഷദ്വീപ് മൈക്രോ x ആയിരംകാച്ചിയും (ടാൾxഡ്വാർഫ്), ലക്ഷദ്വീപ് ഓർഡിനറി x ആയിരംകാച്ചിയും (ടാൾxഡ്വാർഫ്), മലയൻ യെല്ലോ ഡ്വാർഫ് x ആയിരംകാച്ചിയും (ഡ്വാർഫ്xഡ്വാർഫ്) വിളവിലും നാളികേര ഗുണത്തിലും കേരശ്രീയോട് തുല്യമോ/ മികവുറ്റതോആയ പ്രകടനം കാഴ്ചവെച്ചു. ആയതിനാൽ ഈ ഇനങ്ങളെ വികസിപ്പിച്ചെടുത്താൽ വ്യാവസായികാടിസ്ഥാനത്തിൽ നാളികേരത്തിന് വേണ്ടിയും മറ്റ് മൂല്യവർദ്ധന ഉത്പ്പന്നങ്ങൾ ഉണ്ടാക്കുന്നതിന് വേണ്ടിയും ഉപയോഗിക്കുവാൻ സാധിക്കും. സങ്കരയിനമായ ഫിലിപ്പൈൻസ് x ആയിരംകാച്ചി നാളികേരത്തിന്റെ ഗുണമേന്മയിൽ മറ്റ് ഇനങ്ങളെക്കാളും ഉയർന്ന അളവ് രേഖപ്പെടുത്തി (നാളികേരത്തിന്റെ ഭാരം 1057.400 ഗ്രാം, കൊപ്രയുടെ അളവ് 211.020 ഗ്രാം). കൊച്ചിൻ ചൈന x ആയിരംകാച്ചി എന്ന ഇനം പ്രതിവർഷ നാളികേരത്തിന്റെ എണ്ണത്തിൽ കേരശ്രീ ഇനത്തെക്കാളും അധിക ഉത്പ്പാദനം ഉണ്ടായിരുന്നുവെങ്കിലും നാളികേരത്തിന്റെ ഗുണമേന്മയിൽ കുറഞ്ഞ അളവുകൾ രേഖപ്പെടുത്തി. എന്നാൽ കൊച്ചിൻ ചൈന x ആയിരംകാച്ചി ഇനത്തിന്റെ മാതൃസസ്യമായ കൊച്ചിൻ ചൈന

ഇളനീരിന്റെ ഉപയോഗത്തിന് യോജിച്ചതായതിനാൽ ഈ സങ്കരഇനത്തേയും ഇളനീരിന്റെ ഉപയോഗ്യതക്ക് വേണ്ടി പഠനം നടത്താവുന്നതാണ്.

തെങ്ങിനങ്ങളുടെ ഡി-എൻ-എ അടിസ്ഥാനമാക്കിയുള്ള ജനിതകപരമായ മൂല്യനിർണയത്തിനായി സിമ്പിൾ സീകവൻസ് റിപ്പീറ്റ് (എസ്-എസ്-ആർ) മാർക്കറുകളാണ് ഉപയോഗിച്ചത്. 34 എണ്ണം മാർക്കറുകളിൽ 15 എണ്ണം (CAC02, CAC11, CNZ40, CNZ44, CnCirA9, CnCirB12, CnCirE2, CnCirC7, CnCirH4, CnCirE12, CnCirC3, CnCirF2, CnCirH7, CnCirG11and CnCirC5) മാർക്കറുകൾ ഉപയോഗിച്ചപ്പോൾ മാതൃസസ്യങ്ങൾ തമ്മിലുള്ള ജനിതകപരമായ വ്യത്യാസം കണ്ടെത്താൻ സാധിച്ചു. ആയതിനാൽ ഭാവിയിൽ ഈ മാർക്കറുകൾ സങ്കരയിനങ്ങളിലെ ജനിതക പഠനത്തിനായി ഉപയോഗിക്കാവുന്നതാണ്.