MORPHO-MOLECULAR CHARACTERISATION OF D x D HYBRIDS DEVELOPED USING 'ANNUR', THE DWARF ECOTYPE OF WCT COCONUT (Cocos nucifera L.)

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2021

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by

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE Faculty of Agriculture Kerala Agricultural University



DEPARTMENT OF PLANT BREEDING AND GENETICS COLLEGE OF AGRICULTURE PADANNAKKAD, KASARAGOD 671314 KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled "Morpho-molecular characterisation of D x D hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (Cocos nucifera L.)" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Place: Padannakkad Date: 13/04/2021

CERTIFICATE

Certified that this thesis, entitled "Morpho-molecular characterisation of D x D hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (Cocos nucifera L.)" is a record of research work done independently by Mr. Anuprasad T. E (2018-11-153) 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 him.

1000M

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ACKNOWLEDGEMENT

First and foremost, I bow my head before the **Al mighty God** for making me complete my thesis work.

I am in dearth of words to express my love towards **my beloved Parents and Sister** for their boundless affections, moral support, eternal love, deep concern, prayers and personal sacrifices which gave me enough mental strength and perseverance to get through all odds and tedious circumstances.

It is with immense pleasure I avail this opportunity to express my deep sense of whole hearted gratitude and indebtedness to my major advisor **Dr. Sujatha. R** for her expert advice, inspiring guidance, valuable suggestions, constructive criticisms, constant encouragement, affectionate advice and above all, the extreme patience, understanding and whole hearted cooperation rendered throughout the course of my study. I really consider it my greatest fortune in having her guidance for my research work.

I consider it as my privilege to express my deep- felt gratitude to **Dr. Namboodiri Raji Vasudevan**, Assistant Professor, Department of Plant Breeding and Genetics, CoA, Padannakkad and member of my advisory committee for her constant support, valuable suggestions and critical scrutiny of the manuscript.

I express my gratitude to **Dr. Suresh P. R,** Professor and Associate Dean, CoA, Padannakkad and member of my advisory committee for his immense help and assistance provided for constituting the manuscript.

I sincerely thank **Dr. K.N. Satheesan**, Professor and Associate Director of Research, RARS, Pilicode and member of my advisory committee for his precious suggestions, generous support and constructive criticisms during my entire study.

I express my heartfelt thanks to **Dr. Vaijayanthi P.V**, Assistant Professor, Plant Breediing and Genetics, CoA, Padannakkad for her immense help, precious suggestions and critical scrutiny of the manuscript.

I express my sincere thanks to **Pratheesh P. Gopinath**, Assistant Professor Agricultural Statistics, CoA, Vellayni for his immense support and valuable suggestions for data analysis. I am deeply obliged to the teaching assistants and non- teaching staffs at CoA, Padannakkad for their invaluable help, guidance and critical assessment throughout the period of work. I thank them for all the help and cooperation they has extended to me.

I duly acknowledge the encouragement, moral support, precious suggestions and timely persuasions by my dear seniors, not only in my research work but also throughout my PG programe. I express my sincere thanks to my classmates for their affection and kind help offered during my thesis work. I have infinite pleasure to express whole hearted thanks to my loving juniors for their love, innumerable help and support.

I sincerely thank the authority and laborers at **RARS Pilicode** *especially* **Mr. Ajith** *sir for the timely help, support, and valuable suggestions.*

I owe special thanks to Librarian, CoA, Padannakkad, **Dr. Ajthakumari** and all other staff members of library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.

I express my deep sense of gratitude to Kerala Agricultural University for financial and technical support for persuasion of my study and research work. It would be impossible to list out all those who have helped me in one war or other in the successful completion of this work. I once again express my heartfelt thanks to all those who helped me in completing this venture.

My word of apology to those I have not mentioned in person for the successful completion of this endeavor.

ANUPRASAD T E

TABLE OF CONTENTS

Sl. No.	Title	Page No.
1	INTRODUCTION	1 - 4
2	REVIEW OF LITERATURE	5 - 20
3	MATERIALS AND METHODS	21 - 36
4	RESULTS	37 – 106
5	DISCUSSION	107 - 120
6	SUMMARY	121 - 124
7	REFERENCES	i - xiv
8	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Plant materials of Annur (<i>interse</i>) and its hybrids in field of RARS, Pilicode	22
2	Details of coconut specific SSR markers used for molecular characterisation of parental and check palms	22
3	Mean value for vegetative characters of Annur <i>interse</i> and hybrids in comparison with parental cultivars and checks in coconut	39
4	Mean value for reproductive and yield characters of Annur <i>interse</i> and hybrids in comparison with parental cultivars and checks in coconut	42
5	Mean value for nut characters of Annur <i>interse</i> and hybrids in comparison with parental cultivars and checks in coconut	44
6	Palm to palm variations within Annur (<i>interse</i>) ecotype of WCT coconut	53
7	Palm to palm variations within Annur x GB hybrids	56
8	Palm to palm variations within Annur x MYD hybrids	57
9	Genotypic correlation of yield and yield contributing characters of coconut	82
10	Direct and indirect effects of different characters on yield	87
11	Genetic parameters of morphological characters under study	91
12	Relative heterosis, economic heterosis and heterobeltiosis of hybrid Annur x MYD	94
13	Relative heterosis, economic heterosis and heterobeltiosis of hybrid Annur x GB	95
14	Details on qualitative characters of hybrids, check and parents	96
15	Details of reproductive characters related to pollinating behaviour	97
16	Quantity and quality of isolated genomic DNA	98
17	Screening of 34 SSR markers	101
18	Amplification details of 34 SSR primers	103
19	Grouping of accessions based on SSR data using DARwin ver 6.0 software	106
20	Dice dissimilarity matrix	106

LIST OF FIGURES

Fig.	Title	Pages
No.		Between
1	Layout of experimental field (N8 block) RARS, Pilicode	22&23
2	Mean performance of height of palm of Annur <i>interse</i> and hybrids in comparison with other cultivars	38&39
3	Mean performance of girth of palm of Annur <i>interse</i> and hybrids in comparison with other cultivars	38&39
4	Mean performance of petiole length of Annur <i>interse</i> and hybrids in comparison with other cultivars	38&39
5	Mean performance of leaf length of Annur <i>interse</i> and hybrids in comparison with other cultivars	38&39
6	Mean performance of internode length of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
7	Mean performance of number of green leaves of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
8	Mean performance of rate of leaf production of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
9	Mean performance of total number of inflorescence at the time of observation of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
10	Mean performance of no. of opened inflorescence undergoing pollination of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
11	Mean performance of no. of inflorescence in which pollination is over and seed setting started of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
12	Mean performance of no. of female flowers per inflorescence of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
13	Mean performance of no. of female flowers one month after pollination of Annur <i>interse</i> and hybrids in comparison with other cultivars	40&41
14	Mean performance of no. of nuts per palm per year of Annur <i>interse</i> and hybrids in comparison with other cultivars	42&43
15	Mean performance of no. of nuts per bunch of Annur <i>interse</i> and hybrids in comparison with other cultivars	42&43
16	Mean performance of size of unhusked nut of Annur <i>interse</i> and hybrids in comparison with other cultivars	42&43
17	Mean performance of fruit weight (with husk) of Annur <i>interse</i> and hybrids in comparison with other cultivars	42&43

18	Mean performance of nut weight (without husk) of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
19	Mean performance of volume of fruit of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
20	Mean performance of shell and meat weight (without water) of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
21	Mean performance of kernel thickness of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
22	Mean performance of quantity of liquid endosperm of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
23	Mean performance of quantity of copra content of Annur <i>interse</i> and hybrids in comparison with other cultivars	44&45
24	GCV and PCV of 28 characters in coconut cultivars	90&91
25	Heritability and Genetic advance of 28 characters in coconut cultivars	90&91
26	Dendrogram of SSR markers using parental and check coconut palms	104&105

LIST OF PLATES

Plate. No.	Title	Pages
		Between
1	Annur (<i>interse</i>) palms	38&39
2	Annur x MYD hybrid palms	38&39
3	Annur x GB hybrid palms	38&39
4	Comparison of nuts of parents, hybrids and check	98&99
5	Eriophyid mite attack	98&99
6	Rhinoceros beetle attack	98&99
7	Gel profile of primers with two distinct	100&101
	polymorphic amplicons	
8	Gel profile of primers having single polymorphic amplicon	100&101
9	Gel profile of primers with two amplicons - one polymorphic and one monomorphic	100&101
10	Gel profile of primers with monomorphic amplicon	100&101

LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	Analysis of Variance
Avg	Average
via	by means of
cm	Centi metre
СТАВ	Cetyl Trimethyl Ammonium Bromide
CGD	Chavakkad Green Dwarf
et al.	Co- workers/ Co-authors
CD	Critical difference
°Bx	degree brix value
°C	Degree celcius
D x D	Dwarf x Dwarf
EDTA	Ethylene Diamine Tetraacetic Acid
Fig.	Figure
GB	Gangabondam
GCV	Genotypic Coefficient of Variation
g	Gram
KG	Keraganga
KAU	Kerala Agricultural University
KS	Kerasree
MYD	Malayan Yellow Dwarf
m	Metre
μg	Micro gram
μl	Micro litre
μΜ	Micro molar
ml	Milli litre
mm	Milli metre

mM	Milli molar
viz.	Namely
nm	Nano metre
No.of	Number of
OD	Optical density
%	Percent
PCV	Phenotypic Coefficient of Variation
pM	Pico molar
PVP	Polyvinyl pyrrolidone
pH	Potential of hydrogen
Tm	Primer melting temperature
RARS	Regional Agricultural Research Station
rpm	Revolution per minute
SSR	Simple sequence repeats
SDS	Sodium dodecyl sulphate
Sd	Standard deviation
i.e.	that is
UV	Ultra violet
WCT	West Coast Tall

INTRODUCTION

1. INTRODUCTION

Coconut is one of the most widely cultivated perennial oil crops in the world and plays an important role in the socio-economic life of more than 80 tropical countries. It provides farmers with a potential source of employment and income. All parts of the coconut tree are useful including coconut leaf, stem, and nut. It is also known as "Kalpavriksha", "Tree of heaven", "King of palm" and "Tree of life".

Coconut (*Cocos nucifera* L.) is a diploid (2n=2x=32) monocotyledonous plant belonging to the family Arecaceae (Palmae). *Cocos* is a monotypic genus which indicates that no close botanical relatives are available for this palm. However, the species exhibit high variability and the cultivars are broadly classified into tall (variety *typica*) and dwarf (variety *nana*). Tall coconut palms are usually outcrossing in nature and grow up to a height of 18 m or above. It generally starts flowering seven to eight years after planting. Tall coconut palms are high yielding and produce good quality nuts. Generally, dwarf palms show self-pollinating behavior due to the concordance of phases (male and female phases). Three to four years after planting it starts blooming. It grows up to a height of six to seven meters.

Short statured palms are nowadays receiving more attention due to various socio-economic factors. However, the poor quality of nuts for various processing aspects and high susceptibility of dwarf palms to pest and diseases makes them unsuitable for large scale cultivation. The solution to this problem is the development of suitable hybrids by combining the desirable features of tall and dwarf palms.

In 1928, the first hybridization programme was started in Fiji. However, due to the economic crisis, the programme was discontinued. Later in 1932 at Coconut Research Station, Nileswaram a hybridization programme was initiated. It included three intravarietal crosses and one intervarietal cross. The cross between tall and dwarf palm *viz*. WCT x CGD showed hybrid vigor (Patel, 1938) and was the milestone in the history of the coconut improvement programme. After that, all over the world, different hybrids *viz*. Tall x Dwarf (T x D), Dwarf x Tall (D x T), Tall x Tall (T x T) and Dwarf x Dwarf (D x D)

were generated. In Kerala, Regional Agricultural Research Station (RARS) Pilicode, Kasaragod and Central Plantation Crop Research Institute (CPCRI), Kasaragod have released different hybrids.

West Coast Tall (WCT) is the most widely cultivated variety in Kerala and several ecotypes of WCT were reported. Ecotypes are variants within a cultivar that are grown for a long period to be superior to other local cultivars by the farmers. In a study initiated in 2005 at RARS Pilicode, an ecotype of WCT was detected in Annur, a place in Kannur district which showed dwarfing nature with nut qualities similar to WCT. The Annur ecotype was crossed in 2007-08 with Malayan Yellow Dwarf (MYD) and Gangabondam (GB). The hybrid seed nuts along with *interse* seed nuts of Annur were planted at RARS Pilicode in 2009 and first flowering was recorded in 2014.

The present study entitled "Morpho-molecular characterization of D x D hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (*Cocos nucifera* L.)" aims at evaluation of plant stature, yield, nut quality, and other important morphological traits of Annur and the hybrids developed from it. With the development of molecular markers, the characterisation of genotypes at genetic level became a standard procedure. Among the various types of molecular markers available, SSR (Simple Sequence Repeat) markers are PCR-based, codominant markers with highly reproducible nature. Hence in the present investigation, the molecular characterization using SSR markers was also carried out to supplement the morphological data. The study is expected to estimate the inheritance of dwarfing nature and nut qualities in the hybrids and identification of hybrid with heterotic vigor.

The research programme was undertaken with the following objectives

 Morphological characterisation of coconut hybrids produced by crossing the dwarf coconut ecotype 'Annur' with Gangabondam (GB) and Malayan Yellow Dwarf (MYD). 2. Screening of reported SSR markers for polymorphism between the three parental genotypes and one check cultivar and selection of polymorphic primers for future characterisation of hybrids.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Coconut (*Cocos nucifera* L.) is a monotypic genus belonging to the family Arecaceae. Coconut is generally cultivated as an oil crop and the cultivation of coconut as a plantation crop commenced in 1840.

Although coconut was cultivated in Srilanka by about 300BC, Martius (1850) suggested that the centre of origin of coconut was west coast of Central America. Hill (1929) discovered the fossils of *Cocos* from New Zealand. Later, the *Cocos* fossils were identified in Rajasthan by Kaul (1951). Purseglove (1972) and Dahlgren *et al.* (1985) suggested that the center of origin of *Cocos* was Central or South America.

2.1 VARIETAL CLASSIFICATION

Narayana and John (1949) made the first systematic classification of coconut palms. They classified whole coconut palms into two broad groups *viz*. Tall and Dwarf. Tall coconut groups comprising of three types *viz*. typica, spicata, and androgena. The dwarf group include two types, nana, and javanica. The nana varieties were delicate in nature and bears after three years of planting whereas the javanica varieties were vigorous in nature and bears after four years of planting.

Liyanage (1958) classified the coconut palms in Ceylon into three categories *viz*. variety typica, variety nana, variety aurantiaca. Spicata and androgena varieties from earlier system of classification was omitted and included the new variety aurantiaca. The variety typica is tall with late flowering and outcrossing characters. The dwarf, inbreeding coconut, variety nana shows early flowering characters. The semi-tall variety aurantiaca shows inbreeding and late-flowering characters.

Fremond *et al.* (1966) made the third system of classification on the basis of pollination characteristics. The dwarf palms showed self-pollinating behavior and the tall palms showed the cross-pollinating behavior. However, another report mention that in plantation with the dwarf palms surrounded by tall palm showed opposite pollinating characteristics as dwarf being allogamous and tall autogamous (Rognon, 1976).

2.1.1 Tall

They are slow maturing and flower 6-10 years after planting. They are long-lived with an economic life of about 60-70 years, although much older palms are known to exist and yield well. They are normally cross-pollinating and therefore considered to be heterozygous. They generally grow to a height of 15 m to 18 m or above. The nut size varies from very big to very small. The leaves and bunches are compactly attached than dwarf palms. The main characteristic of the tall palm is the absence of overlap between the male and female phases. West Coast Tall, East Coast Tall, Laccadive Ordinary, and Andaman Ordinary are the predominant tall coconut palms grown in India.

2.1.1.1. West coast tall (WCT)

West Coast Tall is a tall coconut variety that grows mainly in the coastal regions of Kerala and Karnataka. It yields economically for about 75 years. A mature palm produces 36 functional leaves and 12 to 13 inflorescences per year. The crown is spherical or semi-spherical in shape. Under favorable conditions, WCT palm comes to bear in about six to seven years. It yields on an average of 80 nuts per palm per year. Copra content and oil content in WCT is 176 g per nut and 68 per cent respectively (Ratnambal, 2001).

2.1.2 Dwarf

The dwarf palms generally grow up to a height of seven meters. They show selfpollinated behavior due to the overlapping of male and female phases. They are early bearers *ie.*, initial flowering occurs three to four years after planting. Nut size varied from small to medium range. They have generally thin cylindrical trunk without a swollen base or bole. Chowghat Green Dwarf (CGD), Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Malayan Green Dwarf (MGD), Malayan Orange Dwarf (MOD), Gangabondam Green Dwarf (GBGD) are the predominantly cultivated dwarf coconut palms in India. Menon and Pandalai (1958) suggested that the dwarf palms originated through mutation from tall palms whereas Swaminathan and Nambiar (1961) believed that the inbreeding of tall palms lead to the origin of dwarf palm.

2.1.2.1. Gangabondam Green Dwarf (GBGD)

Gangabondam green dwarf also called Gangabondam (GB) is a dwarf coconut palm which is mainly cultivated for tender coconut water. The stem is short and narrow in nature. The papaya shaped nuts are the characteristic feature of GBGD. The GBGD palms start bearing about three to four years after planting. The average yield of the palm is 67 nuts per palm per year. The copra content of the palm is 153 g per nut and the oil content is 67 per cent. In hybrid production, GBGD is being used as a male parent. Lakshaganga, Anandaganga, and Keraganga are examples of the hybrids produced using GBGD as a male parent (Ratnambal, 2001).

2.1.2.2. Malayan Yellow Dwarf (MYD)

Malayan Yellow Dwarf is an introduced dwarf coconut palm which is mainly used for hybrid production. It starts to bear about four years after planting. The average yield of the palm is 66 nuts per palm per year. The copra content and oil content are 140 g per nut and 66 per cent respectively. MYD is more homogeneous compared to other dwarf palms (Ratnambal, 2001).

2.1.2.3. Annur

Annur coconuts are the dwarf ecotypes of WCT coconut. It was found that palms are having dwarfing nature but nut qualities similar to WCT. Rajesh *et al.* (2014) compared two ecotypes *viz.* Bedakam and Annur with WCT using SSR markers and found that the Annur ecotypes were closer to WCT.

2.1.3 Hybrids

Hybrid palms are the result of intervarietal and intravarietal cross between two different palm varieties. There are mainly four types of coconut hybrids *viz*. intervarietal

[Dwarf x Tall (D x T) and Tall x Dwarf (T xD)] and intravarietal [Tall x Tall (T x T), and Dwarf x Dwarf (D x D)]. Kerasree, and Keraganga are some of the popular hybrids released by KAU. Chandra Sankara, Chandra Laksha and Kear Sankara are some of the popular hybrids released by CPCRI.

2.1.3.1. Keraganga

Keraganga is a Tall x Dwarf hybrid released in 1988 by KAU. The female parent of the hybrid is West Coast Tall whereas the male parent is Gangabondam. The hybrid starts bearing in about four to five years after planting. The average annual yield of the palm is 100 nuts per palm per year. The copra content and oil content of the palm is 201 g per nut and 69 per cent respectively (Ratnambal, 2001).

2.1.3.2. Kerasree

Kerasree is a Tall x Dwarf hybrid released in 1992 by KAU. The female parent and the male parent of the hybrids are West Coast Tall and Malayan Yellow Dwarf respectively. Medium-sized green nuts are the characteristics of the palm. It starts flowering after five years of planting. The average yield of the palm is 130 nuts per palm per year. The copra content and oil content of the palm is 216 g per nut and 66 per cent respectively (Ratnambal, 2001).

2.2 ECOTYPES OF COCONUT

Ecotypes are morphologically similar individuals or groups of individuals grown in similar environmental conditions (Ohler, 1999). Thampan (1999) mentioned coconut ecotypes such as Komadan, Jappanam, and Kuttiyadi Tall.

Komadan is an ecotype of WCT. Gopimony (1984) analyzed nine quantitative characters of Komadan. He found that WCT was inferior to Komadan for the quantitative characters.

Parthasarathi *et al.* (2005) analyzed the molecular diversity present in two ecotypes *viz.* WCT and Assam Tall. They found that the ecotypes differed only in climatic

adaptations. They also reported that Assam Tall and WCT were genetically similar when tested with DNA markers.

Samsudeen *et al.* (2013) compared two ecotypes of WCT *viz.* Bedakkam and Kuttiyadi ecotypes. They compared 20 palm characters including 14 fruit characters of these two ecotypes and found superior performance of Kuttiyadi ecotypes compared to Bedakkam ecotypes. Similarly, Rajesh *et al.* (2014) conducted a comparative study between WCT and its ecotypes Annur and Bedakkam. Seventeen SSR primers were used for the study and got 100% polymorphism. From the cluster analysis, they revealed that Annur ecotype was closer than Bedakkam ecotype with WCT coconut.

Manjula *et al.* (2014) studied the similarities and variations present in the West Coast Tall coconut and Kuttiyadi ecotypes. However, the similarity coefficient of these palms ranged from 0.20 to 0.97 with the West Coast Tall coconut.

2.3 MORPHOLOGICAL CHARACTERS

2.3.1 Vegetative characters

The number of functional leaves in tall and dwarf palms were analysed by Bhaskaran and Leela (1963). They observed a higher number of functional leaves in dwarf palms (24) compared to tall palms (20).

Liyanage (1967) confirmed a positive significant correlation between number of green leaves and copra yield. Similarly, Namboothiri *et al.* (2007) reported a positive significant correlation between total number of green leaves produced in the palm and nut yield. They also confirmed a negative significant correlation between the petiole length and nut yield. Natarajan *et al.* (2010) reported that petiole length, leaf length, and number of functional leaves had a positive significant correlation with nut yield. They also observed negative significant correlation between number of functional leaves had a positive significant correlation with nut yield.

Satyabalan and Mathew (1984) confirmed a positive significant correlation between the number of leaves produced during ten months after planting and five to nine months after planting. They also found a positive significant correlation between collar girth during the fifth month and the tenth month. From this they suggested that seedling selection can be done from fifth month onwards.

Manjula *et al.* (2014) conducted a comparative study between West Coast Tall and Kuttiyadi ecotypes. They selected palms of age between 40 to 50 years. They analyzed a total of 38 characters among 200 palms including nine vegetative characters, eight reproductive characters, and 21 fruit characters. Significant variation was observed for characters *viz.* height of the plant, girth of the palm, petiole length and number of opened leaves between West Coast Tall and Kuttiyadi ecotypes.

Oyoo *et al.* (2015) conducted studies for the morphological characterization of coconut palms grown in the coastal area of Kenya. They collected data from tall, dwarf, and hybrid palms of the region. Principal Component Analysis (PCA) was done. A 65.54 percent variation occurred for green leaves present in principal component 1 (PC1). Principal Component 2 (PC2) accounted for 19.71 percent variation. The stem height was associated with total variation in PC2.

An intra varietal variability study was conducted by Sathishkumar (2016) in Komadan coconut palms. He found that number of leaves at a one-year age showed maximum coefficient of variation and high variability and he also recorded positive correlation between number of nuts per palm per year and number of green leaves, number of inflorescence per palm per year and number of female flowers per inflorescence.

2.3.2 Reproductive characters

Palm to palm variation in the number of female flowers in coconut was observed by Patel (1938). He also suggested that the age of bearing, environmental conditions, manuring *etc.* were influencing the female flower production. Menon and Pandalai (1958) reported that the tall palms produced less number of female flowers than dwarf palms. According to Bai and Ramadasan (1982), the highest number of female flower production was from March to April. Similarly, Vanaja and Amma (2002) observed varying numbers of female flowers with the season. They also pointed out that season is not influencing the average nut production per bunch.

Liyanage (1949) confirmed that the first male flower blooms one week after the spathe opening in dwarf coconut palms. Whereas in tall palms, the beginning of the blooms of male flowers starts immediately after the spathe opening.

Fremond and Brunin (1966) found a positive association between early growth and earliness in flowering. They also suggested a method to detect early bearing palms by analyzing the number of leaves in the first two years. Nandi and Sugata (2000) analyzed the Komadan coconut inflorescence and found an early flowering pattern.

Ninan and Satyabalan (1964) reported an average setting per cent of 35.6 per cent and copra yield of 157.6 g per nut for West Coast Tall. Nambiar and Nambiar (1970) reported a higher heritability value for per cent flower set in WCT coconut.

Pandin (2009) analyzed the inbreeding depression of selfed Mapanget tall coconut no. 32 (DMT-32) among the four generations. He analyzed the inbreeding depression based on the vegetative and reproductive characters. In fourth-generation, reproductive characters showed 29.02 % to 85.92 % inbreeding depression. He also noted an increasing inbreeding depression with generations.

2.3.3 Yield and nut characters

Child and Nathanel (1950) conducted studies on liquid endosperm and found one per cent of sugar in initial stages. It increased up to five per cent and then decreased to two per cent when the nut reaches maturity. Nathanael (1966) reported drastic reduction of moisture content from 94% in the tender nut to 43% in a ripe nut. While, oil content increases with an increase in maturity and dry matter production increases from 0.34 g to 184 g.

Satyabalan *et al.* (1970) conducted studies on T x D hybrids of WCT coconut. They found that all the hybrids showed heterosis in characters such as husked nut weight, kernel weight, and weight of liquid endosperm.

Mantriratne (1972) analyzed the dwarf palms and compared the performance with tall palms in Ceylon. He recorded the yield of dwarf yellow, dwarf green, and dwarf red palms and found a lower yield compared to tall varieties.

Raveendra *et al.* (1987) studied nut characters of different genotypes of coconut including Gangabondam and found lower value of nut characters compared to other tall coconut palms.

Ratnambal and Nair (1994) reported several nut characters among different cultivars of coconuts including Kerasree, Keraganga, and West Coast Tall. They found higher copra yield per nut and oil content in hybrids *viz*. Kerasree (201 g per nut, 69 percent) and Keraganga (201 g per nut, 69 percent) compared to WCT (176 g per nut, 68 percent).

Zizumbo-Villarreal and Pinero (1998) analysed the morphological variation and diversity using 17 fruit characters among the 41 coconut populations. From the dendrogram studies, they classified the 41 population into four morphotypes *viz*. Atlantic tall, Pacific tall 2, and Malayan Dwarf morphotypes.

Nampoothiri *et al.* (1999) conducted studies on nine coconut genotypes including West Coast Tall. Among these genotypes, diallel crosses were made. They also studied the number of nuts per annum of genotypes and hybrids. The average number of nuts per annum for hybrid WCT x LO was higher compared to WCT whereas the hybrid between WCT and other seven genotypes showed less number of nuts per annum compared to WCT.

Shinde *et al.* (2018) compared the yield performance of twelve hybrids with three varieties. The highest yield (127.6 nuts per palm) was recorded for the hybrid between Gangabondam and East Coast Tall (GB x ECT).

Suchitra and Paramaguru (2018) analyzed the vegetative, reproductive, yield, and nut characters among 14 genotypes including 11 tall and three dwarf palms. They found a positive significant correlation between nut yield per palm with other characters *viz*.

number of female flowers per year, number of nuts per bunch, kernel thickness, copra content, and husk weight.

Pieries (1934) found a positive significant correlation between the number of female flowers and the number of nuts per bunch. Studies conducted by Liyanage and Sakai (1960) showed a positive significant correlation between copra yield and nut yield whereas a negative correlation was found between copra yield and flowering period. They also reported higher heritability values for copra yield and weight of husked nut. Lower heritability values were obtained for the flowering period.

Fernando (1996) studied genetic control of husked nut weight using full diallel cross of coconuts in Srilanka and reported a heritability of 0.45 for this character, and suggested improvement in this trait through mass selection is limited.

Selvaraju (2008) conducted studies on six coconut cultivars including WCT. They found that number of nuts, the weight of husked and unhusked nuts had higher heritability and genetic advance.

Jayabose *et al.* (2008) conducted studies on ten parents and their 16 hybrids and analysed the heterosis pattern present in economically important traits such as yield, copra content, oil content, and kernel weight. The heterosis was found to be varying among the economically important characters.

2.4 PEST OF COCONUT

Murphy and Briscoe (1999) reported that Red palm weevil was controlled using natural enemies such as endoparasitic nematode *Praecocilenchus ferruginophorus*, *Pseudomonas aeruginosa*, and Cytoplasmic polyhedrosis virus. Kiruba *et al.* (2006) conducted studies on pests of coconut and their management using traditional practices. The major pests reported were Rhinoceros beetle (*Oryctes rhinoceros*), Red palm weevil (*Rhynchophorus ferrugineus*), and Coconut leaf eating caterpillar (*Opisina arenosella*). Fly ash along with lime prevents pests like the Rhinoceros beetle and Red palm weevil. *Coleus amboinicus* prevents the egg laying of Rhinoceros beetle in nurseries. *Coleus aromaticus* also have similar properties. They also pointed out that sand traps are effective against the Rhinoceros beetle.

Nair (2000) reported that factors such as size, shape and colour of nut and perianth characters influence degree of eriophyid mite attack in coconut. Heavy mite infestation was found on green oblong WCT nuts than round reddish brown coloured nut. Under severe infestation button shedding and nut malformation leads to economic losses such as reduction of copra and malformed fibers (Nair *et al.*, 2005).

Levin and Mammooty (2003) reported minimum mite damage in Strait Settlement (8.30 per cent) followed by Cochin China (9.90 per cent) among exotic cultivars, and Lakshaganga (19.40 per cent) among hybrids, and Laccadive Micro (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.

2.5 DNA ISOLATION

Couch and Fritz (1990) prepared a DNA isolation protocol for plants with high polyphenolics content. The formation of oxidized polyphenolic compounds were prevented and separation of nuclei and cytoplasmic components was possible through this method.

Aitchitt *et al.* (1993) isolated high-quality DNA samples from date palms (*Phoenix dactylifera* L.) and coconut leaves. They used Cetyl Trimethyl Ammonium Bromide (CTAB) based buffer. They isolated 800 µg of DNA from one gram of leaf sample. The procedure was the modified combination of Murray and Thompson (1980), Saghai-Maroof *et al.* (1984) and Webb and Knapp (1990). They increased the concentration of CTAB, Chloroform-isoamyl alcohol was used for single extraction and used sodium acetate and ethanol for the additional precipitation of DNA.

Al-Shyji *et al.* (1994) isolated total genomic DNA using a different protocol in date palm. They prepared a modified protocol and found efficient than other protocols of Dellaporta *et al.* (1983), Murray and Thompson (1980), and Callahan and Mehta (1991).

Proteinase K and RNase were used for homogenization and PVP and potassium meta bisulphate in the extraction buffer. They also added potassium acetate and Sodium Dodecyl Sulphate (SDS) in the homogenate. The modified protocol was reported to be efficient in yielding a higher amount and good quality DNA.

Everard *et al.* (1996) isolated DNA from young leaves of tall and dwarf palms and their F2 progenies. They made modifications to the protocol developed by Dellaporta *et al.* (1983). Instead of using 15 ml of extraction buffer, 2.5% PVP was also included in the extraction buffer.

Perera *et al.* (1998) analyzed the genetic relationship among the indigenous coconut accessions from Srilanka. The fresh young coconut leaves were used for DNA isolation. A modified version of the miniprep protocol by Dellaporta *et al.* (1983) was used. DNA was purified by using a chloroform-isoamyl alcohol mixture.

Upadhyay *et al.* (1999) proposed a new protocol for yielding good quality genomic DNA from young coconut leaves. They used SDS and CTAB detergents at different pH and concentrations. SDS gave good quality DNA using 1 percent conc. and pH of 8.

Meerow *et al.* (2003) analyzed the population structure and genetic diversity of coconut germplasm in Florida. They isolated DNA from fully expanded leaves. Either 200 mg of a fresh sample or 30 mg of silica gel dried sample was used. They extracted the DNA using the Fast DNA Kit and quantified the DNA.

Ramirez *et al.* (2004) isolated genomic DNA of coconut using CTAB protocol developed by Doyle and Doyle (1990).

Angeles *et al.* (2005) extracted DNA using different parts of a coconut palm. They got poor quality DNA samples using solid endosperm while using young leaves of the first emergence, they were able to isolate good quality DNA. They improved the protocol of Dellaporta *et al.* (1983). The addition of polyvinylpolypyrrolidone and the use of higher concentration salt in the extraction buffer (2 M concentration instead of 0.5 M) were the modifications in the protocol.

Rajesh *et al.* (2013) reported a rapid isolation procedure of DNA from coconut spindle leaves. Liquid nitrogen was used for grinding one gram of leaf sample. One ml of 10 percent SDS, 50 μ l of β - mercaptoethanol, and RNase were used in the protocol.

Aina *et al.* (2015) isolated the DNA samples from coconut leaf and estimated the DNA level in the leaf sample. They used 30 μ g of leave sample. Lyase buffer and morning fresh detergents were used for chemical homogenisation. They got good quality DNA samples.

2.6 SSR MARKERS IN COCONUT

A study was conducted by Perera *et al.* (2000) in 130 coconut palms to assess the genetic diversity using eight SSR markers. Among 130 coconut palms, 75 were tall palms and 55 were dwarf in nature. They detected 50 alleles in tall palms whereas dwarf palms possessed only 26 alleles. They also detected a higher average diversity value in tall palms compared to the dwarf palms.

Teulat *et al.* (2000) analysed the genetic diversity among 14 coconut populations using 37 SSR primers. They detected a total of 339 alleles in 31 individuals. They found genetic diversity between 0.47 and 0.90.

A study was conducted in fifteen selfed and reciprocal crossed progenies of Laccadive tall and Gangobondam dwarf using ten SSR markers (Manimekalai *et al.*, 2005). They found a total of 42 alleles. The number of alleles per locus ranged from two to seven with a mean of 4.2 alleles per primer locus. Jaccard's similarity coefficient varied from 0.136 to 1.000 with an average of 0.590.

Manimekalai *et al.* (2006) compared the effectiveness of three markers *viz.* Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), and Random Amplified Polymorphic DNA (RAPD) to identify the polymorphism among the coconut accessions. They found 100 per cent polymorphism, Polymorphic information content (PIC) of 0.78, and a wide range of similarity for SSR markers. These three values were higher for SSR

markers compared to the other two markers. They found that SSR markers are more efficient among three markers used for the study.

Rajesh *et al.* (2008a) analyzed the genetic diversity of coconut palms in the Andaman and Nicobar Islands. They examined the 26 accessions using 14 SSR markers. The average expected and observed heterozygosity was 0.66 and 0.29 respectively. They identified a total of 103 alleles. The average alleles per locus obtained were 7.35.

Rajesh *et al.* (2008b) conducted studies on ten coconut landraces using simple sequence repeat (SSR). They used 14 SSR markers and identified a total of 90 alleles. The polymorphic information content (PIC) was found between 0.41 and 0.89 with an average of 0.61. A mean of 6.42 alleles per locus was detected. Dwarf palms showed less heterozygosity whereas the tall palms show higher heterozygosity.

Dasanayaka *et al.* (2009) conducted studies on 43 coconut accessions to assess the genetic relationships using sixteen SSR markers. Compared to the dwarf palms, tall palms showed higher genetic diversity and Polymorphic Information Content.

Kumar *et al.* (2011) characterized 14 accessions of coconut using eight SSR primers. They detected 24 polymorphic alleles. An average similarity index of 0.4231 was identified. Using the dendrogram, the accessions were grouped into three clusters.

Liu *et al.* (2011) conducted studies in ten coconut accessions in china using 21 SSR markers to assess the genetic diversity among the cultivars. A total of 180 alleles were detected and found an average polymorphism information content of 0.575.

Rajesh *et al.* (2012) detected that 17 SSR markers from a set of 50 SSR markers, were able to distinguish between the parents used in the D x T hybrids. By the use of these markers, the seedling purity of D x T hybrids were assessed.

Rajesh *et al.* (2014) conducted studies using parental West Coast Tall coconuts and their progenies. They used 15 SSR markers and identified a total of 41 alleles. An average

of 2.7 alleles per locus was found. They confirmed that the percentage of similarities were varied from 55 to 74 percent.

A total of 309 SSR primer sets were detected by Xia *et al.* (2014) and characterized 191 polymorphic SSR markers. They detected 615 alleles with an average of 3.22 alleles per locus. They also detected average heterozygosity of 0.385.

Perera *et al.* (2016) revealed that single codominant genes were responsible for the presence of bole in dwarf palms using SSR markers. The inheritance of heights was also depending on the single codominant gene. They also revealed that inheritance of height was not strongly associated with the presence of bole.

Rasam *et al.* (2016) conducted studies in five coconut varieties including Gangabondam Green Dwarf. They characterized the varieties using 14 Simple Sequence Repeat (SSR) and 18 Inter Simple Sequence Repeat (ISSR) markers. They found ISSR markers were inferior to SSR markers.

2.7 SSR MARKERS IN OTHER PALMS

Elshibi *et al.* (2008) conducted studies on 60 accessions of date palm (*Phoenix dactylifera*) from Sudan to assess genetic diversity using SSR primers. They detected 343 alleles using 16 SSR primers. Sudan cultivars showed a higher level of heterozygosity.

Pintaud *et al.* (2010) conducted studies on 308 accessions of *Phoenix* including 12 species using SSR markers. They revealed that wild populations of *Phoenix dactylifera* were the ancestors of modern date palms. There were only secondary genetic contributions from other species.

Elmeer *et al.* (2011) analyzed the genetic diversity of date palms including 11 genotypes using 30 SSR primers. Among 30 SSR primers, only ten were polymorphic in nature. They detected 77 alleles and found an average of 7.7 alleles per locus. They found an average gene diversity of 0.80. The highest gene diversity of 0.90 was observed using DP157 and DP175 markers.

Khierallah *et al.* (2011) assessed the genetic diversity of date palms present in Iraq using 22 SSR primers. They analyzed 30 cultivars including 24 females and six male cultivars. They detected a total of 188 alleles and average heterozygosity of 0.503. Principal coordinate analysis and dendrogram exhibited similar clusters of cultivars.

Arabnezhad *et al.* (2012) conducted studies on 16 date palm cultivars using 22 polymorphic SSR markers. They found a total of 106 alleles. The heterozygosity and PIC were 0.719 and 0.668 respectively. The African cultivars were clearly distinguished from the Iranian and Iraqi cultivars using cluster analysis.

Bodian *et al.* (2012) observed genetic diversity in 11 cultivars of date palm (*Phoenix dactylifera*) from Morocco using SSR markers. A total of 107 alleles and an average percentage polymorphism of 96.11% were detected.

Zaki *et al.* (2012) studied *Elaeis oleifera* and characterized 20 SSR markers from the genomic library (gSSR). They found that average Polymorphism Information Content (PIC) was 0.402. Compared with the *Elaeis guineensis, Elaeis oleifera* shows a lower value of heterozygosity. The genetic diversity in *Elaeis* genus is potentially revealed by the use of *Elaeis oleifera* gSSR markers.

Racchi *et al.* (2014) characterized 18 Libyan date palms using 16 polymorphic SSR markers. They detected a total of 110 alleles. They analyzed a higher level of polymorphism among the cultivars.

Taeprayoon *et al.* (2015) conducted studies on 121 oil palms (*Elaeis guineensis*) belongs to three populations using 96 SSR markers. Among these markers, they found 20 SSR markers were polymorphic in nature. 109 alleles were detected and polymorphic information content ranges from 0.45 to 0.87. Variation among the population was 33% and the variation of individuals within the populations was 67%.

Zhou *et al.* (2015) analyzed two populations of Oil palms (*Elaeis guineensis*) using SSR markers. Two population includes the germplasm collections from china and introduced collections from Malaysia. They observed average heterozygosity of 0.3859.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study on 'Morpho-molecular characterization of DxD hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (*Cocos nucifera*)' was undertaken at the Department of Plant Breeding and Genetics, College of Agriculture, Padannakad during 2018-2020. The morphological data were recorded from the field at the Regional Agricultural Research Station, Pilicode and the molecular characterization was done at the Department of Plant Breeding and Genetics and Department of Plant Biotechnology, College of Agriculture, Padannakkad.

3.1 MATERIALS

3.1.1 Plant materials

The plant materials include ten-year-old palms from *interse* of Annur (34 no.), hybrid palms of cross Annur x Malayan Yellow dwarf (11 no.), and Annur x Gangabondam (13 no.) planted at RARS Pilicode (N8 block) in 2009. Morphological observations on vegetative, reproductive, yield and nut characters were recorded from these 58 palms. Morphological observations of Malayan Yellow Dwarf (MYD), Gangabondam Green Dwarf (GB) and the check varieties Kerasree and Keraganga were recorded from T block of RARS Pilicode. The West Coast Tall (WCT) coconut palm was used as check palm for Annur (*interse*) and its observations were recorded from G block of RARS pilicode. There were 74 palms as detailed in Table 1.

3.1.2. SSR markers

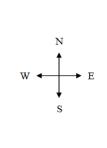
Thirty four primer pairs of SSR reported earlier for coconut were used for molecular characterization. The features of different primers are listed in Table 2.

		-	
S1.	Genotypes	No. of	Palm no. in the field
No.		palms	
1	Annur <i>interse</i> (Annur x	34	1,2,3,4,5,6,8,9,10,11,12,14,15,16,19,
	Annur)		20,21,22,23,24,25,26,27,28,30,31,32,
			34, 36, 37, 38, 39, 40, 41
2	Annur x Malayan	11	42,43,44,46,47,48,49,50,68,71,72
	Yellow Dwarf (MYD)		
3	Annur x	13	51,52,53,55,56,57,58,59,60,62,63,64,65
	Gangabondham (GB)		
4	West Coast Tall (WCT)	2	11, 14
	(As check variety for		
	Annur)		
5	MYD (Male parent)	2	57, 58
6	GB (Male parent)	4	250, 257, 259, 284
7	Kerasree (Check for	4	71, 79, 80, 88
	Annur x MYD)		
8	Keraganga (Check for	4	47, 56, 62, 86
	Annur x GB)		
	Total	74	

Table 1: Plant materials of Annur (interse) and its hybrids in field of RARS, Pilicode

Table 2. Details of coconut specific SSR primer pairs used for molecular characterisation of parental and check palms

Sl no.	Primer name	F/R	Primer sequence $(5' \rightarrow 3')$	GC content (%)
1	CAC 02	F	AGCTTTTTCATTGCTGGAAT	35
1	C/1C 02	R	CCCCTCCAATACATTTTTCC	45
2	CAC 03	F	GGCTCTCCAGCAGAGGCTTAC	61.9
2	CAC 05	R	GGGACACCAGAAAAAGCC	55.6
3	CAC 04	F	CCCCTATGCATCAAAACAAG	45
5	CAC 04	R	CTCAGTGTCCGTCTTTGTCC	55
4	CAC 06	F	TGTACATGTTTTTTGCCCAA	35
+	CAC 00	R	CGATGTAGCTACCTTCCCC	57.9
5	CAC 08	F	ATCACCCCAATACAAGGACA	45
5	CAC 00	R	AATTCTATGGTCCACCCACA	45



72 71 68 65 64 63 62 65 64 63 62 59 58 57 56 55 50 49 48 47 46
41 40 39 38 37 36 34
32 31 30 28 27 26 25 24 23 22 21 20 19 16 15 14 12 11 10 9 8 6 5 4 3 2 1
 a) Annur (<i>interse</i>) - 1 to 41 b) Annur x GB - 51 to 65 c) Annur x MYD - 42 to 50 and 68 to 72

Fig. 1. Layout of experimental field (N8 block), RARS, Pilicode

6	CAC 10	F	GGAACCTCTTTTGGGTCATT	45
0	CAC IU	R	GATGGAAGGTGGTAATGCTG	50
7	CAC 11	F	GATCTTCGGCGTTCCTCA	55.6
/	CACII	R	TCTCCTCAACAATCTGAAGC	45
8	CnCirA9	F	AATGTTTGTGTGTCTTTGTGCGTGTGT	40
0			TCCTTATTTTTCTTCCCCTTCCTCA	40
9	CnCirB12	F	F GCTCTTCAGTCTTTCTCAA	
7	CIICIIDIZ	R	CTGTATGCCAATTTTTCTA	31.6
10	CnCirC12	F	ATACCACAGGCTAACAT	41.2
10		R	AACCAGAGACATTTGAA	35.3
11	CnCirE2	F	TCGCTGATGAATGCTTGCT	47.4
11	CIICIIE2	R	GGGGCTGAGGGATAAACC	61.1
12	CNZ 04	F	TATATGGGATGCTTTAGTGGA	38.1
12	CINZ 04	R	CAAATCGACAGACATCCTAAA	38.1
13	CNZ 05	F	CTTATCCAAATCGTCACAGAG	42.9
15	CINZ 05	R	AGGAGAAGCCAGGAAAGATTT	42.9
14	14 CNZ 06	F	ATACTCATCATCATACGACGC	42.9
14	CINZ 00	R	CTCCCACAAAATCATGTTATT	33.3
15	CAC65	F	GAAAAGGATGTAATAAGCTGG	38
15	CACOJ	R	TTTGTCCCCAAATATAGGTAG	38
16	CNZ10	F	CCTATTGCACCTAAGCAATTA	38
10	CNZIU	R	AATGATTTTCGAAGAGAGGTC	38
17	CNZ12	F	TAGCTTCCTGAGATAAGATGC	43
17	CNZ12	R	GATCATGGAACGAAAACATTA	33
18	CNZ40	F	CTTGATTGCTATCTCAAATGG	38
10	CINZ40	R	CTGAGACCAAATACCATGTGT	43
19	CNZ44	F	CATCAGTTCCACTCTCATTTC	43
17	UNZ44	R	CAACAAAAGACATAGGTGGTC	43
20	CNZ46	F	TTGGTTAGTATAGCCATGCAT	38
20	UNZ40	R	AACCATTTGTAGTATACCCCC	43
21	CnCir01	F	TTGGTCTATTGCATGTTC	39
<i>∠</i> 1		R	TGGCATTGAGAGGGT	53
22	CnCirC5	F	ACCACCAAAGCCAGAGC	59
22	CnCirC5	R	GCAGCCACTACCTAAAAAG	47

			- 1	
23	CnCirHll	F	TCATTCAGAGGACAAAAGTT	35
25	chentin	R	TAAAAATTCATAAAGGTAAAA	14
24	24 CnCir51		TCTCGTGGATCTCGTC	56
	CIICIIJI	R	GCTCTTCCAGTTACGTTT	44
25	CnCir A3	F	AATCTAAATCTACGAAAGCA	30
23	CIICII AS	R	AATAATGTGAAAAAGCAAAG	25
26	CnCir B6	F	GAGTGTGTGAGCCAGCAT	56
20	CIICIT BO	R	ATTGTTCACAGTCCTTCCA	42
27	CnCin C21	F	AGAAAGCTGAGAGGGAGATT	45
27	CnCir C3'	R	GTGGGGCATGAAAAGTAAC	47
28	CnCir C7	F	ATAGCATATGGTTTTCCT	33
28	CIICIPC/	R	TGCTCCAGCGTTCATCTA	50
20	Cr Cir E10	F	TGGGTTCCATTTCTTCTCTCATC	43
29	CnCir E10	R	GCTCTTTAGGGTTCGCTTTCTTAG	46
20	Cr Cir E12	F	TCACGCAAAAGATAAAACC	37
30	CnCir E12	R	ATGGAGATGGAAAGAAAGG	42
21	Cr Cir E2	F	GGTCTCCTCTCCCTCCTTATCTA	52
31	CnCir F2	R	CGACGACCCAAAACTGAACAC	52
32	CnCir G11	F	AATATCTCCAAAAATCATCGAAAG	29
32	CIICIT GI I	R	TCATCCCACACCCTCCTCT	58
22	Cr Ch H4	F	TTAGATCTCCTCCCAAAG	44
33	CnCir H4'	R	ATCGAAAGAACAGTCACG	44
24	CrCin UZ	F	GAGATGGCATAACACCTA	44
34	CnCir H7	R	TGCTGAAGCAAAAGAGTA	39
	1			

3.1.2. Laboratory chemicals and equipment

The existing reagents, laboratory chemicals, equipment and machinery in the Department of Plant Biotechnology lab were used for the study

3.2 METHODS

3.2.1 Morphological characterization

3.2.1.1 Vegetative characters

3.2.1.1.1 Age of the palm at first flowering (year): It is the period between the date of the planting of palm and the date of first flowering. It is expressed in years.

3.2.1.1.2 Shape of the crown: The shape of the crown is recorded as spherical, hemispherical, X-shaped, or V-shaped.

3.2.1.1.3 Height of palm (m): It is measured between the base of the palm above ground level and the base of the crown.

3.2.1.1.4 Girth of palm at 1.5m height (cm): It was measured on the trunk above 1.5 metres.

3.2.1.1.5 Internode length: The length of ten internodes was measured and the average length of each internode was calculated.

3.2.1.1.6 Number of green leaves: It is the total number of fully opened functional leaves at the time of observation (excluding dried leaves).

3.2.1.1.7 *Rate of leaf production*: Total number of leaves produced on the crown per annum.

3.2.1.1.8 Petiole colour: Colour of a portion of leaves without leaflet recorded using visual observation.

3.2.1.1.9 Petiole length (cm): Petiole length is measured between the base of the petiole and portions where leaflets begin.

3.2.1.1.10 Leaf length (cm): It was measured from portions where leaflets begin to the tip of the leaves.

3.2.1.2 Reproductive characters

3.2.1.2.1 Total number of inflorescence in the crown at the time of observation: It is the total number of inflorescence including opened, unopened, bearing inflorescence in the crown at the time of observation

3.2.1.2.2 Number of unopened inflorescence: It is the number of unopened inflorescence in the crown

3.2.1.2.3 Number of opened inflorescence undergoing pollination: It is the number of opened inflorescence in the crown undergoing pollination

3.2.1.2.4 Number of inflorescence in which pollination is over and seed setting started: It is the total number of inflorescence which were in between pollinating and seed setting stages.

3.2.1.2.5 Total inflorescence per palm per year: It is the total number of inflorescence produced in a palm within a year.

3.2.1.2.6 Period between emergence and opening: It is the time gap in days between emergence and opening of an inflorescence.

3.2.1.2.7 Male phase (days): It is the period between the first male flower opening and the dropping of the last male flower.

3.2.1.2.8 Female phase (days): It is the period between the day of receptivity of first female flower and drying of stigma of last female flower.

3.2.1.2.9 Concordance of phase, if any (days): It is the percentage of open male flowers at the time when female flowers are receptive.

3.2.1.2.10 Number of female flowers per inflorescence: It is counted from fully opened inflorescence.

3.2.1.2.11 Number of female flowers one month after pollination: It is the number of female flowers in the inflorescence after one month of pollination.

3.2.1.3 Yield characters

3.2.1.3.1 Number of bunches per palm per year: It is the number of bunches produced in the palm in a year.

3.2.1.3.2 Number of nuts per bunch: It is the number of nuts produced in the bunch

3.2.1.3.3 Number of nuts per palm per year: It is the total number of nuts produced in a palm over a year.

3.2.1.4 Nut characters

3.2.1.4.1 Fruit colour: The colour of fruit is recorded by visual observation

3.2.1.4.2 Size of unhusked nut (equatorial and pole to pole circumference) (cm): It was measured in terms of equatorial and pole to pole circumference.

3.2.1.4.3 Fruit weight (with husk) (g): The fruit weight was measured using weighing balance, without removing the husk.

3.2.1.4.4 Nut weight (without husk) (g): The weight was calculated by removing the husk

3.2.1.4.5 Shell and meat weight (without water) (g): It was calculated by removing the liquid portions in the nuts.

3.2.1.4.6 Kernel thickness at maturity (mm): It was measured using a measuring scale and an average thickness of five nuts was calculated.

3.2.1.4.7 Quantity of liquid endosperm (ml): The amount of liquid endosperm is calculated using a measuring cylinder.

3.2.1.4.8 Sugar content (Bx): Sugar content is calculated using a refractometer.

3.2.1.4.9 Copra content (g): The kernel was sun dried for three weeks and recorded the weight in gram

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

3.2.1.5 Pest and disease incidence if any

The pest and disease attacks of the coconut palms were recorded

3.2.2. Statistical analysis

3.2.2.1. Analysis of variance (ANOVA)

It was done to evaluate variation present between the genotypes (Panse and Sukhatme, 1967). One-way ANOVA was carried out for vegetative, reproductive and yield characters and for nut characters two-way ANOVA was done. WASP software was used for calculation of ANOVA.

3.2.2.2. Estimation of genetic parameters

1) Coefficient of variation

Phenotypic and genotypic coefficient of variation was calculated using following formula.

Phenotypic coefficient of variation (PCV['].) = $(\sigma p / mean) \times 100$

Genotypic coefficient of variation (GCV[']/_.) = (σ g/ mean) x 100

Where, $\sigma p =$ phenotypic standard deviation

 σ g = genotypic standard deviation

Classification of range of variation by Sivasubramanian and Menon (1973):

Low	: less than 10%
Moderate	: 10 - 20%
High	: more than 20%

2) Heritability (Broad sense)

Heritability in the broad sense is the ratio of genotypic variance to phenotypic variance and can be estimated using the formula given below.

Heritability (h²) = $(\sigma^2 p / \sigma^2 g) \times 100$

Where, $\sigma^2 p$ = Phenotypic variance

 $\sigma^2 g$ = Genotypic variance

Classification of range of variability by Johnson et al. (1955):

Low	: less than 30%
Medium	: 30 - 60%
High	: more than 60%

3) Genetic advance

Genetic advance was estimated using the formula proposed by Johnson *et al.* (1955).

$$GA = k x h^2 x \sigma p$$

Where, k = standardized selection differential (2.06 at 5% selection intensity)

4) Genetic gain

Genetic advance expressed in percentage of mean is Genetic gain.

Classification of range of GA as per cent of mean by Johnson et al. (1955):

Moderate : 10 - 20%

High : more than 20%

5) Correlation analysis

Degree and direction of relationship between two or more variables were calculated by correlation analysis. OPISTAT software was used for correlation analysis.

6) Path coefficient analysis

Path coefficient analysis was used to partition the correlation coefficient into measures of direct and indirect effects.

Classification of direct and indirect effects by Lenka and Mishra (1973):

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

3.2.2.3. Heterosis

Heterosis was estimated over mid parent, better parent and standard check using the following formulae and standard error was estimated to find significance.

Relative heterosis (over mid parent) = $\overline{F1} - \overline{MP} \times 100$ \overline{MP} Heterobeltiosis (over better parent) = $\overline{F1} - \overline{BP} \times 100$ \overline{BP} Standard heterosis (over check) = $\overline{F1} - \overline{Check} \times 100$ \overline{Check}

3.2.3 Molecular characterization

3.2.3.1 Isolation of genomic DNA

The middle portion of the spindle leaves was used for the extraction of genomic DNA. The CTAB method (Roger and Bendich, 1985) modified by Chethana (2016) was used for DNA isolation. RNase was applied for reducing RNA contamination during the isolation.

Reagents

- Extraction buffer (pH 8)
 2% CTAB
 100 mM Tris
 20 mM EDTA
 1.4 M NaCl
- 2. β mercaptoethanol
- 3. PVP
- 4. Chloroform: Isoamyl alcohol (24:1)
- 5. Isopropanol
- 6. Ethanol (70%)
- 7. RNase

Steps involved in DNA extraction

- Preheating of 10 ml CTAB buffer was done at 60-65° C in a water bath. The midrib and side portions of young leaves were removed and cut into small pieces.
- One gram of leaf sample was ground along with 40 µl of β- mercaptoethanol, 20µl of PVP (20%), and a pinch of sodium metabisulphate in the presence of liquid N₂ in sterilized mortar and pestle.
- The ground material was transferred to the extraction buffer and mixed by inverting several times.
- The ground material along with extraction buffer was incubated in a waterbath at 65°C for 30 minutes and inverted 2 to 3 times.

- Two by third volume of Chloroform: Isoamyl alcohol (24:1) was added to the incubated mixture and mixed by inverting several times. The centrifugation was done at 12000 rpm for 15 minutes.
- The upper aqueous phase of the centrifuged mixture was collected in a fresh tube and 2µl of RNase was added and incubated at 37°C for 30 minutes.
- Two by third volume of Chloroform: Isoamyl alcohol (24:1) was added to the tube and mixed well. The centrifugation was done at 12000 rpm for 15 minutes.
- The upper aqueous phase was transferred to the centrifuge tube. 1/6th volume of cold isopropanol was added and inverted the tube several times slowly. Centrifugation was done at 12000 rpm for 15 minutes.
- The solution was decanted leaving the pellet and washed it with 70% alcohol for 2 to 3 times.
- Then the DNA pellet was air dried at room temperature and dissolved in water.

Modified protocol

- Preheat 10 ml extraction buffer instead of 14 ml for 1g sample
- Treated with RNase $(2 \mu l)$ and incubated for 30 minutes at 30°C
- DNA pellet was dissolved in TE buffer or ultrapure water

3.2.3.2 Quantification of genomic DNA

The spectrophotometer was used for the quantification of DNA. Ultrapure water $(50 \ \mu l)$ was used as blank. The reading of blank was adjusted to zero. DNA sample $(1 \ \mu l)$ was put into a clean cuvette with 49 μl of water. The sample was mixed well using a micropipette. The optical density (OD) reading was taken at 260 nm and 280 nm. The best quality DNA had A260/A280 ratio of 1.8 to 2.0. The OD value obtained at 260 nm was used for DNA quantity calculation.

3.2.3.3 Agarose gel electrophoresis

Agarose gel electrophoresis is the method to analyse the quality of DNA. DNA molecules are negatively charged. So, they move towards the positive anode. The DNA molecules separated based on their conformation and size. Bromophenol blue is the tracking dye used for locating the DNA and to determine the rate of movement. Ethidium Bromide is used to visualize DNA in an agarose gel under UV light. The horizontal gel electrophoresis unit (BIORAD) is used for Agarose gel electrophoresis.

Reagents and equipment

- 1. Agarose 0.8%
- 2. 50X TAE buffer (pH 8.0) Tris buffer

-Glacial acetic acid

- 0.5 mM EDTA

- 3. Loading Dye Bromophenol blue
- 4. Ethidium bromide
- 5. Electrophoresis unit, power pack, gel casting tray, comb
- 6. BIO-RAD Gel documentation and analysis system

Steps

- Agarose gel (0.8%) was prepared. 0.8 g of agarose powder was dissolved in 100 ml of 1X TAE buffer and heated until complete melting of agar.
- The melted solution was allowed to air cool. When the solution reaches around 60°C, ethidium bromide was added to the gel for visualization of DNA.
- It was poured into a casting tray where both the ends were sealed and fitted with a comb. Then it was allowed to solidify at room temperature.
- After solidification, combs were removed. It was inserted into electrophoresis chamber. The 1X TAE buffer was poured until get complete immersion of gel.
- DNA (5µl) was mixed with 2µl of loading dye. These were loaded into the wells. The voltage was set to 90 volts.

• The power was switched off when the tracking dye reaches 3/4th of gel. Then the gel was taken out carefully and documented using the BIO-RAD gel documentation system.

3.2.3.4 Primer dilution

The primer tubes were centrifuged at 8000 rpm for 30 seconds to get all the lyophilized DNA to the bottom of the tube. These were diluted 10 times the value of nmol of specific primers with water to make it 100 μ M stock solution. These were allowed to sit for two minutes and kept in a water bath at 60° C for 10 minutes. The centrifugation was done at 8000 rpm for 30 seconds. Then the working solution was prepared by adding 90 μ l of water to 10 μ l stock solution. So the solution becomes 10 μ M. The working solution was used for the PCR reactions.

3.2.3.5 Standardisation of PCR conditions for SSR primers

PCR analysis of SSR markers was carried out using EPPENDORF and HI-MEDIA PCR machines. The following reactions were carried out in the lab. Two per cent agarose gel was used for loading the PCR products.

Reaction mixture were set up for all the 34 SSR primers. The annealing temperatures were finalized by establishing a gradient thermal profile. Gradient was setup at temperatures based on the lowest melting temperature ($Tm\pm5^{\circ}C$) among forward and reverse primers of all 34 primers.

Thirty four SSR markers were screened for polymorphism in coconut genotypes. The mastermix concentration and thermal profile were concluded after analyzing the various altered parameters. The standardised PCR condition was setup for characterising six parental and check cultivars of coconut.

Mastermix (20 µl)	Thermal profile				
DNA template	Initial denaturation: 94°C- 5 min				
10X PCR buffer with MgCl ₂ – 2 μ l	Denaturation: 94°C- 1 min				
10 mM dNTPs – 1.5 μl	Annealing: X°C- 1 min >35 cycles				
Taq polymerase (3U) – 0.1 µl	Extension: 72°C- 2 min				
Forward primer (10 pM) $- 1 \mu l$	Final extension: 72°C- 5 min				
Reverse primer (10 pM) $- 1 \mu l$	Hold: 4°C				
Sterile distilled water					

(X°C – Finalized annealing temperature of each primer)

3.2.3.6. Data analysis

Manual scoring of gel images were done. Distinct and clear images were scored as presence (1) and others were scored as absent (0). The datas were analysed using DARwin ver. 6.0. software.

RESULTS

4.RESULT

The experiment entitled "Morpho-molecular characterisation of D x D hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (*Cocos nucifera* L.)" was conducted during 2018-2020 at College of Agriculture, Padannakkad, Kasaragod and Regional Agriculture Research Station, Pilicode, Kasaragod. Eight coconut genotypes consisting of Annur, Annur x MYD, Annur x GB, the two dwarf parents and three check varieties were evaluated in the field and observations were recorded. The molecular characterization of parental and check palms was done at College of Agriculture, Padannakkad. Statistical analysis of the data was conducted and result of the study are presented in this chapter.

4.1. MORPHOLOGICAL CHARACTERISATION OF GENOTYPES

Mean performance on morphological characters of eight coconut genotypes consisting of Annur (Plate 1), Annur x MYD (Plate 2), Annur x GB (Plate 3) were calculated in comparison with male parents and check cultivars.

4.1.1. Height of palm (m)

A significant variation was observed among genotypes for palm height (Table 3). Height of the palm ranged from 2.065 m (Annur x GB) to 16.373 m (WCT) with an average of 7.15 m (Fig. 2). Among parents and hybrids, the highest value was recorded in Gangabondam (8.885 m) and lowest in Annur x GB (2.065 m) which was on par with Annur x MYD (2.251 m) and *interse* of Annur (2.725 m).

4.1.2. Girth of the palm at 1.5 m height (cm)

It was observed that the girth of the palm at 1.5 m height had significant variations among the eight genotypes (Table 3). Girth ranged from 57.250 cm (MYD) to 83 cm (WCT) with an average of 76.508 cm (Fig. 2). Among parents and hybrids, the highest value was recorded by GB (82.25 cm) which was on par with *interse* of Annur (76.507

cm), Annur x GB (75.827 cm) and Annur x MYD (74.977 cm). The lowest value was obtained for MYD (57.250 cm).

4.1.3. Girth of the palm at 20 cm height (cm)

The genotypes differed significantly for girth of the palm at 20 cm height (Table 3). Girth ranged from 59.625 cm (MYD) to 116.75 cm (WCT) with an average of 101.074 cm (Fig. 3). Among parents and hybrids, the highest value was recorded by *interse* of Annur (110.06 cm) which was statistically on par with Annur x GB and Annur x MYD. The lowest value was obtained by MYD (59.625 cm).

4.1.4. Petiole length (cm)

Petiole length differed significantly among the genotypes and ranged between 92.25 cm (MYD) and 140.941 cm (*interse* of Annur) with an average of 123.84 cm (Table 3; Fig.4). Among parents and hybrids, the highest value was observed for *interse* of Annur (140.941 cm) which was on par with GB (140 cm), Annur x MYD (131.5 cm) and Annur x GB (126.538 cm). Lowest value was recorded for MYD (92.25 cm) and was statistically on par with Annur x GB (126.538 cm).

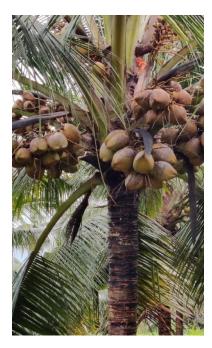
4.1.5. Leaf length (cm)

The genotypes differed significantly for leaf length. It ranges from 324.75 cm (MYD) to 516.044 cm (*interse* of Annur) with an average of 473.161 cm (Table 3; Fig. 5). Among parents and hybrids, the highest value was observed for *interse* of Annur (516.044 cm) which was on par with GB (507.5 cm), Annur x GB (476.346 cm) and Annur x MYD (476.273 cm). Lowest value was recorded for MYD (324.75 cm).

4.1.6. Internode length (cm)

It was observed that internode length had significant variations among the eight genotypes (Table 3). Internode length ranged from 2.925 cm (GB and MYD) to 6.472 cm (*interse* of Annur) with an average of 4.458 cm (Fig. 6). Among parents and hybrids,

$ANNUR-A \ \ local \ ecotype \ of \ West \ Coast \ Tall \ (WCT) \ coconut$



Palm no. 1



Palm no. 2



Plate 1. Annur (interse) palms

D x D Hybrids from ANNUR (ecotype of WCT) crossed with Malayan Yellow Dwarf and Gangabodam cultivars of coconut



Plate 2a. Palm no. 44



Plate 2b. Palm no. 49

Plate 2. Annur x MYD hybrid palms



Palm no. 52

Palm no. 55

Plate 3. Annur x GB hybrids palms

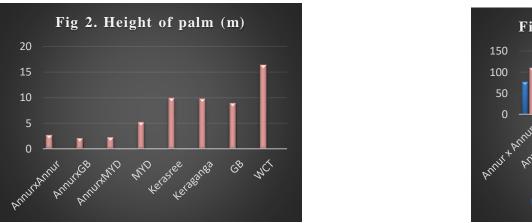
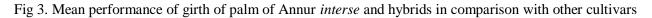
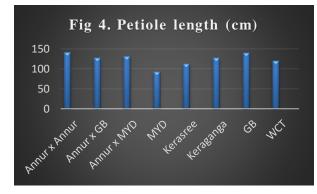




Fig 2. Mean performance of height of palm of Annur interse and hybrids in comparison with other cultivars





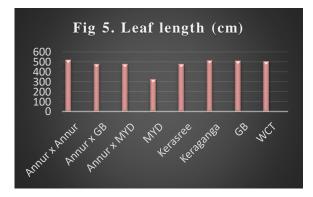


Fig 4. Mean performance of petiole length of Annur interse and hybrids in comparison with other cultivars

Fig 5. Mean performance of leaf length of Annur interse and hybrids in comparison with other cultivars

Genotypes	HOP (m)	G150 (cm)	G20 (cm)	PL (cm)	LL (cm)	IL (cm)	NGL	RLP
AnnurxAnnur	2.725	76.507	110.06	140.941	516.044	6.472	21.386	1.956
AnnurxGB	2.065	75.827	99.423	126.538	476.346	5.531	19.952	1.467
AnnurxMYD	2.251	74.977	103.409	131.5	476.273	5.564	20.898	1.477
MYD	5.200	57.250	59.625	92.25	324.75	2.925	24.813	1.14
Kerasree	9.915	83	113.575	112.25	475	3.5	28.5	1.438
Keraganga	9.786	79.25	100.75	127.25	509.375	3.7	30.469	1.375
GB	8.885	82.25	105	140	507.5	2.925	30.188	1.25
WCT	16.373	83	116.75	120	500	5.05	22.438	1.195
Mean	7.15	76.508	101.074	123.84	473.161	4.458	24.83	1.412
CD	1.499	11.566	34.68	34.62	83.083	2.896	5.012	0.72

Table 3. Mean value for vegetative characters of Annur *interse* and hybrids in comparison with parental cultivars and checks in coconut

HOP- Height of the palm, G150- Girth of the palm at 1.5m height, G20- Girth of the palm at 20cm height, PL- Petiole length, LL- Leaf length, IL- Internode length, NGL- Number of green leaves, RLP- Rate of leaf production

MYD (15.333) showed highest value and was on par with Annur x MYD (14.293), GB (14.125), *interse* of Annur (13.414) and Annur x GB (12.917) and lowest was observed for GB and MYD (2.925 cm), which was on par with Annur x GB (5.531 cm) and Annur x MYD (5.564 cm).

4.1.7. Number of green leaves

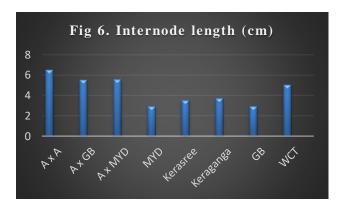
The number of green leaves differed significantly among eight genotypes (Table 3). It ranges from 19.952 (Annur x GB) to 30.469 (keraganga) with an average of 24.83 (Fig. 7). The highest value was observed for GB (30.188) among the parents and hybrids and lowest value was observed for Annur x GB (19.952) which was on par with Annur x MYD (20.898) and *interse* of Annur (21.386).

4.1.8. Rate of leaf production

A significant variation was observed among genotypes for rate of leaf production (Table 3). It ranged from 1.14 (MYD) to 1.956 (Annur x Annur) with an average of 1.412 (Fig. 8). Annur (*interse*) (1.956) showed the highest value which was on par with Annur x MYD (1.477) and Annur x GB (1.467). Lowest value was observed for MYD (1.14) and was on par with GB (1.250).

4.1.9. Total number of inflorescence at the time of observation

It was observed that the total number of inflorescence at the time of observation had a significant variation among eight genotypes (Table 4). It ranged from 6.045 (Annur x MYD) to 14.25 (GB) with an average of 9.68 (Fig. 9). Highest value was observed for GB (14.25) and the lowest value was observed for Annur x MYD (6.045) which was on par with Annur x GB (6.548), MYD (6.75) and *interse* of Annur (8.768).



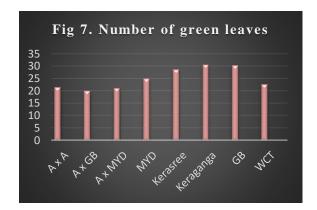
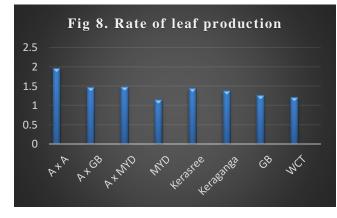


Fig 6. Mean performance of internode length of Annur interse and hybrids in comparison with other cultivars

Fig 7. Mean performance of number of green leaves of Annur interse and hybrids in comparison with other cultivars



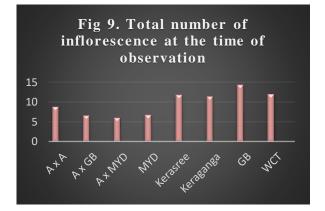


Fig 8. Mean performance of rate of leaf production of Annur interse and hybrids in comparison with other cultivars

Fig 9. Mean performance of total number of inflorescence at the time of observation of Annur *interse* and hybrids in comparison with other cultivars

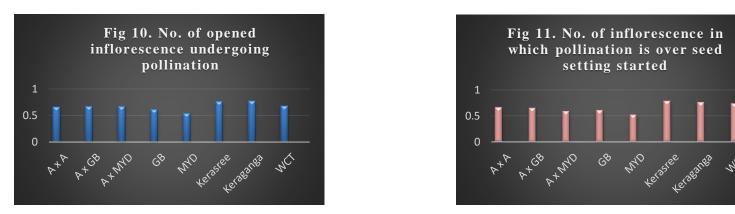


Fig 10. Mean performance of no. of opened inflorescence undergoing pollination of Annur interse and hybrids in comparison with other cultivars

Fig 11. Mean performance of no. of inflorescence in which pollination is over and seed setting started of Annur *interse* and hybrids in comparison with other cultivars

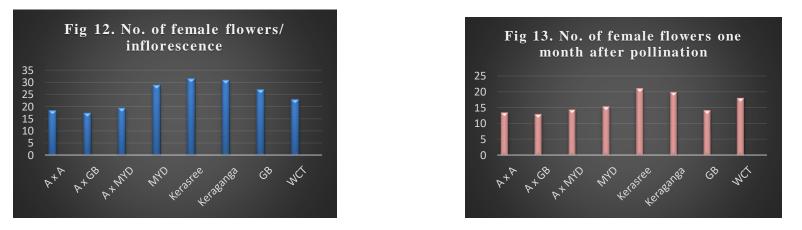


Fig 12. Mean performance of no. of female flowers per inflorescence of Annur interse and hybrids in comparison with other cultivars

Fig 13. Mean performance of no. of female flowers one month after pollination of Annur interse and hybrids in comparison with other cultivars

4.1.10. Number of opened inflorescence undergoing pollination

A significant variation was observed among genotypes for number of opened inflorescence undergoing pollination (Table 4). It ranged from 0.542 (MYD) to 0.778 (Keraganga) with an average of 0.67 (Fig. 10). Among parents and hybrids, Annur x MYD and Annur x GB (0.667) showed highest value and was on par with *interse* of Annur (0.653) and GB (0.611). The lowest value was observed for MYD (0.542) and was on par with GB (0.611).

4.1.11. Number of inflorescence in which pollination is over and seed set started

A significant variation was observed among genotypes for the number of inflorescence in which pollination is over and seed set started. It ranged from 0.528 (MYD) to 0.792 (Kerasree) with an average of 0.669 (Table 4; Fig. 11). Among parents and hybrids, *interse* of Annur (0.667) showed highest value and was on par with Annur x GB (0.653), GB (0.611) and Annur x MYD (0.597). The lowest value was observed for MYD (0.528) and was on par with Annur x MYD (0.597), GB (0.611) and Annur x GB (0.653).

4.1.12. Number of female flowers per inflorescence

It was observed that the number of female flowers per inflorescence had a significant variation among genotypes. It ranged from 17.267(Annur x GB) to 31.563 (Kerasree) with an average of 24.55 (Table 4; Fig. 12). Among parents and hybrids, the highest value was obtained for MYD (28.813) which was on par with GB (26.938). Annur x GB (17.267) showed lowest value and was on par with *interse* of Annur (18.418) and Annur x MYD (19.304).

4.1.13. Number of female flower one month after pollination

A significant variation was observed among genotypes for number of female flower one month after pollination. It ranged from 12.917 (Annur x GB) to 21 (Kerasree)

Genotypes	NITO	NUI	OIUP	POSS	TI	FF	FF1M	BPY	NPY	N/B
AnnurxAnnur	8.768	1.705	0.653	0.667	11.059	18.418	13.414	9.212	58.212	6.097
AnnurxGB	6.548	1.519	0.667	0.653	9.615	17.267	12.917	8	34.3	4.097
AnnurxMYD	6.045	1.548	0.667	0.597	9.909	19.304	14.293	9.3	55.4	5.835
MYD	6.75	1.5	0.542	0.528	8.5	28.813	15.333	7	49.5	7.071
Kerasree	11.781	2	0.764	0.792	13.75	31.563	21	10.75	104.25	9.809
Keraganga	11.344	1.938	0.778	0.764	13	31.094	19.8	10.5	101.5	9.664
GB	14.25	1.563	0.611	0.611	9	26.938	14.125	8.5	58	6.861
WCT	11.938	1.75	0.679	0.743	11	23.063	18	9.5	91.5	9.6
Mean	9.68	1.69	0.67	0.669	10.73	24.55	16.11	9.095	69.08	7.38
CD	5.203	NS	0.105	0.103	NS	6.082	5.245	NS	56.717	3.818

Table 4. Mean value for reproductive and yield characters of Annur *interse* and hybrids in comparison with parental cultivars and checks in coconut

NITO- Total number of inflorescence in the crown at the time of observation, NUI- Number of unopened inflorescence, OIUP- Number of opened inflorescence undergoing pollination, **POSS**- Number of inflorescence in which pollination is over and seed setting started, **TI**- Total inflorescence per palm per year, **FF**- Number of female flowers per inflorescence, **FF1M**- Number of female flowers one month after pollination, **BPY**- Number of bunches per palm per year, **NPY**- Number of nuts per palm per year, **N/B**- Number of nuts per bunches

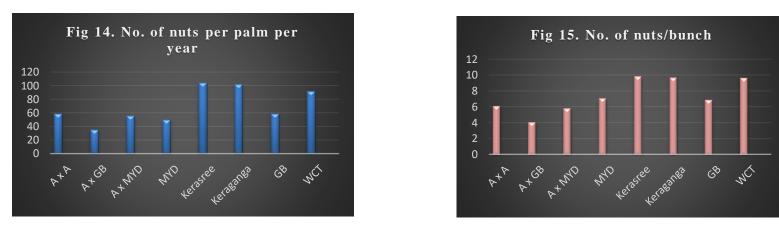


Fig 14. Mean performance of no. of nuts per palm per year of Annur interse and hybrids in comparison with other cultivars

Fig 15. Mean performance of no. of nuts per bunch of Annur interse and hybrids in comparison with other cultivars

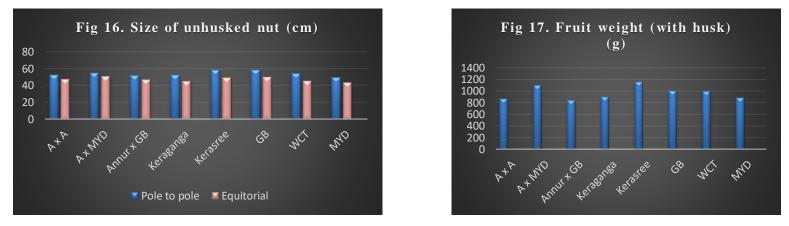


Fig 16. Mean performance of size of unhusked nut of Annur interse and hybrids in comparison with other cultivars

Fig 17. Mean performance of fruit weight (with husk) of Annur interse and hybrids in comparison with other cultivars

with an average of 16.11 (Table 4; Fig. 13). Among parents and hybrids, MYD (15.333) showed highest value and was on par with Annur x MYD (14.293), GB (14.125), *interse* of Annur (13.414) and Annur x GB (12.917).

4.1.14. Number of nuts per palm per year

Number of nuts per palm per year showed significant variation among genotypes. It showed a range of 34.3 (Annur x GB) to 104.25 (Kerasree) with an average of 69.08 (Table 4; Fig 14). Annur (*interse*) (58.212) showed highest value among parents and hybrids which was on par with GB (58), Annur x MYD (55.4), MYD (49.5) and Annur x GB (34.3).

4.1.15. Number of nuts per bunch

A significant variation was observed among genotypes for number of nuts per bunch. It ranged from 4.092 (Annur x GB) to 9.809 (Kerasree) with an average of 7.38 (Table 4; Fig. 15). Among parents and hybrids, MYD (7.071) showed highest value and was on par with GB (6.861), *interse* of Annur (6.097), Annur x MYD (5.835) and Annur x GB (4.092).

4.1.16. Size of unhusked nut (pole to pole circumference) (cm)

A significant variation was observed among genotypes forsize of unhusked nut (pole to pole circumference). It ranged from 49 cm (MYD) to 58.2 cm (GB) with an average of 53.725 cm (Table 5; Fig. 16). Among parents and hybrids, lowest value was observed for MYD (49 cm) was on par with Annur x GB (51.8 cm) and *interse* of Annur (52.2 cm).

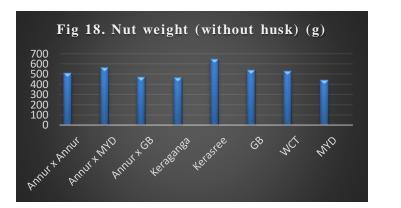
4.1.17. Size of unhusked nut (equitorial circumference) (cm)

It was observed that size of unhusked nut (equatorial circumference) had significant variation among genotypes. It ranged from 43.08 cm (MYD) to 50.2 cm (Annur x MYD) with an average of 46.935 cm (Table 5; Fig. 16). Among parents and hybrids,

Genotypes	PP(cm)	EQ(cm)	FWH(g)	VF(ml)	FW(g)	SMW(g)	KT(mm)	QLE(ml)	SC(°B)	CC(g)
AnnurxAnnur	52.2	47	861	805.56	510.8	414.2	10.9	96	7.82	162.412
AnnurxMYD	54.4	50.2	1090.8	1065	562	431.2	12.4	130	5.5	161.432
AnnurxGB	51.8	46.6	835	808.89	469.8	394.8	11.3	76.4	5.78	147.324
Keraganga	52.2	44.8	893	868.33	464.48	360.2	10.5	101	6.9	167.12
Kerasree	58	49	1149	1117.67	647.2	473.8	12	170.2	6.2	193
GB	58.2	49.8	999.2	968.78	539.1	429.6	10.34	105	6.98	157.84
WCT	54	45	990.8	947.44	530.6	381	11.8	149.4	5.86	169.84
MYD	49	43.08	881.2	848.33	438.4	308	10.2	129.6	6.46	120.6
Mean	53.725	46.935	962.5	928.75	520.298	399.1	11.18	119.7	6.437	159.946
CD	3.712	2.986	170.73	92.375	96.371	64.50	1.278	44.38	NS	21.86

Table 5. Mean value for nut characters of Annur interse and hybrids in comparison with parental cultivars and checks in coconut

PP- Size of unhusked nut pole to pole circumference (cm), **EQ**- Size of unhusked nut equatorial circumference (cm), **FWH**- Fruit weight with husk (g), **VF**- Fruit volume (ml), **FW**- Nut weight without husk (g), **SMW**- Shell and meat weight without water (g), **KT**- Kernel thickness at maturity (mm), **QLE**- Quantity of liquid endosperm (ml), **SC**- Sugar content (°Brix), **CC**- Copra content (g)



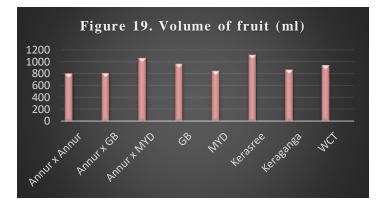
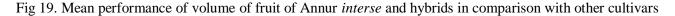
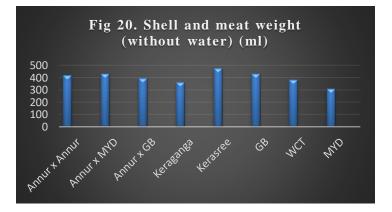


Fig 18. Mean performance of nut weight (without husk) of Annur interse and hybrids in comparison with other cultivars





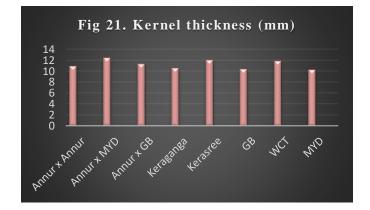


Fig 20. Mean performance of shell and meat weight (without water) of Annur interse and hybrids in comparison with other cultivars

Fig 21. Mean performance of kernel thickness of Annur interse and hybrids in comparison with other cultivars

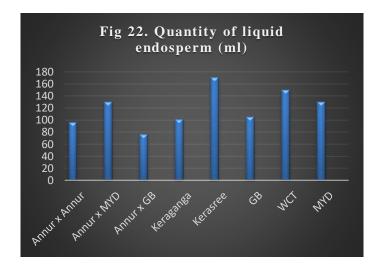


Fig 22. Mean performance of quantity of liquid endosperm of Annur *interse* and hybrids in comparison with other cultivars

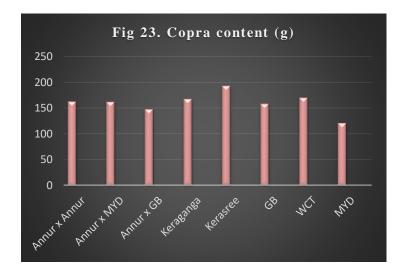


Fig 23. Mean performance of quantity of copra content of Annur *interse* and hybrids in comparison with other cultivars

Annur x GB (50.2 cm) showed highest value and was on par with GB (49.8 cm).

4.1.18. Fruit weight (with husk) (g)

A significant variation was observed among genotypes for fruit weight (with husk). It ranged from 835 g (Annur x GB) to 1149 g (Kerasree) (Table 5; Fig. 17). Among parents and hybrids, Annur x MYD (1090.8 g) showed highest value and was on par with GB (999.2 g). Lowest value was shown by Annur x GB (835 g) which was on par with Annur (*interse*) (861 g) and MYD (881.2 g).

4.1.19. Fruit volume (ml)

A significant variation was observed among genotypes for fruit volume. It ranged from 805.56 ml (Annur x Annur) to 1117.67 ml (Kerasree) with an average of 928.75 ml (Table 5; Fig. 19). Among parents and hybrids, Annur x MYD (1065 ml) showed highest value. Lowest value was shown by Annur (*interse*) (805.56 ml) which was on par with Annur x GB (808.89 ml) and MYD (848.33 ml).

4.1.20. Nut weight (without husk) (g)

It was observed that nut weight (without husk) had significant variation among genotypes. It ranged from 438.4 g (MYD) to 647.2 g (Kerasree) with an average of 520.298 g (Table 5; Fig. 18). Among parents and hybrids, Annur x MYD (562 g) showed highest value which was on par with GB (539.1 g), *interse* of Annur (510.8 g) and Annur x GB (469.8 g). Lowest value was observed for MYD (438.4 g) and was on par with *interse* of Annur (510.8 g) and Annur x GB (469.8 g).

4.1.21. Shell and meat weight (without water) (g)

Shell and meat weight (without water) differed significantly among eight genotypes (Table 3). It ranged from 308 g (MYD) to 473.8 g (Kerasree) with an average of 399.1 g (Fig. 20). Among parents and hybrids, Annur x MYD (431.2 g) showed highest value which was on par with GB (429.6 g), *interse* of Annur (414.2 g), and Annur x GB (394.8 g).

4.1.22. Kernel thickness (mm)

A significant variation was observed among genotypes for kernel thickness (Table 5). It ranged from 10.2 mm (MYD) to 12.4 mm (Annur x MYD) with an average of 11.18 mm (Fig. 21). Among parents and hybrids, Annur x MYD (12.4 mm) showed highest value and was on par with Annur x GB (11.3 mm). Lowest value was shown by MYD (10.2 mm) and was on par with GB (10.34 mm).

4.1.23. Quantity of liquid endosperm (ml)

It was observed that quantity of liquid endosperm had significant variation among genotypes for quantity of liquid endosperm (Table 5). It ranged from 76.4 ml (Annur x GB) to 170.2 ml (Kerasree) with an average of 119.7 ml (Fig. 22). Among parents and hybrids, Annur x MYD (130 ml) had highest value and was on par with MYD (129.6 ml), GB (105 ml) and Annur (*interse*) (96 ml). Lowest value was shown by Annur x GB (76.4 ml) and was on par with Annur (*interse*) (96 ml) and GB (105 ml).

4.1.24. Copra content (g)

A significant variation was observed among genotypes for copra content (Table 3). It ranged from 120.6 g (MYD) to 193 g (Kerasree) with an average of 159.946 g (Fig. 23). Among parents and hybrids, highest value was shown by Annur (*interse*) (162.412 g) and was on par with Annur x MYD (161.432 g), GB (157.84 g) and Annur x GB (147.324 g). 4.2 COMPARISON OF ANNUR (*INTERSE*) AND ITS HYBRIDS WITH RESPECTIVE CHECK VARIEITES

4.2.1. Comparison of Annur x Annur with WCT

Among 28 characters studied, variation with respect to 22 characters *viz*. girth of palm at 150 cm, girth of palm at 20 cm, petiole length, internode length, leaf length, number of green leaves, total number of inflorescence in the crown at the time of observation, number of unopened inflorescence, number of opened inflorescence undergoing pollination, number of inflorescence in which pollination is over and seed setting started,

total inflorescence per palm per year, number of female flowers per inflorescence, number of bunches per palm per year, number of nuts per palm per year, size of unhusked nut (pole to pole circumference), size of unhusked nut (equitorial circumference), fruit weight (with husk), nut weight (without husk), shell and meat weight (without water), kernel thickness at maturity, sugar content, copra content were found to be non significant whereas other six characters *viz*. height of the palm, rate of leaf production, number of female flowers one month after pollination, number of nuts per bunch, volume of fruit, quantity of liquid endosperm showed significant variation between the two genotypes.

4.2.1.1. Height of the palm

A significant variation was observed between Annur (*interse*) and WCT for palm height. Among these, higher value was observed for WCT (16.373 m) compared to Annur (*interse*) (2.725 m).

4.2.1.2. Rate of leaf production

It was observed that Annur (*interse*) had significant variation with WCT for rate of leaf production. Among these, higher value was observed for Annur (*interse*) (1.956) compared to WCT (1.195).

4.2.1.3. Number of female flowers one month after pollination

Number of female flowers one month after pollination had significant variation among Annur (*interse*) and WCT. The higher value was observed for WCT (18) compared to Annur (*interse*) (13.414).

4.2.1.4. Number of nuts per bunch

Number of nuts per bunch had significant variation among Annur (*interse*) and WCT. The higher value was observed for WCT (9.6) compared to Annur (*interse*) (6.097).

4.2.1.5. Volume of fruit (ml)

It was observed that Annur (*interse*) had significant variation with WCT for volume of fruit. The higher value was observed for WCT (947.44 ml) compared to Annur (*interse*) (805.56 ml).

4.2.1.6. Quantity of liquid endosperm (ml)

A significant variation was observed between Annur (*interse*) and WCT for quantity of liquid endosperm. WCT (149.4 ml) showed higher value compared to Annur (*interse*) (96 ml).

4.2.2. Comparison of Annur x GB with Keraganga

Number of characters used for study was 28. Among these, eight characters *viz*. height of the palm, number of green leaves, total number of inflorescence in the crown at the time of observation, number of female flower per inflorescence, number of female flowers one month after pollination, number of bunches per palm per year, number of nuts per palm per year, number of nuts per bunch were found significant.

4.2.2.1. Height of the palm (m)

A significant variation was observed between Annur x GB and Keraganga for palm height. The higher value was observed for Keraganga (9.786 m) compared to Annur x GB (2.065 m).

4.2.2.2. Number of green leaves

It was observed that Annur x GB had significant variation with Keraganga for number of green leaves. Keraganga (30.469) showed higher value compared to Annur x GB (19.952).

4.2.2.3. Total number of inflorescence in the crown at the time of observation

A significant variation was observed between Annur x GB and Keraganga for total number of inflorescence in the crown at the time of observation. Keraganga (11.344) showed higher value compared to Annur x GB (6.548).

4.2.2.4. Number of female flower per inflorescence

It was observed that Annur x GB had significant variation with Keraganga for number of female flower per inflorescence. Keraganga (31.049) showed higher value compared to Annur x GB (17.267).

4.2.2.5. Number of female flowers one month after pollination

A significant variation was observed between Annur x GB and Keraganga for number of female flowers one month after pollination. Keraganga (19.8) showed higher value compared to Annur x GB (12.917).

4.2.2.6. Number of bunches per palm per year

It was observed that Annur x GB had significant variation with Keraganga for number of bunches per palm per year. Keraganga (10.5) showed higher value compared to Annur x GB (8).

4.2.2.7. Number of nuts per palm per year

A significant variation was observed between Annur x GB and Keraganga for number of nuts per palm per year. Keraganga (101.5) showed higher value compared to Annur x GB (34.3).

4.2.2.8. Number of nuts per bunch

A significant variation was observed between Annur x GB and Keraganga for number of nuts per bunch. Keraganga (9.664) showed higher value compared to Annur x GB (4.092).

4.2.3. Comparison of Annur x MYD and Kerasree

Number of characters used for study was 28. Among these, eleven characters *viz.* height of the palm, internode length, number of green leaves, total number of inflorescence in the crown at the time of observation, number of unopened inflorescence, number of inflorescence in which pollination is over and seed setting started, number of female flower per inflorescence, number of female flowers one month after pollination, number of nuts per palm per year, number of nuts per bunch and copra content were found significant.

4.2.3.1. Height of the palm (m)

A significant variation was observed between Annur x MYD and Kerasree for palm height. The higher value was observed for Kerasree (9.915 m) compared to Annur x GB (2.251 m).

4.2.3.2. Internode length (cm)

It was observed that Annur x MYD had significant variation with kerasree for internode length. The higher value was observed for Kerasree (3.5 cm) compared to Annur x GB (5.564 cm).

4.2.3.3. Number of green leaves

It was observed that Annur x MYD had significant variation with Kerasree for number of green leaves. Kerasree (28.5) showed higher value compared to Annur x MYD (20.898).

4.2.3.4. Total number of inflorescence in the crown at the time of observation

A significant variation was observed between Annur x MYD and Kerasree for total number of inflorescence in the crown at the time of observation. Kerasree (11.781) showed higher value compared to Annur x MYD (6.045).

4.2.3.5. Number of unopened inflorescence

It was observed that Annur x MYD had significant variation with Kerasree for number of unopened inflorescence. Kerasree (2) showed higher value compared to Annur x MYD (1.548).

4.2.3.6. Number of inflorescence in which pollination is over and seed setting started

A significant variation was observed between Annur x MYD and Kerasree fornumber of inflorescence in which pollination is over and seed setting started.Kerasree (0.792) showed higher value compared to Annur x MYD (0.597).

4.2.3.7. Number of female flower per inflorescence

It was observed that Annur x MYD had significant variation with Kerasree for number of female flower per inflorescence. Kerasree (31.563) showed higher value compared to Annur x MYD (19.034).

4.2.3.8. Number of female flowers one month after pollination

A significant variation was observed between Annur x MYD and Kerasree for number of female flowers one month after pollination. Kerasree (21) showed higher value compared to Annur x MYD (14.293).

4.2.3.9. Number of nuts per palm per year

A significant variation was observed between Annur x MYD and Kerasree for number of nuts per palm per year. Kerasree (104.25) showed higher value compared to Annur x MYD (55.4).

4.2.3.10. Number of nuts per bunch

A significant variation was observed between Annur x MYD and Kerasree for number of nuts per bunch. Kerasree (9.809) showed higher value compared to Annur x MYD (5.835).

4.2.3.11. Copra content

It was observed that Annur x MYD had significant variation with Kerasree for copra content. Kerasree (193 g) showed higher value compared to Annur x MYD (161.432 g).

4.3. PALM TO PALM VARIATIONS WITHIN EACH CROSS

From among the different individual palms under each cross, better palms were identified based on Sd + Avg method. For all palm characters except height of palm, higher value than Sd + Avg are considered whereas lower value was taken for height of palm. The palms having maximum desirable characters were considered as superior.

Annur (*interse*) consists of 34 palms which were evaluated based on the morphological characters to identify the superior palms. Among these, palm no. 1, 2, 15, 19, 20 and 28 were found to be superior than the rest (Table 6). Out of 13 palms in the group Annur x GB, palm no. 53, 55 and 56 showed superior performance than the rest (Table 7). Among the 11 palms of hybrid group Annur x MYD, four palms *viz.*, palm no. 43, 44, 47 and 49 were identified as superior in performance (Table 8).

4.4. CORRELATION ANALYSIS

The association between twenty-eight characters including morphological, reproductive, yield and nut characters were calculated in all possible combinations. The phenotypic and genotypic correlation coefficients for all characters are presented in Table 9.

4.4.1. Height of the palm (m)

4.4.1.1. With vegetative characters

Height of the palm showed significant positive genotypic correlation with girth of the palm at 150 cm (0.446), girth of palm at 20 cm (0.380) and number of green leaves (0.415) whereas it showed significant negative correlation with internode length (-0.364) followed by rate of leaf production (-0.276).

Palm no	HOP (m)	G150 (cm)	G20 (cm)	PL (cm)	LL (cm)	IL (cm)	NGL	NITO	NUI	TI	FF	FF1M	BPY	NPY	NPB
1	193.5	75.5	141	155	557.5	6.35	23.75	8.875	1.8	9	23.75	13.6	11	65	5.91
2	223.5	68	76	105	487.5	2.85	22.25	7	2.25	18	25.75	18.2	13	159	12.23
3	227.5	88.25	100.5	115	460	4.55	22.38	3.875	1.67	10	16.16	14.33	4	24	6
4	326.5	77.5	161	170	587.5	8.55	21	9.875	1.67	10	15.2	10.33	7	28	4
5	382.5	81.5	100.5	165	547.5	7	18.75	11.625	1.5	9	20.25	11.25	10	57	5.7
6	283	83.25	112.5	135	537.5	7.35	21	9.625	1.33	8	23	12.25	11	43	3.91
8	308	77.5	101	157.5	552.5	8.35	19.875	7	1.28	9	16.5	10	8	43	5.375
9	277	83.5	121	140	542.5	8	22.375	12.75	2	14	15.625	11.2	14	90	6.43
10	252.5	69.5	118	132.5	527.5	8.15	21.125	8	1.57	11	14.857	14.75	6	19	3.17
11	281.5	70.75	94	132.5	502.5	5.25	22.875	12.375	2	14	18.285	12.5	9	58	6.44
12	261.5	74.75	101	142.5	497.5	5.5	23.875	11.75	1.86	13	18.143	11	8	48	6
12	324.5	77.25	118.5	137.5	552.5	7.2	21.375	8	2.375	19	18.714	9.75	10	29	2.9
I4 Sd+Avg	285.51	77.407	113.50	143.99	522.87	6.729	21.373	8 9.257	1.78	11.83	19.054	13.832	9.63	63.997	6.464

Table 6. Palm to palm variations within Annur (interse) ecotype of WCT coconut

HOP – Height of palm, G150 – girth of palm at 150 cm, G20- Girth of palm at 20 cm, PL – Petiole length, LL- Leaf length, IL – Internode length, NGL – No. of green leaves, NITO – No. of inflorescence at the time of observation, NUI – No. of unopened inflorescence, TI – Total no. of inflorescence per palm per year, FF – No. of female flower per inflorescence, FF1M – No. of female flowers 1 month after pollination, BPY- No. of bunches per palm per year, NPY – No. of nuts per palm per year, NPB – No. of nuts per bunch

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Palm no	HOP (m)	G150 (cm)	G20 (cm)	PL (cm)	LL (cm)	IL (cm)	NGL	NITO	NUI	TI	FF	FF1M	BPY	NPY	NPB
15	327	83.5	120.5	167.5	525	6.25	25.375	12.5	2.25	18	16.5	14.75	10	78	7.8
16	408	79.5	115.5	152.5	555	9.3	21	7.875	2.125	17	16.5	11.75	8	40	5
19	359	76.5	110.5	167.5	547.5	7.25	25.75	11.625	1.625	13	28.83	19	12	102	8.5
20	247	76.25	88.5	150	487.5	4.45	22.875	10.625	1	3	22.28	17	12	104	8.67
21	283	77.5	97.5	145	487.5	7.05	18.625	6.5	1.6	8	16.43	13	9	40	4.44
22	241.5	73.5	101	155	504	5.75	16.75	5.5	1.6	8	-	-	-	-	-
23	283	72.75	97	137.5	493	6.05	25.875	11.375	1.857	13	18.83	14.75	12	62	5.167
24	307	74	122.5	142.5	543	6.85	21.25	7.25	1.125	9	18	14	8	55	6.875
25	213	64.5	94	112.5	442.5	6.05	23.625	12.25	2.125	17	17.5	12.75	8	50	6.25
26	135	80.75	161	142.5	523	7.05	26	5.75	2	6	15.167	10.66	6	29	4.833
27	154.5	68.5	77.5	132.5	430	2.85	16	6	1.2	6	10.5	14.5	6	38	6.333
28	348.5	81.25	131	132.5	535	7.65	24.25	10.875	1.5	6	25	17.6	14	160	11.43
Sd+Avg	285.51	77.407	113.50	143.99	522.87	6.729	21.86	9.257	1.78	11.83	19.054	13.832	9.63	63.997	6.464

HOP – Height of palm, G150 – girth of palm at 150 cm, G20- Girth of palm at 20 cm, PL – Petiole length, LL- Leaf length, IL – Internode length, NGL – No. of green leaves, NITO – No. of inflorescence at the time of observation, NUI – No. of unopened inflorescence, TI – Total no. of inflorescence per palm per year, FF – No. of female flower per inflorescence, FF1M – No. of female flowers 1 month after pollination, BPY- No. of bunches per palm per year, NPY – No. of nuts per palm per year, NPB – No. of nuts per bunch

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Dalmana	НОР	G150	G20	PL	LL	IL	NGL	NITO	NUI	TI	FF	FF1M	BPY	NPY	NPB
Palm no	(m)	(cm)	(cm)	(cm)	(cm)	IL (cm)	NGL	NIIO	NUI	11	ГГ	FF INI	DF 1		NED
30	438	75.5	118.5	145	547.5	5.65	16.875	6.875	2.5	15	18.714	14	10	76	7.6
31	398	69.25	92.5	157.5	512.5	5.05	19.125	9.625	1.857	13	14.57	11.4	10	35	3.5
32	149	78.5	131	165	527.5	6.95	19.5	2.5	1	4	16.6	13.67	7	43	6.14
34	137.5	75.5	105.5	112.5	435	5.25	16.75	2	1	4	17.5	13	8	60	7.5
36	243	84.25	122.5	142.5	525	7.45	20	8.125	1.5	12	16.2	11	8	31	3.875
37	327.5	80.5	115.5	132.5	520	7.55	21.5	10.875	1.75	14	18.167	14.67	11	49	4.45
38	218	73.5	89	125	475	4.75	23.5	10.125	2.285	16	20	15.33	7	49	7
39	253	78.5	119	142.5	557.5	7.55	21.75	11.5	1.5	9	18.4	16.33	8	63	7.875
40	194.5	75.5	100.5	102	455	6.95	17.375	8.375	1.25	5	17.2	11.33	10	51	5.1
41	253	74.5	86.5	145	565	7.25	22.75	9.25	2	16	18.67	13	9	43	4.78
Sd+Avg.	285.51	77.407	113.50	143.99	522.87	6.73	21.86	9.257	1.78	11.83	19.054	13.832	9.63	63.997	6.464

HOP – Height of palm, **G150** – girth of palm at 150 cm, **G20**- Girth of palm at 20 cm, **PL** – Petiole length, **LL** - Leaf length, **IL** – Internode length, **NGL** – No. of green leaves, **NITO** – No. of inflorescence at the time of observation, **NUI** – No. of unopened inflorescence, **TI** – Total no. of inflorescence per palm per year, **FF** – No. of female flower per inflorescence, **FF1M** – No. of female flowers 1 month after pollination, **BPY**- No. of bunches per palm per year, **NPY** – No. of nuts per palm per year, **NPB** – No. of nuts per bunch

Palm no	HOP (m)	G150 (cm)	G20 (cm)	PL (cm)	LL (cm)	IL (cm)	NGL	NITO	NUI	TI	FF	FF1M	BPY	NPY	NPB
51	212	75.5	100.5	137.5	452.5	4.45	20	5.625	1.625	13	15.5	10.667	6	21	3.5
52	177	72.5	85.5	85	397.5	2.55	22	5.875	1.57	11	15.8	12.5	7	21	3
53	216.5	75.5	93	82.5	382.5	3.75	21.25	9.375	2.625	21	21	14.75	10	56	5.6
55	218	81	85.5	127.5	465	3.25	21.875	10.375	2.28	16	19.67	17.33	10	68	6.8
56	295.5	72.5	86	132.5	525	7.45	21.25	11.125	1.625	13	21.167	18.66	8	51	6.375
57	178	75.5	100.5	142.5	475	6.35	20.875	7.5	1.75	14	15.167	11.25	6	18	3
58	238	85.5	125.5	155	545	9.05	21.625	7	1.75	14	14.33	10.5	7	15	2.14
59	208	76.5	105.5	137.5	512.5	6.95	19	7.5	1	6	15.4	12.75	11	48	4.36
60	142.5	66.5	95.5	112.5	411	6.45	16.25	2.125	1	4	-	-	_	-	-
62	208.5	77	105.5	135	521.5	5.05	15.625	2.875	1	4	18.8	14.66	7	29	4.14
63	209.5	81.75	116	127.5	514	5	18.625	4.375	1	4	17.6	13	8	16	2
64	198	77.5	98.5	137.5	493	6.05	21.75	7.625	-	-	-	-	_	-	-
65	183	68.5	95	132.5	498	5.55	19.25	3.75	1	5	15.5	6	-	-	-
Sd+Avg.	216.49	77.26	102.70	132.44	490.79	6.029	20.54	7.323	1.677	12.056	18.019	13.96	8.56	40.47	4.628

Table 7. Palm to palm variations within Annur x GB hybrid

Palm no	HOP (m)	G150 (cm)	G20 (cm)	PL (cm)	LL (cm)	IL (cm)	NGL	NITO	NUI	TI	FF	FF1M	BPY	NPY	NPB
68	207.5	75.75	110.5	107.5	473	4.75	21.25	6.875	1	6	17.8	13.25	7	32	4.57
71	293.5	81.5	111	140	467.5	6.75	20.125	7.625	1.33	8	22	15.25	11	54	4.91
72	173.5	81	110.5	135	525	6.85	21.5	5.25	1.5	9	17.66	14	7	32	4.57
42	178	63.5	80.5	107.5	385	3.7	17.5	2.5	1.6	8	-	-	-	-	-
43	217.5	76.75	95.5	127.5	472.5	3.8	20.25	4.2 5	1	5	20	16.25	11	65	5.91
44	208	87	138.5	135	473	3.85	22.375	8.25	1.714	12	19.375	13.6	13	74	5.69
46	218	70.25	98.5	140	426.5	5.05	22	4.5	1.714	12	15	8.33	4	17	4.25
47	223	71	105.5	156.5	541.5	7.85	22	5.125	2	16	22.33	17.25	13	120	9.23
48	301.5	76	100.5	137.5	512.5	6.85	16.75	4.75	1.857	13	19.4	15.75	6	55	9.16
49	248	73.5	101	147.5	527.5	5.75	22.625	11	1.71	12	19.66	15.5	13	64	4.923
50	207	68.5	85.5	112.5	435	6	23.5	6.375	1.6	8	19.8	13.75	8	41	5.125
Sd+Avg	237.46	76.98	108.02	136.37	490.79	5.996	21.53	6.746	1.645	10.91	19.97	15.07	10.34	64.51	6.417

Table 8. Palm to palm variations within Annur x MYD hybrid

HOP – Height of palm, G150 – girth of palm at 150 cm, G20- Girth of palm at 20 cm, PL – Petiole length, LL- Leaf length, IL – Internode length, NGL – No. of green leaves, NITO – No. of inflorescence at the time of observation, NUI – No. of unopened inflorescence, TI – Total no. of inflorescence per palm per year, FF – No. of female flower per inflorescence, FF1M – No. of female flowers 1 month after pollination, BPY- No. of bunches per palm per year, NPY – No. of nuts per palm per year, NPB – No. of nuts per bunch

4.4.1.2. With reproductive characters

It had significant positive correlation with number of inflorescence at the time of observation (0.807), number of unopened inflorescence (0.521), number of opened inflorescence undergoing pollination (0.374), number of inflorescence in which pollination is over seed set started (0.648), number of female flowers per inflorescence (0.526) and number of female flowers one month after pollination (0.734).

4.4.1.3. With yield characters

It was observed that height of palm had positive significant correlation with number of bunches per palm per year (0.295), number of nuts per bunch (0.897) and number of nuts per palm per year (0.767).

4.4.1.4. With nut characters

Height of the palm possessed positive significant correlation with size of unhusked nut (pole to pole circumference) (0.464), fruit weight (with husk) (0.362), volume of fruit (0.311), nut weight (without husk) (0.319), quantity of liquid endosperm (0.564) and copra content (0.530).

4.4.2. Girth of the palm at 150 cm

4.4.2.1. With vegetative characters

Girth of the palm at 150 cm possessed significant positive genotypic correlation with girth of the palm at 20 cm (0.977), height of the palm (0.446), petiole length (0.748), leaf length (0.947), internode length (0.252) and rate of leaf production (0.391).

4.4.2.2. With reproductive characters

It was observed that girth of the palm at 150 cm had positive significant correlation with number of inflorescence at the time of observation (0.679), number of unopened inflorescence (0.515), number of opened inflorescence undergoing pollination (0.809), number of inflorescence in which pollination is over seed set started (0.648), total inflorescence per palm per year (0.656) and number of female flowers one month after pollination (0.341).

4.4.2.3. With yield characters

It had positive significant correlation with number of bunches per palm per year (0.754), number of nuts per bunch (0.402) and number of nuts per palm per year (0.572).

4.4.2.4. With nut characters

It showed positive significant correlation with size of unhusked nut (pole to pole and equatorial circumference) (0.752, 0.580), fruit weight (with husk) (0.470), volume of fruit (0.458), nut weight (without husk) (0.649), shell and meat weight (without water) (0.797), kernel thickness (0.501) and copra content (0.912).

4.4.3. Girth of the palm at 20 cm

4.4.3.1. With vegetative characters

Girth of the palm at 20 cm possessed significant positive genotypic correlation with girth of the palm at 150 cm (0.977), height of the palm (0.380), petiole length (0.746), leaf length (0.955), internode length (0.453) and rate of leaf production (0.498).

4.4.3.2. With reproductive characters

It was observed that girth of the palm at 20 cm had positive significant correlation with number of inflorescence at the time of observation (0.547), number of unopened inflorescence (0.484), number of opened inflorescence undergoing pollination (0.797), number of inflorescence in which pollination is over seed set started (0.787), total inflorescence per palm per year (0.674) and number of female flowers one month after pollination (0.278).

4.4.3.3. With yield characters

It had positive significant correlation with number of bunches per palm per year (0.875), number of nuts per bunch (0.338) and number of nuts per palm per year (0.573).

4.4.3.4. With nut characters

It showed positive significant correlation with size of unhusked nut (pole to pole and equatorial circumference) (0.660, 0.586), fruit weight (with husk) (0.480), volume of fruit (0.469), nut weight (without husk) (0.678), shell and meat weight (without water) (0.806), kernel thickness (0.606) and copra content (0.907).

4.4.4. Petiole length

4.4.4.1. With vegetative characters

Petiole length showed positive significant correlation with leaf length (0.907) followed by girth of palm at 150 cm (0.748), girth of palm at 20 cm (0.746), rate of leaf production (0.493) and internode length (0.463).

4.4.4.2. With reproductive characters

Petiole length possessed positive significant correlation with number of opened inflorescence undergoing pollination (0.307) followed by number of inflorescence at the time of observation (0.273). It showed negative significant correlation with number of female flowers per inflorescence (-0.447) followed by number of female flowers one month after pollination (-0.369) and number of unopened inflorescence (-0.272).

4.4.4.3. With yield characters

It showed positive significant correlation with number of bunches per palm per year (0.385).

4.4.4.4. With nut characters

It was observed that petiole length had positive significant correlation with size of unhusked nut (equatorial circumference) (0.587) followed by shell and meat weight (without water) (0.535), size of unhusked nut (pole to pole circumference) (0.384), copra content (0.359) and sugar content (0.316) whereas it showed negative significant correlation with quantity of liquid endosperm (-0.503).

4.4.5 Leaf length

4.4.5.1 With vegetative characters

Leaf length showed positive significant correlation with girth of the palm at 20 cm (0.955) followed by girth of the palm at 150 cm (0.947), petiole length (0.907), rate of leaf production (0.558) and internode length (0.447).

4.4.5.2 With reproductive characters

It was observed that petiole length had positive significant correlation with number of opened inflorescence undergoing pollination (0.725) followed by number of inflorescence in which pollination is over fruit development (0.646), total inflorescence per palm per year (0.585), number of inflorescence at the time of observation (0.492) and number of unopened inflorescence (0.349).

4.4.5.3. With yield characters

Leaf length possessed positive significant correlation with number of bunches per palm per year (0.726) followed by number of nuts per palm per year (0.395).

4.4.5.4. With nut characters

Leaf length had positive significant correlation with copra content (0.704) followed by shell and meat weight (without water) (0.630), size of unhusked nut (pole to pole, equatorial circumference) (0.498, 0.491), fruit weight (0.386) and kernel thickness (0.335).

4.4.6. Internode length

4.4.6.1. With vegetative characters

It was observed that internode length had positive significant correlation with petiole length (0.463) followed by girth of the palm at 20 cm (0.453), leaf length (0.447), girth of the palm at 150 cm (0.252) and rate of leaf production (0.248).

4.4.6.2. With reproductive characters

Internode length had positive significant correlation with total inflorescence per palm per year (0.339), whereas it had negative significant correlation with with number of female flowers per inflorescence (-0.901) followed by number of unopened inflorescence (-0.502) and number of female flowers one month after pollination (-0.453).

4.4.6.3. With yield characters

Number of bunch per palm per year showed positive significant correlation with internode length (0.419) whereas number of nuts per bunch showed negative significant correlation with internode length (-0.524).

4.4.6.4. With nut characters

It was observed that internode length had positive significant correlation with kernel thickness (0.524) whereas it showed negative significant correlation with size of unhusked nut (pole to pole circumference) (-0.296) followed by quantity of liquid endosperm (-0.293).

4.4.7. Number of green leaves

4.4.7.1. With vegetative characters

It was observed that number of green leaves had positive significant correlation with height of the palm (0.415), whereas it had negative significant correlation with internode length (-0.877).

4.4.7.2. With reproductive characters

Number of green leaves possessed positive significant correlation with number of female flowers per inflorescence (0.912) followed by number of inflorescence at the time of observation (0.717), number of female flowers one month after pollination (0.617), number of unopened inflorescence (0.489), number of opened inflorescence undergoing pollination (0.364) and number of inflorescence in which pollination is over seed set started (0.357).

4.4.7.3. With yield characters

It was observed that number of green leaves had positive significant correlation with number of nuts per bunch (0.719) followed by number of nuts per palm per year (0.545).

4.4.7.4. With nut characters

Number of green leaves showed positive significant correlation with size of unhusked nut (pole to pole circumference) (0.533) followed by sugar content (0.394), copra content (0.388), volume of fruit (0.289) and nut weight (without husk) (0.254), whereas it showed negative significant correlation with kernel thickness (-0.408).

4.4.8. Rate of leaf production

4.4.8.1. With vegetative characters

The rate of leaf production showed positive significant correlation with leaf length (0.558) followed by girth of palm at 20 cm (0.498), petiole length (0.493), girth of palm at 150 cm (0.391) and internode length (0.248), whereas it showed negative significant correlation with height of the palm (-0.276).

4.4.8.2. With reproductive characters

It was observed that rate of leaf production had positive significant correlation with total inflorescence per palm per year (0.786) followed by number of opened inflorescence undergoing pollination (0.662), number of inflorescence in which pollination is over fruit development started (0.605) and number of unopened inflorescence (0.313).

4.4.8.3. With yield characters

The rate of leaf production possessed positive significant correlation with number of bunches per palm per year (0.900) followed by number of nuts per palm per year (0.397).

4.4.8.4. With nut characters

The rate of leaf production had positive significant correlation with sugar content (0.744) followed by copra content (0.565), shell and meat weight (without water) (0.553), nut weight (without husk) (0.393) and size of unhusked nut (equatorial circumference) (0.306).

4.4.9. Total number of inflorescence at the time of observation

4.4.9.1. With vegetative characters

It was observed that total number of inflorescence at the time of observation had positive significant correlation with height of the palm (0.807) followed by number of green leaves (0.717), girth of palm at 150 cm (0.679), girth of palm at 20 cm (0.547), leaf length (0.492) and petiole length (0.273) whereas it showed negative correlation with internode length (-0.502).

4.4.9.2. With reproductive characters

Total number of inflorescence at the time of observation had positive correlation with number of inflorescence in which pollination is over and seed setting started (0.677), number of female flowers per inflorescence (0.572), number of female flowers one month after pollination (0.544), number of unopened inflorescence (0.497) and number of opened inflorescence undergoing pollination (0.415.

4.4.9.3. With yield characters

Total number of inflorescence at the time of observation showed positive significant correlation with number of nuts per bunch (0.785) followed by number of nuts per palm per year (0.66) and number of bunches per palm per year (0.286).

4.4.9.4. With nut characters

Total number of inflorescence at the time of observation showed positive significant correlation with size of unhusked nut (equatorial circumference) (0.75)

followed by copra content (0.651), nut weight (without husk) (0.455), shell and meat weight (without water) (0.412), sugar content (0.386), fruit weight (with husk) (0.354), volume of fruit (0.323) and quantity of liquid endosperm (0.289).

4.4.10. Number of unopened inflorescence

4.4.10.1. With vegetative characters

Number of unopened inflorescence showed positive significant correlation with height of the palm (0.521) followed by girth of palm at 150 cm (0.515), number of green leaves (0.489), girth of palm at 20 cm (0.484), leaf length (0.349) and petiole length (0.313) whereas it showed negative correlation with petiole length (-0.272).

4.4.10.2. With reproductive characters

It was observed that number of unopened inflorescence had positive significant correlation with number of female flowers one month after pollination (0.911) followed by number of inflorescence in which pollination is over and fruit development started (0.831), total inflorescence per palm per year (0.824), number of female flowers per inflorescence (0.711), number of opened inflorescence undergoing pollination (0.582) and total number of inflorescence in the crown at the time of observation (0.497).

4.4.10.3. With yield characters

Number of unopened inflorescence showed positive significant correlation with number of nuts per palm per year (0.999) followed by number of nuts per bunch (0.886) and number of bunches per palm per year (0.617).

4.4.10.4. With nut characters

Number of unopened inflorescence showed positive significant correlation with copra content (0.975) followed by quantity of liquid endosperm (0.572), sugar content (0.524), nut weight (without husk) (0.464), shell and meat weight (without water) (0.277) whereas it show negative significant correlation with size of unhusked nut (equatorial circumference) (-0.386).

4.4.11. Number of opened inflorescence undergoing pollination

4.4.11.1. With vegetative characters

Number of opened inflorescence undergoing pollination showed positive significant correlation with girth of palm at 150 cm (0.809) followed by girth of palm at 20 cm (0.797), leaf length (0.725), rate of leaf production (0.662), height of palm (0.374), number of green leaves (0.364) and petiole length (0.307).

4.4.11.2. With reproductive characters

Number of opened inflorescence undergoing pollination showed positive significant correlation with total inflorescence per palm per year (0.987) followed by number of female flowers one month after pollination (0.814), number of inflorescence in which pollination is over and seed setting started (0.755), number of unopened inflorescence (0.582), total number of inflorescence in the crown at the time of observation (0.415) and number of female flowers per inflorescence (0.388).

4.4.11.3. With yield characters

It was observed that number of opened inflorescence undergoing pollination had positive significant correlation with number of nuts per palm per year (0.941) followed by number of bunches per palm per year (0.902) and number of nuts per bunch (0.648).

4.4.11.4. With nut characters

Number of opened inflorescence undergoing pollination possessed positive significant correlation with copra content (0.967) followed by shell and meat weight (without water) (0.553), nut weight (without husk) (0.509), kernel thickness (0.474), size of unhusked nut (pole to pole circumference) (0.392), volume of fruit (0.368) and fruit weight (with husk) (0.366).

4.4.12. Number of inflorescence in which pollination is over and seed setting started

4.4.12.1. With vegetative characters

It was observed that number of inflorescence in which pollination is over and seed setting started had positive significant correlation with girth of palm at 150 cm (0.826) followed by girth of palm at 20 cm (0.787), height of palm (0.648), leaf length (0.646), rate of leaf production (0.605) and number of green leaves (0.357).

4.4.12.2. With reproductive characters

Number of inflorescence in which pollination is over and seed setting started showed positive significant correlation with number of unopened inflorescence (0.831) followed by number of female flowers one month after pollination (0.822), total inflorescence per palm per year (0.765), number of opened inflorescence undergoing pollination (0.755), total number of inflorescence in the crown at the time of observation (0.677) and number of female flowers per inflorescence (0.424).

4.4.12.3. With yield characters

Number of inflorescence in which pollination is over and seed setting started showed positive significant correlation with number of bunches per palm per year (0.979) followed by number of nuts per palm per year (0.964) and number of nuts per bunch (0.758).

4.4.12.4. With nut characters

Number of inflorescence in which pollination is over and seed setting started showed positive significant correlation with copra content (0.963) followed by nut weight (without husk) (0.502), shell and meat weight (without water) (0.455), size of unhusked nut (pole to pole circumference) (0.425), quantity of liquid endosperm (0.362), fruit weight (with husk) (0.325), kernel thickness (0.315) and volume of fruit (0.298).

4.4.13. Total inflorescence per palm per year

4.4.13.1. With vegetative characters

Total inflorescence per palm per year showed positive significant correlation with rate of leaf production (0.786) followed by girth of palm at 20 cm (0.674), girth of palm at 150 cm (0.656), leaf length (0.585) and internode length (0.339).

4.4.13.2. With reproductive characters

Total inflorescence per palm per year showed positive significant correlation with number of opened inflorescence undergoing pollination (0.987) followed by number of unopened inflorescence (0.824), number of inflorescence in which pollination is over and seed setting started (0.765) and number of female flowers one month after pollination (0.516).

4.4.13.3. With yield characters

It was observed that total inflorescence per palm per year had positive significant correlation with number of bunches per palm per year (0.872) followed by number of nuts per palm per year (0.617) and number of nuts per bunch (0.278).

4.4.13.4. With nut characters

Total inflorescence per palm per year possessed positive significant correlation with copra content (0.796) followed by shell and meat weight (without water) (0.470), nut weight (without husk) (0.379) and kernel thickness (0.351).

4.4.14. Number of female flowers per inflorescenc

4.4.14.1. With vegetative characters

It was observed that number of female flowers per inflorescence had positive significant correlation with number of green leaves (0.912) followed by height of the palm

(0.526) whereas it showed negative significant correlation with internode length (-0.901) and petiole length (-0.447).

4.4.14.2. With reproductive characters

Number of female flowers per inflorescence showed positive significant correlation with number of female flowers per inflorescence (0.826) followed by number of unopened inflorescence (0.711), total number of inflorescence in the crown at the time of observation (0.572), number of inflorescence in which pollination is over and seed setting started (0.424) and number of opened inflorescence undergoing pollination (0.388).

4.4.14.3. With yield characters

It was observed that number of female flowers per inflorescence had positive significant correlation with number of nuts per bunch (0.849) followed by number of nuts per palm per year (0.675).

4.4.14.4. With nut characters

Number of female flowers per inflorescence showed positive significant correlation with quantity of liquid endosperm (0.528) followed by size of unhusked nut (pole to pole circumference) (0.391), fruit weight (with husk) (0.388), volume of fruit (0.378), copra content (0.354) and nut weight (without husk) (0.284) whereas it showed negative significant correlation with kernel thickness (-0.277).

4.4.15. Number of female flowers one month after pollination

4.4.15.1. With vegetative characters

It was observed that number of female flowers one month after pollination had positive significant correlation with height of the palm (0.734) followed by number of green leaves (0.617), girth of palm at 150 cm (0.341) and girth of palm at 20 cm (0.278) whereas it showed negative significant correlation with internode length (-0.453) followed by petiole length (-0.369).

4.4.15.2. With reproductive characters

Number of female flowers one month after pollination showed positive significant correlation with number of unopened inflorescence (0.911) followed by number of female flowers per inflorescence (0.826), number of inflorescence in which pollination is over and seed setting started (0.822), number of opened inflorescence undergoing pollination (0.814), total number of inflorescence in the crown at the time of observation (0.544) and number of female flowers one month after pollination (0.516).

4.4.15.3. With yield characters

It was observed that number of female flowers one month after pollination had positive significant correlation with number of nuts per bunch (0.956) followed by number of nuts per palm per year (0.947) and number of bunch per palm per year (0.617).

4.4.15.4. With nut characters

Number of female flowers one month after pollination possessed positive significant correlation with quantity of liquid endosperm (0.737) followed by copra content (0.707), fruit weight (with husk) (0.575), volume of fruit (0.552), nut weight (without husk) (0.487) and size of unhusked nut (pole to pole circumference) (0.443).

4.4.16. Number of bunches per palm per year

4.4.16.1. With vegetative characters

Number of bunches per palm per year showed positive significant correlation with rate of leaf production (0.900) followed by girth of palm at 20 cm (0.875) girth of palm at 150 cm (0.754), leaf length (0.726), internode length (0.419) and height of the palm (0.295).

4.4.16.2. With reproductive characters

Number of bunches per palm per year possessed positive significant correlation with number of inflorescence in which pollination is over and seed setting started (0.979) followed by number of opened inflorescence undergoing pollination (0.902), total inflorescence per palm per year (0.872), number of unopened inflorescence (0.617), number of female flowers one month after pollination (0.617) and total number of inflorescence in the crown at the time of observation (0.286).

4.4.16.3. With yield characters

It was observed that number of bunches per palm per year had positive significant correlation with number of nuts per palm per year (0.813) followed by number of nuts per bunch (0.574).

4.4.16.4. With nut characters

Number of bunches per palm per year showed positive significant correlation with copra content (0.996) followed by nut weight (without husk) (0.777), shell and meat weight (without water) (0.719), volume of fruit (0.653), fruit weight (with husk) (0.651), kernel thickness (0.638), quantity of liquid endosperm (0.527), size of unhusked nut (pole to pole circumference) (0.478) and size of unhusked nut (equatorial circumference) (0.433).

4.4.17. Number of nuts per palm per year

4.4.17.1. With vegetative characters

It was observed that number of nuts per palm per year had positive significant correlation with height of the palm (0.767) followed by girth of the palm at 20 cm (0.573), girth of the palm at 150 cm (0.572), number of green leaves (0.545), rate of leaf production (0.397) and leaf length (0.395).

4.4.17.2. With reproductive characters

Number of nuts per palm per year showed positive significant correlation with number of unopened inflorescence (0.999) followed by number of inflorescence in which pollination is over and seed setting started (0.964), number of female flowers one month after pollination (0.947), number of opened inflorescence undergoing pollination (0.941), number of female flowers per inflorescence (0.675), total number of inflorescence in the crown at the time of observation (0.660) and total inflorescence per palm per year (0.617).

4.4.17.3. With yield characters

It was observed that number of nuts per palm per year had positive significant correlation with number of nuts per bunch (0.939) followed by number of bunches per palm per year (0.813).

4.4.17.4. With nut characters

Number of nuts per palm per year possessed positive significant correlation with copra content (0.866) followed by quantity of liquid endosperm (0.680), nut weight (without husk) (0.565), fruit weight (with husk) (0.552), volume of fruit (0.526), size of unhusked nut (pole to pole circumference) (0.483), shell and meat weight (without water) (0.328) and kernel thickness (0.262).

4.4.18. Number of nuts per bunch

4.4.18.1. With vegetative characters

It was observed that number of nuts per bunch had positive significant correlation with height of the palm (0.897) followed by number of green leaves (0.719), girth of palm at 150 cm (0.402) and girth of palm at 20 cm (0.338) whereas it showed negative significant correlation with internode length (-0.525).

4.4.18.2. With reproductive characters

Number of nuts per bunch possessed positive significant correlation with number of female flowers one month after pollination (0.956) followed by number of unopened inflorescence (0.86), number of female flowers per inflorescence (0.849), total number of inflorescence in the crown at the time of observation (0.785), number of inflorescence in which pollination is over and seed setting started (0.758), number of

opened inflorescence undergoing pollination (0.648) and total inflorescence per palm per year (0.278).

4.4.18.3. With yield characters

Number of nuts per bunch showed positive significant correlation with number of nuts per palm per year (0.939) followed by number of bunches per palm per year (0.574).

4.4.18.4. With nut characters

It was observed that number of nuts per bunch had positive significant correlation with copra content (0.668) followed by quantity of liquid endosperm (0.676), size of unhusked nut (pole to pole circumference) (0.492), fruit weight (with husk) (0.486), volume of fruit (0.452) and nut weight (without husk) (0.417).

4.4.19. Size of unhusked nut (pole to pole circumference)

4.4.19.1. With vegetative characters

It was observed that size of unhusked nut (pole to pole circumference) had positive significant correlation with girth of palm at 150 cm (0.752) followed by girth of palm at 20 cm (0.660), number of green leaves (0.533), leaf length (0.498), height of the palm (0.464) and petiole length (0.384).

4.4.19.2. With reproductive characters

Size of unhusked nut (pole to pole circumference) showed positive significant correlation with total number of inflorescence in the crown at the time of observation (0.750) followed by number of female flowers one month after pollination (0.443), number of inflorescence in which pollination is over and seed setting started (0.425), number of opened inflorescence undergoing pollination (0.392) and number of female flowers per inflorescence (0.391).

4.4.19.3. With yield characters

It was observed that size of unhusked nut (pole to pole circumference) had positive significant correlation with number of nuts per bunch (0.492) followed by number of nuts per palm per year (0.483) and number of bunch per palm per year (0.478).

4.4.19.4. With nut characters

Size of unhusked nut (pole to pole circumference) possessed positive significant correlation with nut weight (without husk) (0.872) followed by shell and meat weight (without water) (0.857), fruit weight (with husk) (0.834), volume of fruit (0.827), size of unhusked nut (equatorial circumference) (0.783), copra content (0.762), quantity of liquid endosperm (0.486) and kernel thickness (0.474).

4.4.20. Size of unhusked nut (equatorial circumference)

4.4.20.1. With vegetative characters

It was observed that size of unhusked nut (equatorial circumference) had positive significant correlation with petiole length (0.587) followed by girth of palm at 20 cm (0.586), girth of palm at 150 cm (0.580), leaf length (0.491) and rate of leaf production (0.306).

4.4.20.2. With reproductive character

Size of unhusked nut (equatorial circumference) possessed negative significant correlation with number of unopened inflorescence (-0.386).

4.4.20.3. With yield characters

Size of unhusked nut (equatorial circumference) showed positive significant correlation with number of bunches per palm per year (0.433).

4.4.20.4. With nut characters

It was observed that size of unhusked nut (equatorial circumference) had positive significant correlation with shell and meat weight (without water) (0.923) followed by nut weight (without husk) (0.789), size of unhusked nut (pole to pole circumference) (0.783), volume of fruit (0.767), fruit weight (with husk) (0.744), kernel thickness (0.645) and copra content (0.532).

4.4.21. Fruit weight (with husk)

4.4.21.1. With vegetative characters

Fruit weight (with husk) showed positive significant correlation with girth of palm at 20 cm (0.480) followed by girth of palm at 150 cm (0.470), height of the palm (0.362) and number of green leaves (0.290).

4.4.21.2. With reproductive characters

It was observed that fruit weight (with husk) had significant positive correlation with number of female flowers one month after pollination (0.575) followed by number of female flowers per inflorescence (0.388), number of opened inflorescence undergoing pollination (0.366), total number of inflorescence in the crown at the time of observation (0.354) and number of inflorescence in which pollination is over and seed setting started (0.325).

4.4.21.3. With yield characters

It was observed that fruit weight (with husk) had positive significant correlation with number of bunches per palm per year (0.651) followed by number of nuts per palm per year (0.552) and number of nuts per bunch (0.486).

4.4.21.4. With nut characters

Fruit weight (with husk) possessed positive significant correlation with volume of fruit (0.999) followed by nut weight (without husk) (0.929), size of unhusked nut (pole

to pole circumference) (0.834), quantity of liquid endosperm (0.797), kernel thickness (0.751), shell and meat weight (without water) (0.750), size of unhusked nut (equatorial circumference) (0.744) and copra content (0.716) whereas it show negative significant correlation with sugar content (-0.378).

4.4.22. Volume of fruit

4.4.22.1. With vegetative characters

It was observed that volume of fruit had positive significant correlation with girth of palm at 20 cm (0.469) followed by girth of palm at 150 cm (0.458), height of the palm (0.311) and number of green leaves (0.289).

4.4.22.2. With reproductive characters

Volume of fruit possessed positive significant correlation with number of female flowers one month after pollination (0.552) followed by number of female flowers per inflorescence (0.378), number of opened inflorescence undergoing pollination (0.368), total number of inflorescence in the crown at the time of observation (0.323) and number of inflorescence in which pollination is over and seed setting started (0.298).

4.4.22.3. With yield characters

It was observed that volume of fruit had positive significant correlation with number of bunches per palm per year (0.653) followed by number of nuts per palm per year (0.526) and number of nuts per bunch (0.452).

4.4.22.4. With nut characters

Volume of fruit showed positive significant correlation with fruit weight (with husk) (0.999) followed by nut weight (without husk) (0.929), size of unhusked nut (pole to pole circumference) (0.827), quantity of liquid endosperm (0.777), size of unhusked nut (equatorial circumference) (0.767), shell and meat weight (without water) (0.763), kernel

thickness (0.751) and copra content (0.706) whereas it showed negative significant correlation with sugar content (-0.378).

4.4.23. Nut weight (without husk)

4.4.23.1. With vegetative characters

Nut weight (without husk) possessed positive significant correlation with girth of palm at 20 cm (0.678) followed by girth of palm at 150 cm (0.649), rate of leaf production (0.393), leaf length (0.386), height of the palm (0.319) and number of green leaves (0.254).

4.4.23.2. With reproductive characters

It was observed that nut weight (without husk) had positive significant correlation with number of opened inflorescence undergoing pollination (0.509) followed by number of inflorescence in which pollination is over and seed setting started (0.502), number of female flowers one month after pollination (0.487), number of unopened inflorescence (0.464), total number of inflorescence in the crown at the time of observation (0.455), total inflorescence per palm per year (0.379) and number of female flowers per inflorescence (0.284)

4.4.23.3. With yield characters

It was observed that nut weight (without husk) had positive significant correlation with number of bunches per palm per year (0.777) followed by number of nuts per palm per year (0.565) and number of nuts per bunch (0.417).

4.4.23.4. With nut characters

Nut weight (without husk) showed positive significant correlation with fruit weight (with husk) (0.929) followed by volume of fruit (0.929), shell and meat weight (without water) (0.901), size of unhusked nut (pole to pole circumference) (0.872), copra

content (0.838), size of unhusked nut (equatorial circumference) (0.789), kernel thickness (0.723) and quantity of liquid endosperm (0.714).

4.4.24. Shell and meat weight (without water)

4.4.24.1. With vegetative characters

Shell and meat weight (without water) showed positive significant correlation with girth of palm at 20 cm (0.806) followed by girth of palm at 150 cm (0.797), leaf length (0.630), rate of leaf production (0.553) and petiole length (0.535).

4.4.24.2. With reproductive characters

Shell and meat weight (without water) possessed positive significant correlation with number of opened inflorescence undergoing pollination (0.553) followed by total inflorescence per palm per year (0.470), number of inflorescence in which pollination is over and seed setting started (0.455), total number of inflorescence in the crown at the time of observation (0.412) and number of unopened inflorescence (0.277).

4.4.24.3. With yield characters

It was observed that shell and meat weight (without water) had positive significant correlation with number of bunches per palm per year (0.719) followed by number of nuts per palm per year (0.328).

4.4.24.4. With nut characters

Shell and meat weight (without water) showed positive significant correlation with nut weight (without husk) (0.901) followed by size of unhusked nut (equatorial circumference) (0.923), size of unhusked nut (pole to pole circumference) (0.857), copra content (0.784), volume of fruit (0.763), fruit weight (with husk) (0.750), kernel thickness (0.650) and quantity of liquid endosperm (0.341).

4.4.25. Kernel thickness

4.4.25.1. With vegetative characters

It was observed that kernel thickness had positive significant correlation with girth of the palm at 20 cm (0.606) followed by internode length (0.524), girth of the palm at 150 cm (0.501) and leaf length (0.335) whereas it showed negative significant correlation with number of green leaves (-0.408).

4.4.25.2. With reproductive characters

Kernel thickness showed positive significant correlation with number of opened inflorescence undergoing pollination (0.474) followed by total inflorescence per palm per year (0.351) and number of inflorescence in which pollination is over seed setting started (0.315) whereas it showed negative significant correlation with number of female flowers per inflorescence (-0.277).

4.4.25.3. With nut characters

It was observed that kernel thickness had positive significant correlation with number of bunches per palm per year (0.636) and number of nuts per palm per year (0.262).

4.4.25.4. With yield characters

Kernel thickness showed positive significant correlation with volume of fruit (0.751) followed by nut weight (without husk) (0.751), fruit weight (with husk) (0.723), shell and meat weight (without water) (0.650), size of unhusked nut (equatorial circumference) (0.645), copra content (0.569), quantity of liquid endosperm (0.542) and size of unhusked nut (pole to pole circumference) (0.474) whereas it showed negative significant correlation with sugar content (-0.792).

4.4.26. Quantity of liquid endosperm

4.4.26.1. With vegetative characters

It was observed that quantity of liquid endosperm had positive significant correlation with height of the palm (0.564) whereas it showed negative significant correlation with petiole length (-0.503) and internode length (-0.293).

4.4.26.2. With reproductive characters

Quantity of liquid endosperm showed positive significant correlation with number of female flowers one month after pollination (0.737) followed by number of unopened inflorescence (0.572), number of female flowers per inflorescence (0.526), number of inflorescence in which pollination is over seed setting started (0.362) and number of inflorescence at the time of observation (0.289).

4.4.26.3. With yield characters

It was observed that quantity of liquid endosperm had positive significant correlation with number of nuts per palm per year (0.680) followed by number of nuts per bunch (0.676) and number of bunches per palm per year (0.527).

4.4.26.4. With nut characters

Quantity of liquid endosperm had positive significant correlation with fruit weight (with husk) (0.797) followed by volume of fruit (0.777), nut weight (without husk) (0.714), copra content (0.546), kernel thickness (0.542), size of unhusked nut (pole to pole circumference) (0.486) and shell and meat weight (without water) (0.341) whereas it had negative significant correlation with sugar content (-0.262).

4.4.27. Copra content

4.4.27.1. With vegetative characters

It was observed that copra content had positive significant correlation with girth of palm at 150 cm (0.912) followed by girth of palm at 20 cm (0.907), leaf length (0.704), rate of leaf production (0.565), height of the palm (0.530), number of green leaves (0.386) and petiole length (0.359).

4.4.27.2. With reproductive characters

Copra content showed positive significant correlation with number of unopened inflorescence (0.975) followed by number of opened inflorescence undergoing pollination (0.967), number of inflorescence in which pollination over seed setting started (0.963), total inflorescence per palm per year (0.796), number of female flowers one month after pollination (0.707), number of inflorescence at the time of observation (0.651) and number of female flowers per inflorescence (0.354).

4.4.27.3. With yield characters

Copra content had positive significant correlation with number of bunches per palm per year (0.996) followed by number of nuts per palm per year (0.866) and number of nuts per bunch (0.668).

4.4.27.4. With nut characters

It was observed that copra content had positive significant correlation with nut weight (without husk) (0.838), shell and meat weight (without water) (0.784), size of unhusked nut (pole to pole circumference (0.762), fruit weight (with husk) (0.716), volume

	НОР	G150	G20	PL	LL	IL	NGL	RLP	NITO	NUI	OIUP	POSS
HOP	1											
G150	0.446**	1										
G20	0.380**	0.977**	1									
PL	-0.117 ^{NS}	0.748**	0.746**	1								
LL	0.225 ^{NS}	0.947**	0.955**	0.907**	1							
IL	-0.364**	0.252*	0.453**	0.463**	0.447**	1						
NGL	0.415**	0.212 ^{NS}	-0.002 ^{NS}	-0.011 ^{NS}	0.080^{NS}	-0.877**	1					
RLP	-0.276*	0.391**	0.498**	0.493**	0.558**	0.248*	0.179 ^{NS}	1				
NITO	0.807**	0.679**	0.547**	0.273*	0.492**	-0.502**	0.717**	0.118 ^{NS}	1			
NUI	0.521**	0.515**	0.484**	-0.272*	0.349**	-0.121 ^{NS}	0.489**	0.313**	0.497**	1		
OIUP	0.374**	0.809**	0.797**	0.307**	0.725**	0.182 ^{NS}	0.364**	0.662**	0.415**	0.582**	1	
POSS	0.648**	0.826**	0.787**	0.168 ^{NS}	0.646**	0.075 ^{NS}	0.357**	0.605**	0.677**	0.831**	0.755**	1
TI	0.110 ^{NS}	0.656**	0.674**	0.226 NS	0.585**	0.339**	0.088 ^{NS}	0.786**	0.187 ^{NS}	0.824**	0.987**	0.765**
FF	0.526**	-0.006 ^{NS}	-0.158 ^{NS}	-0.447**	-0.226 ^{NS}	-0.901**	0.912**	0.067 ^{NS}	0.572**	0.711**	0.388**	0.424**
FF1M	0.734**	0.341**	0.278*	-0.369**	0.064^{NS}	-0.453**	0.617**	0.101 ^{NS}	0.544**	0.911**	0.814**	0.822**
BPY	0.295*	0.754**	0.875**	0.385**	0.726**	0.419**	0.153 ^{NS}	0.900**	0.286*	0.617**	0.902**	0.979**
NPY	0.767**	0.572**	0.573**	-0.030 ^{NS}	0.395**	-0.194 ^{NS}	0.545**	0.397**	0.660**	0.999**	0.941**	0.964**
N/B	0.897**	0.402**	0.338**	-0.187 ^{NS}	0.183 ^{NS}	-0.525**	0.719**	0.088 ^{NS}	0.785**	0.886**	0.648**	0.758**
PP	0.464**	0.752**	0.660**	0.384**	0.498**	-0.296*	0.533**	0.132 ^{NS}	0.750**	0.137 ^{NS}	0.392**	0.425**
EQ	-0.141 ^{NS}	0.580**	0.586**	0.587**	0.491**	0.140^{NS}	0.123 ^{NS}	0.306**	0.198 ^{NS}	-0.386**	0.186 ^{NS}	0.037 ^{NS}
FWH	0.362**	0.470**	0.480**	0.009^{NS}	0.192 ^{NS}	-0.185 ^{NS}	0.290*	0.066 ^{NS}	0.354**	0.120 ^{NS}	0.366**	0.325**
VF	0.311**	0.458**	0.469**	0.023 ^{NS}	0.189 ^{NS}	-0.177 ^{NS}	0.289*	0.092 ^{NS}	0.323**	0.107 ^{NS}	0.368**	0.298*
FW	0.319**	0.649**	0.678**	0.170^{NS}	0.386**	-0.047 ^{NS}	0.254*	0.393**	0.455**	0.464**	0.509**	0.502**
SMW	0.067 ^{NS}	0.797**	0.806**	0.535**	0.630**	0.145 ^{NS}	0.200 NS	0.553**	0.412**	0.277*	0.553**	0.455**
КТ	0.090 ^{NS}	0.501**	0.606**	0.136 ^{NS}	0.335**	0.524**	-0.408**	-0.015 ^{NS}	-0.113 ^{NS}	-0.044 ^{NS}	0.474**	0.315**
QLE	0.564**	0.115 ^{NS}	0.173 ^{NS}	-0.503**	-0.183 ^{NS}	-0.293*	0.179 ^{NS}	-0.035 ^{NS}	0.289*	0.572**	0.217 ^{NS}	0.362**
CC	0.530**	0.912**	0.907**	0.359**	0.704**	0.051 ^{NS}	0.388**	0.565**	0.651**	0.975**	0.967**	0.963**

Table 9. Genotypic correlation of yield and yield contributing characters of coconut

Table 9 (contd...)

	TI	FF	FF1M	BPY	NPY	N/B	PP	EQ	FWH	VF	FW	SMW	KT	QLE
TI	1													
FF	0.096 ^{NS}	1												
FF1M	0.516**	0.826**	1											
BPY	0.872**	0.186 ^{NS}	0.617**	1										
NPY	0.617**	0.675**	0.947**	0.813**	1									
N/B	0.278*	0.849**	0.956**	0.574**	0.939**	1								
PP	0.141 ^{NS}	0.391**	0.443**	0.478**	0.483**	0.492**	1							
EQ	0.008 ^{NS}	-0.086 ^{NS}	-0.048 ^{NS}	0.433**	0.030 ^{NS}	-0.092 ^{NS}	0.783**	1						
FWH	0.090 ^{NS}	0.388**	0.575**	0.651**	0.552**	0.486**	0.834**	0.744**	1					
VF	0.093 ^{NS}	0.378**	0.552**	0.653**	0.526**	0.452**	0.827**	0.767**	0.999**	1				
FW	0.379**	0.284*	0.487**	0.777**	0.565**	0.417**	0.872**	0.789**	0.929**	0.929**	1			
SMW	0.470**	0.030 ^{NS}	0.189 ^{NS}	0.719**	0.328**	0.124 ^{NS}	0.857**	0.923**	0.750**	0.763**	0.901**	1		
КТ	0.351**	-0.277*	0.231 ^{NS}	0.638**	0.262*	0.022 ^{NS}	0.474**	0.645**	0.751**	0.751**	0.723**	0.650**	1	
QLE	0.084 ^{NS}	0.528**	0.737**	0.527**	0.680**	0.676**	0.486**	0.213 ^{NS}	0.797**	0.777**	0.714**	0.341**	0.542**	1
CC	0.796**	0.354**	0.707**	0.996**	0.866**	0.668**	0.762**	0.532**	0.716**	0.706**	0.838**	0.784**	0.569**	0.546**

**Significant at 1% *Significant at 5%

HOP- Height of the palm, G150- Girth of the palm at 1.5m height, G20- Girth of the palm at 20cm height, PL- Petiole length, LL- Leaf length, IL-Internode length, NGL- Number of green leaves, RLP- Rate of leaf production, NITO- Total number of inflorescence in the crown at the time of observation, NUI- Number of unopened inflorescence, OIUP- Number of opened inflorescence undergoing pollination, POSS- Number of inflorescence in which pollination is over and seed setting started, TI- Total inflorescence per palm per year, FF- Number of female flowers per inflorescence, FF1M- Number of female flowers one month after pollination, BPY- Number of bunches per palm per year, NPY- Number of nuts per palm per year, N/B- Number of nuts per bunches, PP- Size of unhusked nut pole to pole circumference, EQ- Size of unhusked nut equatorial circumference, FWH- Fruit weight with husk, VF- Fruit volume, FW- Nut weight without husk, SMW- Shell and meat weight without water, KT-Kernel thickness at maturity, QLE- Quantity of liquid endosperm, CC- Copra content of fruit (0.706), kernel thickness (0.569), quantity of liquid endosperm (0.546) and size of unhusked nut (equatorial circumference) (0.532).

4.5. PATH ANALYSIS

Identification of characters showing positive significant correlation with number of nuts per palm per year (yield) was done and estimated the direct and indirect effects of these characters on yield. The values are presented in Table 10. Nuts per palm per year (yield) was taken as the dependent character and the component characters *viz*.height of the palm, girth of palm at 150 cm, girth of palm at 20 cm, leaf length, number of green leaves, rate of leaf production, total number of inflorescence in the crown at the time of observation, number of unopened inflorescence, number of opened inflorescence undergoing pollination, number of inflorescence in which pollination is over and seed setting started, total inflorescence per palm per year, number of female flowers per inflorescence, number of female flowers one month after pollination, number of bunches per palm per year, number of nuts per bunch, size of unhusked nut (pole to pole circumference), fruit weight (with husk), nut weight (without husk), volume of fruit, shell and meat weight (without water), kernel thickness at maturity, quantity of liquid endosperm, copra content were considered as independent character.

4.5.1. Direct effects

A very high direct effect on yield was obtained by fruit weight (with husk) (2.055) followed by number of nuts per bunch (1.378). Size of unhusked nut (pole to pole circumference) (0.874), copra content (0.785), quantity of liquid endosperm (0.703), girth of palm at 20 cm (0.589), shell and meat weight (without water) (0.547) and number of female flowers per inflorescence (0.336) possessed high direct effect on yield. Number of inflorescence in which pollination is over and seed setting started (0.298), rate of leaf production (0.267) and number of bunches per palm per year (0.217) showed moderate direct effect. It was observed that total inflorescence per palm per year (0.115) had low direct effect.

Nut weight (without husk) (-2.523) and volume of fruit (-1.79) showed very high negative direct effect on yield. A high negative direct effect on yield was recorded by number of female flowers one month after pollination (-0.723) followed by height of the palm (-0.461) and girth of palm at 150 cm (-0.345). It was observed that number of green leaves (-0.289) and leaf length (-0.288) had moderate negative direct effect whereas number of opened inflorescence undergoing pollination (-0.111) and kernel thickness (-0.192) showed low negative direct effect on yield.

The direct effects of number of unopened inflorescence and total number of inflorescence in the crown at the time of observation were negative and negligible.

4.5.2. Indirect effect

Highest positive indirect effect was obtained for volume of fruit *via* fruit weight (with husk) (2.057) followed by kernel thickness *via* fruit weight (with husk) (1.949).

Fruit weight (with husk) had positive genotypic correlation (0.565) with number of nuts per palm per year (yield) but negative direct effect. However, it had positive indirect effect *via* fruit weight with husk (1.908) which accounts for the positive genotypic correlation.

Volume of fruit had positive genotypic correlation (0.526) with number of nuts per palm per year (yield) but negative direct effect. However, it had positive indirect effect *via* fruit weight with husk (2.057) which justifies the positive genotypic correlation.

It was observed that number of female flower one month after pollination had high positive correlation with number of nuts per palm per year (yield) (0.947) but high negative direct effect was obtained. Positive indirect effect *via* number of nuts per bunch (1.321) which accounts for the positive genotypic correlation.

Height of the palm had positive genotypic correlation (0.767) with number of nuts per palm per year (yield) but negative direct effect. However, it had positive indirect effect *via* number of nuts per bunch (1.163) which accounts for the positive genotypic correlation.

Girth of palm at 150 cm had positive genotypic correlation (0.572) with number of nuts per palm per year (yield) but negative direct effect. However, it had positive indirect effect *via* fruit weight with husk (0.911) which justifies the positive genotypic correlation.

It was observed that number of green leaves had high positive correlation with number of nuts per palm per year (yield) (0.545) but high negative direct effect was obtained. Positive indirect effect *via* number of nuts per bunch (0.845) which accounts for the positive genotypic correlation.

It was observed that leaf length had high positive correlation with number of nuts per palm per year (yield) (0.395) but high negative direct effect was obtained. Positive indirect effect *via* number of nuts per bunch (0.720) which justifies the positive genotypic correlation.

The direct effect of number of opened inflorescence undergoing pollination and kernel thickness were negative whereas it had positive indirect effect *via* fruit weight (with husk) (0.997 and 1.943 respectively).

Total number of inflorescence at the time of observation showed negligible negative direct effect on yield, but its genotypic correlation was positive (0.66). It was due to its positive indirect effect *via* number of nuts per bunch (1.182).

Number of unopened inflorescence showed negligible negative direct effect on yield, but its genotypic correlation was positive (0.999). It was due to its positive indirect effect *via* volume of fruit (1.104) and number of nuts per bunch (0.911).

The residual effect obtained was 0.00907.

	HOP	G150	G20	LL	NGL	RLP	NITO	NUI	OIUP	POSS	TI	FF	FF1M	BPY	N/B	PP	FWH	VF	FW	SMW	KT	QLE	CC
HOP	-0.461	-0.146	0.258	-0.108	-0.125	-0.117	-0.074	-0.006	0.002	0.194	-0.012	0.160	-0.595	0.082	1.163	0.364	0.513	-0.402	-0.883	0.175	-0.089	0.257	0.578
G150	-0.195	-0.345	0.582	-0.280	-0.107	0.074	-0.057	0.003	-0.062	0.204	0.033	-0.055	-0.536	0.197	0.701	0.663	0.911	-0.796	-1.481	0.452	-0.086	0.141	0.724
G20	-0.201	-0.341	0.589	-0.272	-0.052	0.101	-0.046	0.002	-0.070	0.212	0.027	-0.071	-0.563	0.199	0.754	0.671	1.158	-1.010	-1.749	0.497	-0.115	0.233	0.773
LL	-0.174	-0.337	0.558	-0.288	-0.081	0.123	-0.061	0.001	-0.043	0.137	0.016	-0.045	-0.383	0.235	0.720	0.297	0.261	-0.213	-0.663	0.220	-0.039	0.017	0.463
NGL	-0.201	-0.129	0.108	-0.081	-0.289	-0.048	-0.064	-0.001	-0.020	0.183	0.012	0.280	-0.572	0.120	0.845	0.376	0.305	-0.269	-0.601	0.150	0.027	0.099	0.381
RLP	0.202	-0.095	0.223	-0.132	0.052	0.267	0.020	0.006	-0.072	-0.023	-0.003	-0.074	-0.003	0.174	0.066	-0.210	0.25	-0.236	-0.359	0.055	0.016	0.112	0.053
NITO	-0.391	-0.224	0.309	-0.200	-0.211	-0.061	-0.088	-0.001	0.006	0.252	0.009	0.177	-0.617	0.179	1.182	0.415	0.151	-0.110	-0.706	0.199	-0.002	0.084	0.542
NUI	-0.185	0.074	-0.085	0.031	-0.012	-0.092	-0.007	-0.015	-0.062	0.322	0.141	0.123	-0.407	0.135	0.911	-0.574	-1.264	1.104	0.638	-0.176	0.071	-0.090	0.189
OIUP	0.008	-0.194	0.375	-0.112	-0.054	0.175	0.005	-0.008	-0.111	0.129	0.051	0.066	-0.530	0.176	0.644	0.230	0.997	-0.876	-1.41	0.355	-0.090	0.264	0.550
POSS	-0.299	-0.236	0.419	-0.131	-0.176	-0.021	-0.074	-0.017	-0.048	0.298	0.064	0.211	-0.729	0.215	1.293	0.364	0.595	-0.483	-1.667	0.402	-0.067	0.351	0.733
TI	0.049	-0.099	0.140	-0.040	-0.029	-0.007	-0.006	-0.019	-0.049	0.165	0.115	-0.057	-0.033	0.087	0.114	-0.454	-1.445	1.265	1.172	-0.179	0.083	-0.425	-0.101
FF	-0.220	0.056	-0.126	0.039	-0.240	-0.059	-0.046	-0.005	-0.021	0.168	-0.019	0.336	-0.504	0.038	0.963	0.083	0.388	-0.319	-0.644	0.046	0.018	0.320	0.282
FF1M	-0.379	-0.256	0.459	-0.152	-0.228	0.001	-0.075	-0.008	-0.081	0.301	0.005	0.234	-0.723	0.159	1.321	0.353	0.882	-0.743	-1.485	0.285	-0.095	0.437	0.707
BPY	-0.175	-0.313	0.542	-0.312	-0.159	0.214	-0.072	-0.009	-0.091	0.296	0.046	0.059	-0.531	0.217	1.085	-0.017	-0.063	0.078	-0.577	0.155	0.025	0.079	0.445
N/B	-0.389	-0.175	0.322	-0.150	-0.176	0.012	-0.075	-0.010	-0.052	0.280	0.009	0.234	-0.693	0.171	1.378	0.087	0.275	-0.198	-0.837	0.131	-0.043	0.293	0.578
PP	-0.192	-0.262	0.454	-0.098	-0.124	-0.064	-0.042	0.010	-0.029	0.124	-0.060	0.032	-0.292	-0.004	0.138	0.874	1.665	-1.450	-1.993	0.466	-0.157	0.454	0.567
FWH	-0.115	-0.153	0.332	-0.036	-0.042	0.032	-0.006	0.009	-0.054	0.086	-0.081	0.063	-0.310	-0.006	0.185	0.707	2.055	-1.795	-2.346	0.476	-0.182	0.661	0.574

Table 10. Direct and indirect effects of different characters on yield

Table 10. (contd...)

	HOP	G150	G20	LL	NGL	RLP	NITO	NUI	OIUP	POSS	TI	FF	FF1M	BPY	N/B	PP	FWH	VF	FW	SMW	KT	QLE	CC
VF	-0.105	-0.153	0.333	-0.034	-0.043	0.035	-0.005	0.009	-0.054	0.080	-0.081	0.059	-0.299	-0.009	0.152	0.706	2.057	-1.79	-2.338	0.476	-0.181	0.655	0.563
FW	-0.161	-0.202	0.409	-0.075	-0.068	0.038	-0.024	0.004	-0.062	0.197	-0.053	0.086	-0.425	0.049	0.457	0.690	1.911	-1.662	-2.523	0.533	-0.167	0.672	0.670
SMW	-0.147	-0.285	0.536	-0.115	-0.079	0.027	-0.032	0.005	-0.072	0.219	-0.038	0.028	-0.376	0.062	0.329	0.745	1.790	-1.562	-2.463	0.547	-0.161	0.612	0.664
KT	-0.214	-0.155	0.354	-0.058	0.040	-0.023	-0.001	0.006	-0.052	0.104	-0.050	-0.031	-0.358	-0.028	0.313	0.713	1.949	-1.696	-2.196	0.460	-0.192	0.605	0.639
QLE	-0.168	-0.069	0.196	-0.007	-0.040	0.043	-0.010	0.002	-0.041	0.149	-0.070	0.153	-0.45	0.025	0.575	0.564	1.933	-1.673	-2.414	0.476	-0.165	0.703	0.621
CC	-0.339	-0.318	0.580	-0.169	-0.139	0.018	-0.061	-0.003	-0.077	0.279	-0.015	0.121	-0.651	0.123	1.014	0.631	1.503	-1.286	-2.153	0.463	-0.156	0.557	0.785

HOP- Height of the palm, G150- Girth of the palm at 1.5m height, G20- Girth of the palm at 20cm height, LL- Leaf length, NGL- Number of green leaves, RLP- Rate of leaf production, NITO- Total number of inflorescence in the crown at the time of observation, NUI- Number of unopened inflorescence, OIUP- Number of opened inflorescence undergoing pollination, POSS- Number of inflorescence in which pollination is over and seed setting started, TI- Total inflorescence per palm per year, FF- Number of female flowers per inflorescence, FF1M- Number of female flowers one month after pollination, BPY- Number of bunches per palm per year, NPY- Number of nuts per palm per year, N/B- Number of nuts per bunches, PP- Size of unhusked nut pole to pole circumference, FWH- Fruit weight with husk, VF- Fruit volume, FW- Nut weight without husk, SMW- Shell and meat weight without water, KT- Kernel thickness at maturity, QLE- Quantity of liquid endosperm, CC- Copra content

4.6 GENETIC VARIABILITY PARAMETERS

Genetic parameters *viz.*, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance for 28 characters were estimated and recorded in Table 11.

The characters showing PCV and GCV ranges from 6.172 to 71.546 and 5.186 to 70.571 respectively. The characters viz. height of the palm, number of nuts per palm per year, Internode length, number of nuts per bunch, Quantity of liquid endosperm, total inflorescence per palm per year, number of inflorescence at the time of observation, number of female flower per inflorescence, number of unopened inflorescence, girth of palm at 20 cm, number of bunches per palm per year, number of female flowers one month after pollination, number of inflorescence in which pollination is over seed setting started and sugar content showed high PCV value. The moderate PCV was recorded for number of opened inflorescence undergoing pollination, number of green leaves, nut weight (without husk), petiole length, fruit weight (with husk), volume of fruit, shell and meat weight (without water), copra content, rate of leaf production, leaf length and girth of palm at 150 cm whereas the low PCV was recorded for kernel thickness, pole to pole circumference, equatorial circumference. The characters viz. height of palm, number of nuts per palm per year, internode length, number of nuts per bunch, quantity of liquid endosperm, number of inflorescence at the time of observation and number of female flowers per inflorescence showed high GCV. The moderate GCV was recorded for girth of palm at 20 cm, number of green leaves, number of female flowers one month after pollination, total inflorescence per palm per year, nut weight (without husk), number of bunches per palm per year, leaf length, copra content, shell and meat weight, sugar content, number of inflorescence in which pollination is over seed setting started, fruit weight (with husk), volume of fruit, petiole length and girth of palm at 150 cm whereas the low GCV was recorded for number of opened inflorescence undergoing pollination, rate of leaf production, kernel thickness, pole to pole circumference, number of unopened inflorescence and equatorial circumference.

High heritability was recorded for the characters viz. height of palm, leaf length, number of green leaves, number of female flowers per inflorescence, girth of palm at 150 cm, number of inflorescence at the time of observation, copra content, shell and meat weight and pole to pole circumference. The moderate heritability was observed for characters viz. equatorial circumference, quantity of liquid endosperm, nut weight (without husk), fruit weight (with husk), girth of palm at 20 cm, number of nuts per palm per year, volume of fruit, internode length, petiole length, number of nuts per bunch, number of female flowers one month after pollination, kernel thickness, sugar content, number of inflorescence in which pollination is over seed setting started and number of bunches per palm per year whereas low heritability was shown by the characters viz. rate of leaf production, number of opened inflorescence undergoing pollination, total inflorescence per palm per year and number of unopened inflorescence. The characters viz. height of palm, number of nuts per palm per year, number of inflorescence at the time of observation, internode length, quantity of liquid endosperm, number of nuts per bunch, number of female flowers per inflorescence, number of green leaves, girth of palm at 20 cm, leaf length, number of female flowers 1 month after pollination, copra content, nut weight (without husk) and shell and meat weight showed high genetic advance as per cent of mean. The moderate genetic gain was observed for fruit weight (with husk), volume of fruit, girth of palm at 150 cm, petiole length, sugar content, number of bunches per palm per year, number of inflorescence in which pollination is over seed setting started, total inflorescence per palm per year and kernel thickness whereas opened inflorescence undergoing pollination, pole to pole circumference, equatorial circumference, rate of leaf production and number of unopened inflorescence showed low genetic gain.

PCV, GCV, heritability and genetic gain was highest for height of palm. High heritability coupled with high GA was observed for height of palm, leaf length, number of green leaves, number of female flowers per inflorescence, number of inflorescence at the time of observation, copra content and shell and meat weight.

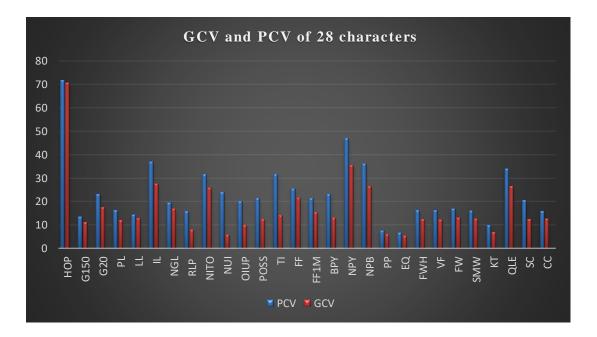


Fig. 24. GCV and PCV of 28 characters in coconut cultivars

HOP- Height of the palm, G150- Girth of the palm at 1.5m height, G20- Girth of the palm at 20cm height, PL- Petiole length, LL- Leaf length, IL-Internode length, NGL- Number of green leaves, RLP- Rate of leaf production, NITO- Total number of inflorescence in the crown at the time of observation, NUI- Number of unopened inflorescence, OIUP- Number of opened inflorescence undergoing pollination, POSS- Number of inflorescence in which pollination is over and seed setting started, TI- Total inflorescence per palm per year, FF- Number of female flowers per inflorescence, FF1M- Number of female flowers one month after pollination, BPY- Number of bunches per palm per year, NPY- Number of nuts per palm per year, N/B- Number of nuts per bunches, PP- Size of unhusked nut pole to pole circumference, EQ- Size of unhusked nut equatorial circumference, FWH- Fruit weight with husk, VF- Fruit volume, FW- Nut weight without husk, SMW- Shell and meat weight without water, KT-Kernel thickness at maturity, QLE- Quantity of liquid endosperm, SC- Sugar content, CC- Copra content

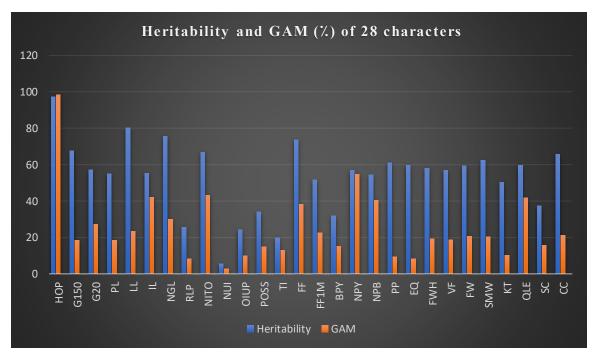


Fig 25. Heritability and Genetic advance of 28 characters in coconut cultivars

HOP- Height of the palm, G150- Girth of the palm at 1.5m height, G20- Girth of the palm at 20cm height, PL- Petiole length, LL- Leaf length, IL- Internode length, NGL- Number of green leaves, RLP- Rate of leaf production, NITO- Total number of inflorescence in the crown at the time of observation, NUI-Number of unopened inflorescence, OIUP- Number of opened inflorescence undergoing pollination, POSS- Number of inflorescence in which pollination is over and seed setting started, TI- Total inflorescence per palm per year, FF- Number of female flowers per inflorescence, FF1M- Number of female flowers one month after pollination, BPY- Number of bunches per palm per year, NPY- Number of nuts per palm per year, N/B- Number of nuts per bunches, PP- Size of unhusked nut pole to pole circumference, EQ- Size of unhusked nut equatorial circumference, FWH- Fruit weight with husk, VF- Fruit volume, FW- Nut weight without husk, SMW- Shell and meat weight without water, KT- Kernel thickness at maturity, QLE- Quantity of liquid endosperm, SC- Sugar content, CC-Copra content

Characters	Mean	PCV	GCV	H ²	GAM(%)
Height of palm	7.143	71.546	70.571	97.292	98.394
Girth of palm (150 cm)	76.597	13.32	10.968	67.801	18.604
Girth of palm (20 cm)	100.519	23.066	17.465	57.332	27.242
Petiole length	123.521	16.217	12.022	54.954	18.358
Leaf length	475.297	14.232	12.749	80.238	23.524
Internode length	4.376	37.044	27.516	55.171	42.102
No. of green leaves	25.062	19.332	16.818	75.682	30.14
Rate of leaf production	1.299	15.575	7.899	25.719	8.252
No. of inflorescence at the time of observation	9.859	31.396	25.685	66.93	43.287
No. of unopened inflorescence	1.736	23.805	5.65	5.634	2.763
No. of opened inflorescence undergoing pollination	0.67	19.937	9.813	24.228	9.95
No. of inflorescence in which pollination is over seed setting started	0.669	21.198	12.362	34.01	14.852
Total inflorescence/ year	11.184	31.578	14.051	19.799	12.879
No. of female flowers	24.511	25.207	21.629	73.627	38.232
Female flowers 1 month after pollination	16.136	21.224	15.265	51.731	22.617
No. of bunches/palm/year	9.063	23.027	12.998	31.866	15.115
No. of nuts/palm/year	69.424	46.776	35.293	56.928	54.855
No. of nuts/bunch	7.509	36.01	26.582	54.492	40.422
Pole to pole circumference	53.329	7.502	5.869	61.195	9.457

Table 11. Genetic parameters of morphological characters under study

Characters	Mean	PCV	GCV	\mathbf{H}^{2}	GAM(%)
Equitorial circumference	46.622	6.712	5.186	59.694	8.253
Fruit weight (with husk)	956.081	16.156	12.301	57.966	19.292
Volume of fruit	928.75	16.044	12.101	56.893	18.803
Nut weight (without husk)	515.236	16.892	13.032	59.522	20.712
Shell and meat weight	395	15.95	12.599	62.392	20.5
Kernel thickness	11.196	9.758	6.918	50.27	10.105
Quantity of liquid endosperm	119.074	33.991	26.248	59.631	41.755
Sugar content	6.461	20.299	12.412	37.388	15.634
Copra content	158.525	15.643	12.682	65.729	21.18

4.7. HETEROSIS

4.7.1. Relative heterosis

The hybrid Annur x GB showed negative significant heterosis over mid parent for height of the palm (-64.42), number of nuts per palm per year (-40.96) and size of unhusked nut (pole to pole circumference) (-6.16) (Table 13).

A positive significant heterosis over mid parent was observed by Annur x MYD for copra content (14.133), fruit weight (with husk) (25.22), nut weight (without husk) (18.41), shell and meat weight (without water) (19.41), size of unhusked nut (pole to pole circumference) (7.5), size of unhusked nut (equitorial circumference) (11.45), kernel thickness (17.5) and volume of fruit (28.78) whereas it showed negative heterosis for plant height (-43.19) (Table 12).

4.7.2. Heterobeltiosis

Annur x GB possessed negative significant heterosis over better parent for characters *viz*. height of the palm (-76.75), number of nuts per palm per year (-41.07), number of nuts per bunch (-40.36), size of unhusked nut (pole to pole circumference) (-10.99), size of unhusked nut (equitorial circumference) (-6.42) and volume of fruit (-16.5) (Table 13).

Annur x MYD showed positive significant heterosis over better parent for characters *viz*. fruit weight (with husk) (23.78), size of unhusked nut (equitorial circumference) (6.8), kernel thickness (13.7) and volume of fruit (25.5) whereas height of palm (-56.72) showed negative heterosis (Table 12).

4.7.3. Economic heterosis (Kerasree as check)

Annur x GB showed negative significant heterosis over standard check (kerasree) for all characters except size of unhusked nut (equitorial circumference) and kernel thickness whereas height of palm (-77.31), number of nuts per palm per year(-46.85), number of nuts per bunch (-40.51) and copra content (-16.35) showed negative significant heterosis over standard check (kerasree) for hybrid Annur x MYD.

4.7.4. Economic heterosis (Keraganga as check)

A negative significant heterosis over standard check (keraganga) was observed by Annur x GB for height of palm (-78.89), number of nuts per palm per year (-66.2), number of nuts per bunch (-57.67) and copra content (-11.84).

The hybrid Annur x MYD possessed positive significant heterosis over standard check (keraganga) for fruit weight (with husk) (22.15), nut weight (without husk) (20.99), shell and meat weight (without water) (19.71), size of unhusked nut (equitorial

Characters	Relative heterosis	Heterobeltiosis	Economic heterosis	Economic heterosis
			(Kerasree as check)	(Keraganga as check)
Height of palm	-43.19*	-56.72**	-77.31**	-77**
Nuts/palm/year	2.87	-4.83	-46.85**	-45.4**
Nuts/bunch	-11.398	-17.48	-40.51**	-39.67**
Copra content	14.133*	-0.602	-16.35**	-3.35
Fruit weight (with husk)	25.22**	23.78**	-5.06	22.15*
Nut weight (without husk)	18.41*	10.02	-13.16	20.99*
Shell and meat weight	19.41*	4.1	-8.99	19.71*
Pole to pole circumference	7.5*	4.21	-6.2	4.21
Equitorial circumference	11.45**	6.8*	2.44	12.05*
Kernel thickness	17.5**	13.7*	3.33	18.09**
Volume of fruit	28.78**	25.5**	-4.7	22.64**

Table 12. Relative heterosis, economic heterosis and heterobeltiosis of hybrid Annur x MYD

**Significant at 1% *Significant at 5%

Characters	Relative heterosis	Heterobeltiosis	Economic heterosis	Economic heterosis
			(Kerasree as check)	(Keraganga as check)
Height of palm	-64.42**	-76.75**	-79.193**	-78.89**
Nuts/palm/year	-40.96**	-41.07**	-67.08**	-66.2**
Nuts/bunch	-36.84	-40.36**	-58.28**	-57.67**
Copra content	-7.994	-9.2	-23.66**	-11.84*
Fruit weight (with husk)	-10.22	-16.43	-27.32**	-6.49
Nut weight (without husk)	-10.505	-12.85	-27.41**	1.14
Shell and meat weight	-6.423	-8.1	-16.67*	9.6
Pole to pole circumference	-6.16*	-10.99**	-10.69**	-0.7
Equitorial circumference	-3.7	-6.42*	-4.89	4.01
Kernel thickness	6.4	3.67	-5.8	7.61
Volume of fruit	-8.82	-16.5**	-27.62**	-6.84

Table 13. Relative heterosis, economic heterosis and heterobeltiosis of hybrid Annur x GB

**Significant at 1% *Significant at 5%

circumference) (12.05), kernel thickness (18.09) and volume of fruit (22.64). Height of palm (-77), number of nuts per palm per year (-45.4) and number of nuts per bunch (-39.67) had negative significant heterosis over standard check (keraganga) for hybrid Annur x MYD.

4.8. QUALITATIVE CHARACTERS

Shape of crown, petiole colour and fruit colour were the qualitative characters under study. Annur (*interse*) and its hybrids *viz*. Annur x MYD and Annur x GB showed hemispherical crown whereas all other coconut genotypes showed spherical crown shape. All genotypes showed green petiole colour except MYD (yellow) and Annur (*interse*) (Greenish orange – 5.88 % individuals). Annur (*interse*) possessed three types of fruit colour *viz*. greenish orange (70.5%), green (23.5%) and orange (5.8%). Green (63.6%) and greenish orange (36.3%) fruit colour was observed among hybrid, Annur x MYD. All other genotypes except MYD (yellow) showed green fruit colour.

Sl. No	Genotype	Shape of crown	Petiole colour	Fruit colour
1	Annur x Annur	Hemispherical	Green and greenish orange	Green, orange and greenish orange
2	Annur x GB	Hemispherical	Green	Green
3	Annur x MYD	Hemispherical	Green	Green and greenish orange
4	Gangabondam	Spherical	Green	Green
5	Malayan yellow dwarf	Spherical	Yellow	Yellow
6	Kerasree	Spherical	Green	Green
7	Keraganga	Spherical	Green	Green
8	West Coast Tall	Spherical	Green	Green

Table 14. Details on qualitative characters of hybrids, check and parents

4.9. REPRODUCTIVE CHARACTERS RELATED TO POLLINATION BEHAVIOR

Some extra reproductive characters related to the pollination behavior of the palms were collected and recorded in Table 15. Annur (*interse*) and its hybrids showed similar age of palms at first flowering (years) (5) compared with checks *viz*. kerasree (4.5) and keraganga (5). Highest value for period between emergence and opening (months) was observed for Keraganga (122) whereas MYD (98) showed the lowest value. Concordance between male and female phases were observed only in Annur (*interse*) and its hybrids similar to dwarf genotypes *viz*. MYD and GB and provide scope for self-pollination. Gap between male and female phases ranged from two (Kerasree) to three (Keraganga, WCT). Thus, there is high chance of cross pollination in these palms.

Genotypes	Age of palm at first flowering (years)	Period between emergence and opening (months)	Male phase (days)	Female phase (days)	Period between phases (days)	Concordance of phases if any (days)
Annur x Annur	5	114	17	7	0	6
Annur x GB	5	112	17	6	0	5
Annur x MYD	5	110	15	6	0	6
Gangabondam	3.5	105	17.9	6.1	0	5.9
Malayan yellow dwarf	4	98	16.3	6.6	0	6.3
Kerasree	5	116	19	5	2	0
Keraganga	4.5	122	18	5	3	0
West coast tall	6.5	120	19	4	3	0

Table 15. Details of reproductive characters related to pollination behavior

4.10. PEST AND DISEASES

Rhinoceros beetle (Plate 6) and eriophyid mite (Plate 5) were the major pests observed in the field. Pest severity of rhinoceros beetle was maximum for Annur x MYD (35.4 %) followed by Annur x GB (31.84 %) and Annur (*interse*) (26.03). No remedial measures were done against eriophyid mites in the field. For management of rhinoceros beetle, field sanitation was done. Cleaning of crown in regular intervals were also done to reduce rhinoceros beetle attack. As a prophylactic measure, equal quantities (250 g) of neem cake and sand were mixed and applied to the innermost 2-3 leaf axils.

4.11. QUANTITY AND QUALITY ASSESSMENT OF DNA

Quality and quantity of DNA was analysed by determining the absorbance at 260 nm using UV spectrophotometer and visual comparison by agarose gel electrophoresis. UV absorbance ratio from spectrophotometer ranged from 1.53 to 1.84-indicating the purity of DNA. The quantity of DNA obtained ranged from 350 to 1300 ng/ μ l.

Genotypes	A260/280	A260/230	DNA concentration (ng/µl)
West Coast Tall	1.62	2.06	1200
Malayan yellow dwarf	1.73	1.28	350
Kerasree	1.53	1.62	500
Keraganga	1.65	1.75	1300
Gangabondam	1.69	1.54	1050
Annur (interse)	1.84	2.27	550

Table 16. Quantity and quality of isolated genomic-DNA

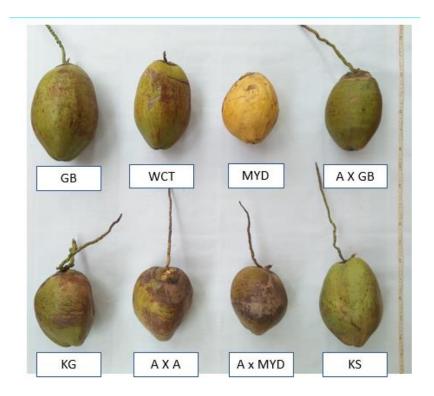


Plate 4: Comparison of nuts of parents, hybrids and check



Plate 5: Eriophyid mite attack



Plate 6: Rhinoceros beetle attack

4.12. SSR MARKER ANALYSIS OF COCONUT GENOTYPES

4.12.1. Standardisation of PCR conditions

For good amplification of different coconut samples, PCR amplification condition were optimised as detailed below. Both the concentration of different components of reaction mixture and the thermal profile of primers used were standardized. The annealing temperature of primers were optimised at 58°C, with an exception of temperature at 51°C (CnCirA3 and CnCirC12) and 56°C (CNZ10 and CAC06).

Mastermix (25	μl)	Thermal profile
DNA template	– 50 ng/µl	Initial denaturation: 94°C - 10 min
10X PCR buffer	– 2.5 µl	Denaturation: 94°C - 1 min
10 mM dNTPs	– 1 µl	Annealing:58°C, 54 °C and 51°C-1 min
Taq polymerase (5U)	$-0.2 \ \mu l$	Extension: $72^{\circ}C - 30$ sec
Forward primer (0.01 mM	I) −1µl	Final extension: 72°C - 7min
Reverse primer (0.01 mM	I) – 1 µl	Hold: 4°C
Ultrapure water		35 cycles

4.12.1. Screening of SSR markers

A total of 34 SSR primers were screened for identifying polymorphism between six coconut genotypes *viz*. Annur (*interse*), WCT, MYD, GB, Kerasree and Keraganga. All the SSR markers used were able to amplify the genomic DNA.

The primers *viz*. CAC 03, CAC 06, CAC 65, CNZ 04, CNZ 06, CNZ 10, CNZ 46, CnCir 01, CnCir 51, CnCirB6, CnCir E10, CnCir A3, CnCir C12 and CnCir HII produced single monomorphic amplicon. Whereas, CAC 04 produced two monomorphic amplicons (Plate 10). As they could not produce distinct amplicons, not selected for future studies.

Primers such as CnCir F2, CnCir H7, CnCir G11, CnCir H4 and CNZ 40 produced single polymorphic amplicon (Plate 8). All of these primers were 100 per cent polymorphic in nature and were selected for future studies.

Primers *viz*. CAC 02, CAC 11, CnCir A9, CnCir B12, CnCir E2, CnCir C7, CnCir E12, CnCir C3 and CnCir C5 produced two distinct polymorphic amplicons (Plate 7). So, these primers were selected and recommended for future studies.

Primers CAC 08, CAC 10, CNZ 05, CNZ 12 and CNZ44 produced two amplicons, one monomorphic and other polymorphic (Plate 9), showing 50 per cent polymorphism

4.12.2. Selection of polymorphic SSR primers

Only those primers which were 100 per cent polymorphic are selected and recommended for future screening of hybrids. Thus, fourteen SSR markers were selected *viz.* CnCir F2, CnCir H7, CnCir G11, CNZ 40, CAC 02, CAC 11, CnCir A9, CnCir B12, CnCir E2, CnCir C7, CnCir H4, CnCir E12, CnCir C3 and CnCir C5. The details of SSR amplification is provided in Table 18.

4.12.2.1. CAC02

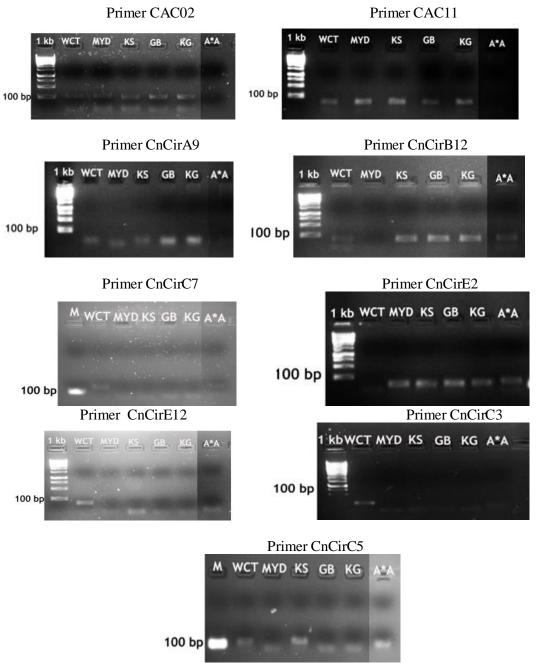
The primer CAC02 could generate two polymorphic amplicons which were 80 bp and 100 bp in size, the bands were faint but distinct. Annur (*interse*) was found to be polymorphic in nature

4.12.2.2. CAC11

Two distinct polymorphic amplicons were produced by the primer CAC11 and were 80 bp and 90 bp in length. The first band of 80 bp was faint and the second band of 90 bp was clear. Both were absent in Annur (*interse*).

4.12.2.3. CNZ40

CNZ40 could generate only one amplicon of size 100 bp by the amplification of coconut genotypes. Only Kerasree and Annur (*interse*) were able to generate bands.



Molecular characterisation of Annur *interse* palms, pollen parents used for hybridisation and varieties used as checks using SSR markers

Plate 7 Gel profile of primers with two distinct polymorphic amplicons

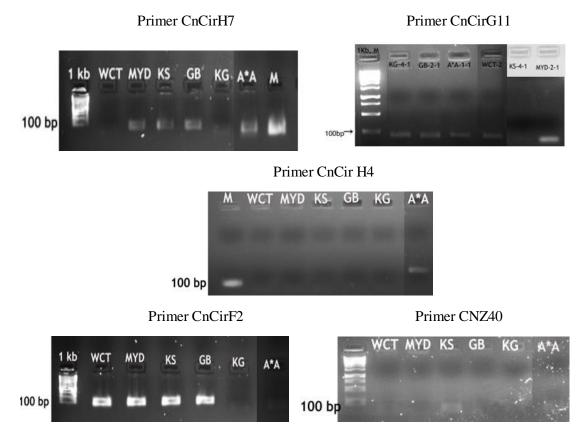
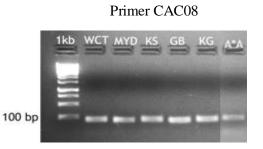
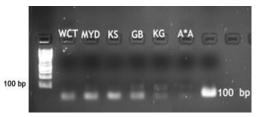


Plate 8. Gel profile of primers having single polymorphic amplicon







Primer CNZ05



Plate 9a. Gel profile of primers with two amplicons - one polymorphic and one monomorphic

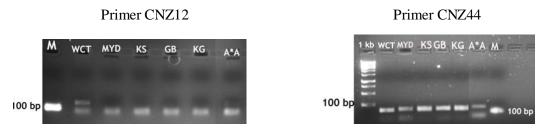


Plate 9b. Gel profile of primers with two amplicons - one polymorphic and one monomorphic

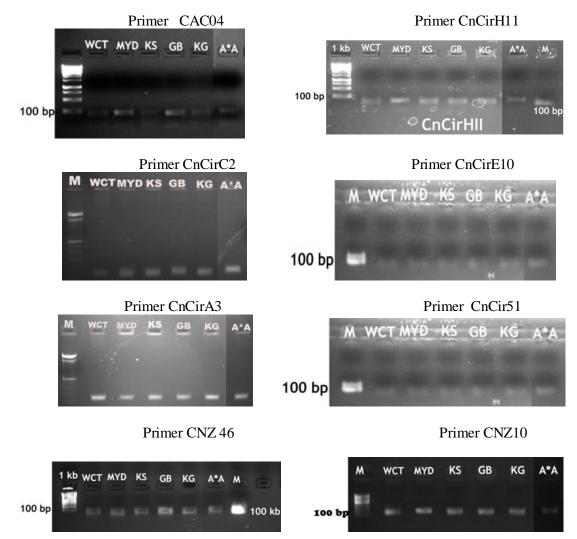
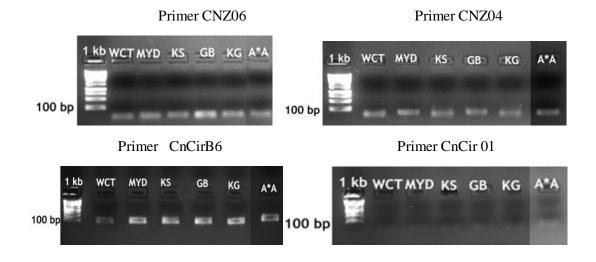


Plate 10a. Gel profile of primers with monomorphic amplicon



Primer CAC65

	1 kb	wст	MYD	KS	GB	KG	A*A
100 Бр	i.i						'n

Primer CAC03

Primer CAC06

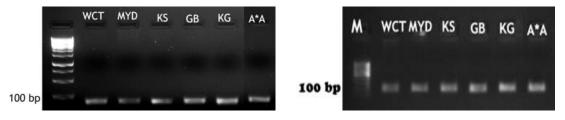


Plate 10b. Gel profile of primers with monomorphic amplicon

4.12.2.4. CnCirA9

CnCirA9 was able to amplify two clear polymorphic amplicons of size 90 bp and 85 bp by the amplification of coconut genotypes. MYD and Annur (*interse*) could not generate bands in 90 bp whereas in 85 bp only MYD was able to generate bands.

4.12.2.5. CnCirB12

Amplification of DNA of six coconut cultivars with the selected primer CnCirB12 produced two clear polymorphic amplicons of size 100 bp and 90 bp. All genotypes except MYD showed bands in 100 bp whereas only Annur (*interse*) and WCT showed amplicons in 90 bp.

		Total number of	Type of amplicons			
Sl no.	Primer	amplicons	High intensity bands	Faint bands		
1	CAC02	2	0	2		
2	CAC03	1	1	0		
3	CAC04	2	1	1		
4	CAC06	1	1	0		
5	CAC08	2	1	1		
6	CAC10	2	1	1		
7	CAC11	2	1	1		
8	CAC65	1	1	0		
9	CNZ04	1	1	0		
10	CNZ05	2	1	1		
11	CNZ06	1	1	0		
12	CNZ10	1	1	0		
13	CNZ12	2	2	0		
14	CNZ40	1	1	0		
15	CNZ44	2	2	0		
16	CNZ46	1	1	0		
17	CnCirA9	2	2	0		
18	CnCir01	1	0	1		
19	CnCir51	1	0	1		

Table 17. Screening of 34 SSR primers

S1.		Total number of	Type of amplicons		
No.	Primer	amplicons	High intensity bands	Faint bands	
20	CnCirE10	1	1	0	
21	CnCirA3	1	1	0	
22	CnCirC12	1	1	0	
23	CnCirB6	1	1	0	
24	CnCirB12	2	2	0	
25	CnCirE2	2	1	1	
26	CnCirC7	2	0	2	
27	CnCirH4	1	1	0	
28	CnCirE12	2	2	0	
29	CnCirC3	2	1	1	
30	CnCirF2	1	1	0	
31	CnCirH7	1	1	0	
32	CnCirG11	1	1	0	
33	CnCirHII	1	1	0	
34	CnCirC5	2	2	0	

4.12.2.6. CnCirC3

Two distinct polymorphic amplicons were produced by the primer CnCirC3 were 80 bp and 90 bp in length. The first band of 80 bp was faint and the second band of 90 bp was clear. In 90 bp, only WCT was able to generate amplicons. Whereas, in 80 bp WCT was unable to produce amplicons.

4.12.2.7. CnCirC5

The primer CnCirC5 generated two clear polymorphic amplicons of size 100 bp and 95 bp by the amplification of coconut genotypes. In 100 bp, WCT and kerasree showed bands. In 95 bp, WCT was unable to show bands.

4.12.2.8. CnCirC7

Amplification of coconut cultivars using SSR primer CnCirC7 produced two faint and distinct amplicons of size 110 bp and 90 bp. Annur (*interse*) and WCT produced bands at 110 bp whereas all genotypes except Annur (*interse*) and WCT produced bands at 90 bp.

4.12.2.9. CnCirE2

The primer CnCirE2 could generate two amplicons of size 105 bp (clear) and 95 bp (faint) by the amplification of coconut genotypes. All genotypes except WCT produced amplicons in 105 bp and only WCT and Annur (*interse*) were able to generate amplicons in 95 bp

4.12.2.10. CnCirE12

Two clear and distinct amplicons were generated by the primer CnCirE12. It produced bands of size 95 bp and 85 bp. Only WCT produced band in 95 bp whereas all genotypes except WCT showed bands in 80 bp.

Sl no.	Primer	Total no. of amplicons	No. of polymorphic amplicons	No. of alleles per locus	Polymor- phism %	Remarks
1	CAC02	2	2	2	100	Selected
2	CAC03	1	0	1	0	-
3	CAC04	2	0	2	0	-
4	CAC06	1	0	1	0	-
5	CAC08	2	1	2	50	-
6	CAC10	2	1	2	50	-
7	CAC11	2	2	2	100	Selected
8	CAC65	1	0	1	0	-
9	CNZ04	1	0	1	0	-
10	CNZ05	2	1	2	50	-
11	CNZ06	1	0	1	0	-

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
12	CNZ10	1	0	1	0	-
13	CNZ12	2	1	2	50	-
14	CNZ40	1	1	1	100	Selected
15	CNZ44	2	1	2	50	-
16	CNZ46	1	0	1	0	-
17	CnCir01	1	0	1	0	-
18	CnCir51	1	0	1	0	-
19	CnCirA3	1	0	1	0	-
20	CnCirA9	2	2	2	100	Selected
21	CnCirB6	1	0	1	0	-
22	CnCirB12	2	2	2	100	Selected
23	CnCirC3	2	2	2	100	Selected
24	CnCirC5	2	2	2	100	Selected
25	CnCirC7	2	2	2	100	Selected
26	CnCirC12	1	0	1	0	-
27	CnCirE2	2	2	2	100	Selected
28	CnCirE10	1	0	1	0	-
29	CnCirE12	2	2	2	100	Selected
30	CnCirF2	1	1	1	100	Selected
31	CnCirG11	1	1	1	100	Selected
32	CnCirH4	1	1	1	100	Selected
33	CnCirH7	1	1	1	100	Selected
34	CnCirH11	1	0	1	0	-
Total	I	49	28	49	-	
Avera	ige	1.44	0.823	1.44	48.5	

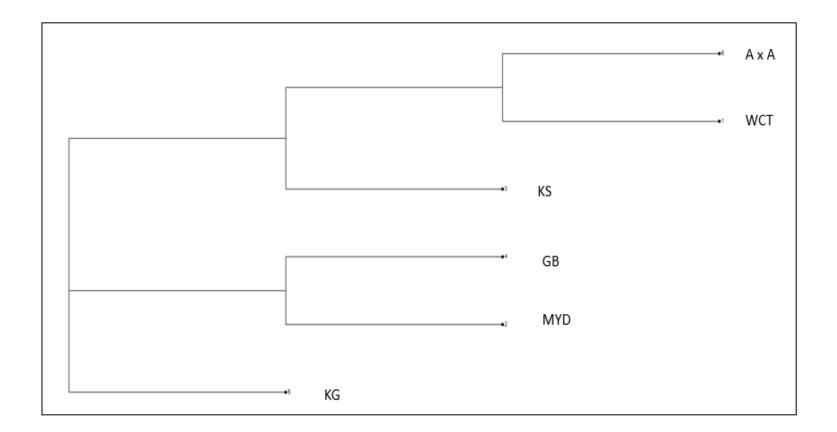


Fig 26. Dendrogram of SSR markers using parental and check coconut palms

4.12.2.11. CnCirF2

Only one amplicon was generated by the primer CnCirF2. It produced band of size 100 bp. Here Keraganga and Annur (*interse*) were unable to produce bands in 100 bp.

4.12.2.12. CnCirG11

CnCirG11 produced only single amplicon of size 100 bp. All genotypes except Keraganga showed bands in 100 bp.

4.12.2.13. CnCirH4

Single amplicon was produced by the primer CnCirH4. It produced clear bands of size 110 bp. In 110 bp, only Annur (*interse*) could generate band.

4.12.2.14. CnCirH7

Only one amplicon of size 100 bp was generated by the primer CnCirH7, which was clear. Only WCT and Keraganga were unable to produce bands in 100 bp.

4.13. DIVERSITY ANALYSIS OF SIX COCONUT CULTIVARS

The 34 SSR primers generated a total of 49 amplicons out of which 28 amplicons were found to be polymorphic. The markers produced an average of 48.5 per cent polymorphism and one to two alleles per locus with a mean of 1.44 alleles per locus (Table 18).

Diversity analysis was done based on presence (1) or absence (0) of bands with respect to six genotypes. Computed the genetic distances using dice coefficient and neighbour joining tree and were calculated using DARwin ver 6.0 software. The bootstrap was 20,000. Dendrogram was constructed by neighbour joining.

The dendrogram showed grouping the coconut genotypes into three major clusters (Fig. 26). Cluster I include three cultivars namely, Annur (*interse*), WCT and Kerasree. Cluster II included two dwarf coconut cultivars namely MYD and GB. Keraganga alone was part of Cluster III. Cluster I again subdivided in to two groups. First group included

Annur (*interse*) and WCT whereas Kerasree included in the second group. This indicated the similarity of Annur (*interse*) and WCT genotypes. The dwarf coconut palms *viz*. MYD and GB grouped in same clusters indicated similarity among these genotypes.

Dissimilarity matrix of six coconut genotypes based on the SSR markers was also generated. Dissimilarity values ranged from 0.04 (Keraganga and GB) to 0.230 (MYD and Annur (*interse*) (Table 21). This indicates most dissimilarity was observed between MYD and Annur (*interse*).

Cluster no.	Number of cultivars in each cluster	Name of cultivars
Cluster I	3	Annur (interse), WCT, Kerasree
Cluster II	2	MYD, GB
Cluster III	1	KG

Table 20. Grouping of accessions based on SSR data using DARwin ver. 6.0 software

Genotypes	WCT	MYD	Kerasree	GB	Keraganga
MYD	0.220				
Kerasree	0.226	0.135			
GB	0.194	0.052	0.108		
Keraganga	0.184	0.066	0.123	0.04	
Annur x Annur	0.189	0.230	0.211	0.179	0.194

DISCUSSION

5. DISCUSSION

Coconut (*Cocos nucifera* L.) cultivars are classified into two major groups *viz*. tall and dwarf palms. The tall coconut palms are widely cultivated owing to their desirable features including yield and nut characters. However, increasing labour charges and unavailability of labourers make them less preferable nowadays. Dwarf palms are early bearer and easy harvesting is possible. However, production of poor quality nuts and higher susceptibility to pest and diseases make dwarf palms less acceptable by farmers. In this context, production of hybrids which contains desirable features of tall and dwarf palms are essential. With this objective, a hybridization programme was initiated in 2005 using local dwarf ecotype of West Coast Tall (WCT) called 'Annur' and the popular dwarf types *viz*. Malayan Yellow Dwarf (MYD) and Gangabondam (GB) to develop D x D hybrids. The present investigation was undertaken to characterise these D x D hybrids using morphological and molecular markers. The results of the study are discussed in this section under different subheadings.

5.1. MORPHOLOGICAL CHARACTERISATION OF GENOTYPES

All the vegetative characters *viz.* height of the palm, girth of palm, petiole length, leaf length, internode length, number of green leaves and rate of leaf production showed variation among eight genotypes which include fifty-eight *interse* or hybrids, corresponding parents and check varieties (16 no.). Variability in vegetative characters in different cultivars of coconut was reported earlier in several investigations such as Louis and Chopra (1991) for height of the palm; Balakrishnan and Namboodiri (1987) and Sreelatha (1987) for girth of palm and number of green leaves; Sreelatha (1987), Pillai *et al.*, (1991) and Sindhumole and Ibrahim (2001) for petiole length and leaf length. However, absence of variation among genotypes was observed by Ramanathan (1984) for number of green leaves and Sindhumole and Ibrahim (2001) for height of the palm, Girth of palm and number of green leaves in a group of tall palms.

Annur is the dwarf ecotype of WCT with an average height of 2.725 m which is significantly different from WCT (16.373 m). When it is crossed with other dwarfs, the

two hybrids also exhibited the short stature. Though further reduction of height was observed for hybrid palms compared to the female parent Annur, it was not statistically significant. Girth of palm (20 cm) of Annur (*interse*) and its hybrids showed on par results with WCT. The increased basal girth in palms indicates the presence of bole which is a characteristic feature of tall cultivars like WCT. In the present study, it was found bole character present in Annur, though it is a dwarf, is also transmitted into its hybrids. However, Perera *et al.* (2016) reported that inheritance of height was not strongly associated with the presence of bole.

The girth of palm (150 cm), petiole length, leaf length, internode length and number of green leaves were on par for WCT, Annur (*interse*) and its hybrids. Hybrids and their female parent showed on par result for rate of leaf production and highest value for rate of leaf production among the eight genotypes was shown by Annur (*interse*).

All the reproductive characters except number of unopened inflorescence and total number of inflorescence per palm per year showed variation among the genotypes. Similar observations were reported by Potty *et al.*, (1980), Rajamony *et al.*, (1983) and Sindhumole and Ibrahim (2001) for number of female flowers per inflorescence. Absence of variation among genotypes was reported for total number of inflorescence per palm per year (Sindhumole and Ibrahim, 2001). However, between Annur (*interse*) and its hybrids these characters were on par. When compared with male parents or check palms, they were showing significant variation.

Among eight genotypes, significant variation was observed for number of nuts per palm per year and number of nuts per bunch. The extent of variability in coconut genotypes is reported earlier by many researchers (Satyabalan and Pillai (1977), Potty *et al.*, (1980), Balakrishnan and Kannan (1991) and Sindhumole and Ibrahim (2001)). Nut characters namely size of unhusked nut (pole to pole circumference and equitorial circumference), fruit weight (with husk), nut weight (without husk), volume of fruit, shell and meat weight, kernel thickness, quantity of liquid endosperm and copra content showed significant variation among genotypes. When these characters were compared between Annur and the two hybrids, it was found that number of nuts per palm per year and number of nuts per bunch were on par in Annur and Annur x MYD while that was lower in hybrid Annur x GB. This may be due to non attainment of yield stabilization in these palms. Nut weight and kernel thickness were significantly higher in Annur x MYD whereas these were on par in Annur and Annur x GB. Copra content in Annur and both hybrids were on par. In general, Annur x MYD showed superior performance for nut characters compared to Annur x GB.

5.2. COMPARISON OF ANNUR (*INTERSE*) AND ITS HYBRIDS WITH RESPECTIVE CHECK VARIETIES

Comparison of Annur (*interse*) and WCT revealed that all major characters (22 out of 28 characters studied) showed on par results. Among the six characters which showed significant variation, the most important one was significant reduction in height. This indicates that Annur is the dwarf ecotype of WCT. The hybrid, Annur x GB was compared with the check variety Keraganga which is a released T x D hybrid produced from the cross WCT x GB. Major characters such as height of palm and number of nuts per palm per year showed significant difference among these two genotypes whereas all nut characters of Annur x GB were on par with Keraganga. Comparison of Annur x MYD and Kerasree, a released T x D hybrid produced from the cross WCT x MYD, showed that there are significant differences for major characters *viz*. height of the palm, number of nuts per palm per year and copra content. The significant difference observed by the hybrids of Annur for yield compared to the check varieties was due to non-attainment of stabilized yield.

Age of palm at first flowering (years) was on par among Annur (*interse*), its hybrids and the respective checks which indicate the early flowering nature compared to the tall variety, WCT. Another important observation is that the flowering phases of Annur (*interse*) and its hybrids showed concordance of phases as in dwarf palms. This may be an indication for self- pollinating behavior of Annur (*interse*), Annur x GB and Annur x MYD which needs to be confirmed through further analysis. This may be a significant result in breeding of superior quality D x D hybrids from which more or less true to type progenies can be obtained.

5.3. 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 show significant palm to palm variation. Hence inorder to identify the best progeny combination, Sd + Avg method was used. For all palm characters except height of palm, higher value than Sd + Avg are considered whereas lower value was taken for height of palm. The palms having maximum desirable characters were considered as superior. Palm no. 1, 2, 15, 19, 20 and 28 showed better performance among 34 palms of Annur (*interse*). Three palms, *viz*. palm no. 53, 55 and 56 were better performers among Annur x GB. Out of 11 palms, palm no. 43, 44, 47 and 49 were obtained as better palms among hybrid Annur x MYD. The better performing palms from each cross can be utilized for future study purpose. For molecular characterization of hybrids better palms from each crosses can be utilized.

5.4. CORRELATION STUDIES ON YIELD

When a breeder selects a character from a population the population is not only improved for that character but also improved for all related characters. Analysis of correlation provides information on the degree and nature of the relationship between characters in a group, thereby helping in the selection of two or more characters to be effective and at the same time help in overall improvement. Since the improvement of yield is the primary objective of any breeding programme, information on the association of other yield traits helps to identify the characteristics that could form the basis of selection. In this analysis, the genotypic correlation of twenty-seven characters with nut yield and their interaction with each other was estimated.

The analysis revealed significant positive correlation of number of nuts per palm per year (nut yield) with height of the palm, girth of the palm at 1.5m height, girth of the palm at 20cm height, leaf length, number of green leaves, rate of leaf production, total number of inflorescence at the time of observation, number of unopened inflorescence, number of opened inflorescence undergoing pollination, number of inflorescence in which pollination is over and seed set started, total inflorescence per palm per year, number of female flowers per inflorescence and number of female flowers one month after pollination. It indicates that selection based on these vegetative and reproductive characters is effective in improving the yield characters. Similar results were observed for vegetative and reproductive characters by Sindhumol and Ibrahim (2001).

The traits like number of bunches per palm per year, number of female flower one month after pollination and number of nuts per bunches directly contribute to yield while the nut characters like size of unhusked nut (pole to pole circumference), fruit weight with husk, nut weight without husk, fruit volume, shell and meat weight without water, kernel thickness at maturity, quantity of liquid endosperm and copra content, and number of nuts per bunch can simultaneously improve yield.

Only petiole length and internode length showed non-significant negative correlation with number of nuts per palm per year (nut yield), whereas size of unhusked nut (equitorial circumference) and sugar content showed non-significant positive correlation.

In many previous report also similar results on positive significant correlation of nut yield with plant height (Selvaraju and Jayalekshmi, 2011), girth of stem (Sreejith, 2006), petiole length (Renuga, 1999; Natarajan *et al.*, 2010 and Suchitra and Paramaguru, 2018), number of green leaves (Renuga, 1999; Sreejith, 2006; Natarajan *et al.*, 2010; Selvaraju and Jayalekshmi, 2011; Sathishkumar, 2016 and Suchitra and Paramaguru, 2018), leaf length (Natarajan *et al.*, 2010), number of female flowers per inflorescence (Sreejith, 2006; Selvaraju and Jayalekshmi, 2011; Sathishkumar, 2016 and Suchitra and Paramaguru, 2018), total inflorescence per palm per year (Sreejith, 2006 and Suchitra and Paramaguru, 2018), number of bunches per palm per year (Kalathiya and Sen, 1991; Manju, 1992; Sreejith, 2006; Selvaraju and Jayalekshmi, 2011; Sathishkumar, 2011; Sathishkumar, 2016 and Suchitra and Paramaguru, 2018), number of bunches per palm per year (Kalathiya and Sen, 1991; Manju, 1992; Sreejith, 2006; Selvaraju and Jayalekshmi, 2011; Sathishkumar, 2016 and Suchitra and Paramaguru, 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru, 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru, 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru, 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per bunches (Sreejith, 2006; Selvaraju and Paramaguru), 2018), number of nuts per

Jayalekshmi, 2011; Sathishkumar, 2016 and Suchitra and Paramaguru, 2018), size of unhusked nut (equitorial circumference) (Selvaraju and Jayalekshmi, 2011), size of unhusked nut (pole to pole circumference) (Selvaraju and Jayalekshmi, 2011), fruit weight with husk (Selvaraju and Jayalekshmi, 2011 and Sathishkumar, 2016), nut weight without husk (Selvaraju and Jayalekshmi, 2011 and Sathishkumar, 2016) and copra content (Liyanage and Sakai, 1960) were observed.

Number of nuts per bunch showed positive significant correlation with number of female flowers per inflorescence (Pieries, 1934), total inflorescence per palm per year (Abeywardena, 1976), number of green leaves (Satyabalan, 1976 and Abeywardena, 1976). Number of bunches per palm per year possessed positive significant correlation with number of nuts per bunch, number of female flowers per inflorescence (Nampoothiri *et al.*, 1999; Kalathiya and Sen, 1991; Manju, 1992 and Sathishkumar, 2016).

In contrast to these results, a positive non-significant correlation of nut yield with plant height (Satyabalan, 1976), leaf length (Abeywardena, 1976), kernel thickness (Louis, 1983) and number of green leaves (Manju, 1992) were reported. Negative significant correlation was observed among nut yield and stem girth (Renuga, 1999).

5.5. PATH ANALYSIS

Correlation of yield and its component characters alone do not provide accurate information on the contribution of these characteristics to yield and are insufficient to interpret cause and effect. Path coefficient analysis divides the coefficient of correlation into measures of direct and indirect effects of independent characters on yield. Thus, it helps to ensure that the correlation of component characters on yield is due to their direct or indirect effect *via* other characters. There is a true relationship between the component character and the yield if the correlation of that character to the yield is due to the direct effect and the direct selection can be made for that trait. However, some characters will be influencing the yield by their influence on other characters which may not be revealed in a correlation study. Path analysis helps to identify such characters and the path through

which they influence the yield so that selection of these indirectly influencing characters also will improve the yield.

In the present investigation, 23 characters significantly correlated with nut yield were considered for path analysis. Among them, for fruit weight (with husk) maximum positive direct effect on nut yield was observed. Also, volume of fruit and kernel thickness showed a very high positive direct effect indicating the importance of the character in selection programme.

Number of nuts per bunch, size of unhusked nut (pole to pole circumference), copra content, quantity of liquid endosperm, girth of palm at 20 cm, shell and meat weight (without water) and nut weight (without husk) had a high direct effect on yield. It indicates true relationship between yield and these characters. The correlation of these characters to yield is effective and selection of superior palms can be done by directly selecting based on these characters.

Number of inflorescence in which pollination is over and seed setting started, rate of leaf production and number of bunches per palm per year recorded only moderate direct effect. Positive but low direct effect was shown by total inflorescence per palm per year.

Nut weight (without husk) and volume of fruit recorded very high negative direct effect on yield but indirectly these characters influenced number of nuts per palm per year through the high indirect effect through fruit weight with husk. Though a high negative direct effect on yield was shown for number of female flowers one month after pollination, it had positive influence on yield through number of nuts per bunch indicating selection based on this character will be effective.

A moderate negative direct effect was observed for number of green leaves and leaf length which is reported in earlier studies also (Selvaraju, 2008). Number of opened inflorescence undergoing pollination and kernel thickness had low negative direct effect on yield. The direct effects of number of unopened inflorescence and total number of inflorescence in the crown at the time of observation were negative and negligible.

113

Earlier concurrent results have been reported on positive direct effect of nut yield with number of female flower per inflorescence (Louis, 1981; Selvaraju, 2008), Size of unhusked nut (pole to pole circumference) (Selvaraju, 2008), number of nuts per bunch (Sreejith, 2006; Natarajan *et al.*, 2010), number of bunches per palm per year (Sreejith, 2006), fruit weight (with husk) (Selvaraju, 2008).

In contrast to the result, nut yield showed positive direct effect with plant height (Selvaraju, 2008), girth of stem (Sreejith, 2006), number of green leaves (Louis, 1981; Ramanathan, 1984 and Natarajan *et al.*, 2010), kernel thickness (Sreejith, 2006), nut weight (without husk) (Selvaraju, 2008) and also negative direct effect with number of bunches per palm per year (Selvaraju, 2008).

5.6. GENETIC PARAMETERS

Another way of describing the amount of variability is the coefficient of variation which offers information on the extent and magnitude of the variance. It provides information about whether the variations are caused by genetic factors or environmental influences. In this analysis, PCV was higher than GCV for all characters considered, indicating a higher environmental effect on these characters in coconut. Similar patterns have been identified by Renuga (1999), Manju and Gopimony (2001), Sindhumole and Ibrahim (2001) and Jerard (2002) and Natarajan *et al.* (2010). PCV and GCV were highest for height of the palm. But the difference between GCV and PCV was low. This indicates low environmental effect and scope for this character to be improved by hybridization and selection.

High GCV with correspondingly high values of PCV was observed for internode length, total number of inflorescence at the time of observation, number of female flower per inflorescence, number of nuts per palm per year, number of nuts per bunch and quantity of liquid endosperm. Hence selection is more effective based on these characters. The results were consistent with the findings of Louis (1981) and Sindhumol and Ibrahim (2001) for number of female flowers per inflorescence, number of nuts per palm per year; Muluk (1987) for plant height; Patil *et al.*, (1993) for number of nuts per palm per year; Manju and Gopimony (2001) for number of nuts per palm per year and number of nuts per bunch; Selvaraju and Jayalekshmi (2011) for number of nuts per palm per year.

Moderate to high PCV and moderate GCV were found for girth of the palm, petiole length, leaf length, number of green leaves, rate of leaf production, number of opened inflorescence undergoing pollination, number of inflorescence in which pollination is over seed set started, total inflorescence per palm per year, number of female flowers one month after pollination, number of bunches per palm per year, fruit weight (with husk), volume of fruit, nut weight (without husk), shell and meat weight (without water) and copra content indicating a higher environmental effect. Hence selection based on these characters need necessary experimental precautions to eliminate environmental effect like including replicated data over the season or year. The results obtained by Manju and Gopimony (2001) for number of green leaves; Sindhumole and Ibrahim (2001) for copra content and Natarajan *et al.*, (2010) for petiole length and leaf length also are in agreement with present study.

Heritability denotes the inherited portion of the total phenotypic variance present in the population, which provides accurate information on the influence of the environment on the characters as well as the gene action involved in the expression of polygenic traits. The measure of genetic gain under selection is genetic advance. Heritability estimates and genetic gain under selection are more effective than heritability estimates alone (Johnson *et al.*, 1955).

For certain characters, high heritability was estimated, which indicates the highly heritable nature of these characters and the minimal environmental influence in their expression. High heritability estimates recorded for height of the palm, girth of the palm at 1.5m, leaf length, number of green leaves, total number of inflorescence in the crown at the time of observation, number of female flowers per inflorescence, size of unhusked nut (pole to pole circumference), shell and meat weight (without water) and copra content. Medium heritability was recorded for girth of the palm at 20cm, petiole length, internode length, number of inflorescence in which pollination is over and seed setting started,

115

number of female flowers one month after pollination, number of bunches per palm per year, number of nuts per palm per year, number of nuts per bunches, fruit weight (with husk), nut weight (without husk), volume of fruit, kernel thickness, quantity of liquid endosperm and sugar content. The findings were supported by the observations of Liyanage and Sakai (1960) for copra content and number of nuts per palm per year; Meunier *et al.*, (1984) for copra content; Nambiar and Nambiar (1970) for number of female flowers per inflorescence; Manju and Gopimony (2001) for number of nuts per palm per year and number of nuts per bunches.

In contrary, high heritability was reported by Liyanage and Sakai (1960) for fruit weight (with husk); Meunier *et al.*, (1984) for number of nuts per palm per year; Patil *et al.*, (1993) for number of bunches per palm per year and number of nuts per bunch; Selvaraju and Jayalekshmi (2011) for fruit weight (with husk), nut weight (without husk) and number of nuts per palm per year likewise medium heritability was observed by Manju and Gopimony (2001) for number of green leaves.

In the study, high genetic advance was exhibited by height of the palm (98.394%) followed by number of nuts per palm per year, total number of inflorescence at the time of observation, internode length, quantity of liquid endosperm, number of nuts per bunch, number of female flower per inflorescence, number of green leaves, girth of palm at 20cm, leaf length, number of female flower one month after pollination, copra content, nut weight (without husk) and shell and meat weight (without water). The moderate genetic advance was observed for fruit weight (with husk) (19.292%) followed by volume of fruit, girth of palm at 150cm, petiole length, sugar content, number of bunches per palm per year, number of inflorescence in which pollination is over seed set started, total number of inflorescence per palm per year and kernel thickness. This indicates that by selecting five per cent superior individuals the genetic improvement possible for fruit weight (with husk) will be 19.292 per cent.

The results were in conjunction with the observations of Louis (1981), Balakrishnan *et al.*, (1991), Renuga (1999), Selvaraju and Jayalekshmi (2011) and Suchitra and Paramaguru (2018) for number of nuts per palm per year, Manju and Gopimony (2001) for number of nuts per bunch, number of female flowers per inflorescence and number of nuts per bunch; Ganeshamoorthy *et al.*, (2002) for copra content, number of nuts per palm per year and nut weight (without husk) and Selvaraju and Jayalekshmi (2011) for nut weight (without husk).

In contrary, Selvaraju and Jayalekshmi (2011) and Ganesamurthy *et al.*, (2002) reported high genetic advance for fruit weight (with husk) and Manju and Gopimony (2001) concluded low genetic advance for number of green leaves.

High heritability coupled with high genetic advance was observed for height of the palm, leaf length, number of green leaves, number of inflorescence at the time of observation, number of female flowers per inflorescence, copra content and shell and meat weight (without water). Characters exhibiting high heritability along with high genetic advance are most likely governed by additive gene action and hence direct phenotypic selection may be effective for their improvement. Medium heritability coupled with high genetic advance was observed for girth of palm (20 cm), internode length, number of nuts per palm per year, number of nuts per bunch, nut weight (without husk) and quantity of liquid endosperm. These characters are also governed by additive gene action and hence selection is effective for these characters. Size of unhusked nut (pole to pole circumference) showed high heritability and low genetic advance due to non additive gene action. Selection of this character may not produce the expected results.

5.7. HETEROSIS

The hybrids were evaluated over mid parent, better parent and standard check for 11 traits under investigation *viz*. height of palm, number of nuts per palm per year, number of nuts per bunch, size of unhusked nut (pole to pole and equitorial circumference), fruit weight (with husk), nut weight (without husk), volume of fruit, shell and meat weight (without water), kernel thickness and copra content. F1's heterotic response indicates genetic diversity among the involved parents. The performance of the hybrid can not be

assessed on the basis of relative heterosis and heterobeltiosis alone, since acceptable standard heterosis is also a major consideration for commercial acceptance. Therefore heterosis estimation over standard check(standard heterosis) is a stronger parameter to determine the usefulness of the hybrid. The commercial hybrids Kerasree and Keraganga are taken as standard checks for this analysis.

Annur x GB and Annur x MYD showed negative significant heterosis over mid parent, better parent and standard check (both Kerasree and Keraganga) for height of the palm, which were preferable by the farmers. None of the yield and nut characters showed positive significant heterosis over better parent, mid parent and standard check for hybrid, Annur x GB. This may be due to non-attainment of stabilized yield. Whereas, the hybrid, Annur x MYD showed positive significant heterosis over mid parent and better parent for yield and nut characters though as the same age as Annur x GB. However it also showed negative significant standard heterosis (Kerasree as check) for yield, number of nuts per bunch and copra content. But the standard heterosis measured using Keraganga as check for nut characters in Annur x MYD was positive and significant.

Short statured palms with high yield and good quality nuts is the ideal plant type in coconut. However, in the present study, number of nuts per palm per year showed negative significant standard heterosis for both hybrids. This may be because the hybrid palms are only 10 year old and stabilized yield was not attained. Also, for these characters PCV was high showing environmental effect. Hence further observations on yield data needs to be continued for two more years to assess the full potential of these two hybrid combinations.

5.8. SSR MARKERS

5.8.1. Standardisation of annealing temperature

For good amplification of different coconut samples, both reaction mixture component concentration and the thermal profile of primers to be standardised. Annealing temperatures of 30 SSR markers including CnCirB6, CnCirH7, CnCirC7, CnCirE12, CnCirF2 and CnCirGII, were 58°C. This was in agreement with the findings of Rajesh *et*

al., (2008a). The primer CnCirC3 had an annealing temperature of 58°C and similar results were found by Rajesh *et al.*, (2008a) and Renju (2012). Other SSR primers *viz.* CnCirA3 and CnCirC12 got amplification at 51°C. 56°C was the annealing temperature of CNZ10 and CAC06.

5.8.2. Screening of SSR primers

Fourteen sets of SSR markers were selected after screening of 34 primers based on the polymorphism (%). Good amplification patterns were observed by the selected SSR primers in their respective annealing temperature. The selected SSR markers will be useful for molecular characterisation of hybrid palms in future.

5.9. DIVERSITY ANALYSIS OF SIX COCONUT GENOTYPES

All the 14 sets of SSR markers were used for diversity analysis of six coconut genotypes. A total of one to two amplicons were produced per primer with an average of 1.44 amplicons per primer. The molecular weight of markers was in range of 85 bp to 110 bp. The polymorphic bands were 48.5 per cent. Out of 34 SSR markers 27 were found monomorphic between Annur (*interse*) and WCT. This revealed similarity of Annur (*interse*) and WCT.

Diversity analysis was done based on presence (1) or absence (0) of bands with respect to six genotypes. Dissimilarity matrix was constructed using DARwin ver. 6.0 software. From dissimilarity matrix genetic diversity values were calculated. The genetic diversity of Annur (*interse*) and MYD was maximum (GD = 0.230) and that of Annur (*interse*) and GB was 0.179. The increasing genetic diversity between the parents helps in the exploitation of heterosis. This is one of the reasons for superior performance of hybrid Annur x MYD in yield and nut characters compared to Annur x GB. The lowest genetic diversity was observed between Keraganga and GB (GD = 0.04). This indicates that Keraganga (WCT x GB) was more similar with its male parent GB. Similarly, less genetic diversity was observed between kerasree and MYD (GD = 0.135) compared with Kerasree and WCT (GD = 0.226). This gave an idea that Kerasree (WCT x MYD) was less similar

to its female parent compared to its male parent. The genetic diversity of dwarf coconut palms under study *viz*. GB and MYD was low (GD = 0.052).

Dendrogram was also drawn using DARwin ver. 6.0 software. The six coconut accessions were broadly grouped into three clusters. Kerasree, Annur (*interse*) and WCT were included in cluster I. The cluster I subdivided into two groups. First sub group included Annur (*interse*) and WCT. Kerasree alone was in subgroup two. This indicates similarity of Annur (*interse*) and WCT and gave confirmation that Annur is a dwarf ecotype of WCT coconut. The cluster II included dwarf coconut palms GB and MYD. This gave an idea about similarity of these dwarf coconut palms. The last cluster, Cluster III included Keraganga alone.

Molecular analysis of parental and check palm provided an idea about similarity of WCT and its dwarf ecotype Annur (*interse*). The hybridization of ecotype Annur with MYD is found to be superior in yield and nut characters compared to the hybridization with Gangabondam. This was due to higher genetic diversity of its parental palms.

SUMMARY

6. SUMMARY

The present study entitled "Morpho-molecular characterisation of D x D hybrids developed using 'Annur', the dwarf ecotype of WCT coconut (*Cocos nucifera*)" was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Padannakad during 2019-2020. The morphological and molecular evaluations were done at the N8 block of Regional Agricultural Research Station, Pilicode, and College of Agriculture, Padannakkad respectively.

The plant materials included 58 palms from N8 block of RARS, Pilicode, out of which 34 palms were Annur (*interse*), 11 palms were Annur x Malayan Yellow Dwarf and 13 were Annur x Gangabondam. The morphological characters were recorded from these palms and compared with corresponding characters of WCT, Kerasree (WCT x MYD), and Keraganga (WCT x GB). The morphological observations of other parental palms (MYD and GB) and check palms were also done at T block of RARS, Pilicode.

The genotypes were evaluated for 28 characters including vegetative, reproductive, yield, and nut characters. The significant differences among genotypes were revealed by the analysis of variance. All vegetative characters showed significant variation among genotypes. All reproductive characters except the number of unopened inflorescences and total inflorescence per palm per year also showed significant variation. A significant variation was observed for all yield and nut characters except the number of bunches per palm per year and sugar content. The short stature of the Annur ecotype (2.725 m) was transmitted to its hybrids and observed further height reduction in Annur x MYD (2.251 m) and Annur x GB (2.065 m). Bole character, which was a characteristic feature of tall palms was observed in Annur (*interse*) and its hybrids. All other vegetative characters *viz*. petiole length, leaf length, internode length, no. of green leaves, and rate of leaf production of Annur (*interse*) was on par with its hybrids. The number of nuts per palm per year was lower in hybrid Annur x GB (34.3) and the same character was on par in Annur (*interse*) (58.2) and Annur x GB whereas significantly higher values were

observed in Annur x MYD. In general, superior performance of Annur x MYD over Annur x GB was observed for nut characters.

Comparison of Annur (*interse*) and WCT revealed similarity among these two genotypes for all major characters except for height of the palm which was on par in these genotypes and confirmed the statement 'Annur is a dwarf ecotype of WCT coconut'. Analysing the characters of Kerasree and Annur x MYD revealed a significant variation in major characters like the number of nuts per palm per year, copra content, and plant height. Similarly, Keraganga and Annur x GB showed significant variation in major characters like height and number of nuts per palm per year. The significant difference of yield shown by the dwarf hybrids was due to non-attainment of stabilized yield.

Sd + Avg. method was used to detect better palms from each cross and observed that palm no. 1, 2, 15, 19, 20, and 28 were superior performers among Annur (*interse*). The palm no. 53, 55, and 56 of Annur x GB and palm no. 43, 44, 47, and 49 of Annur x MYD were better performers.

Annur (*interse*) and its hybrids showed concordance of phases (male and female phase) which indicates self-pollinating behaviour of these palms. Self-pollinating behaviour of the palms can be utilized for maintainance of characters to next generation.

The degree and nature of the relationship between characters in a group were estimated by correlation analysis. In this analysis, the genotypic correlation of 27 characters with yield was estimated. The association of all characters with each other was also analysed. All major characters *viz*. height of palm, number of green leaves, nut weight, fruit weight, and copra content showed positive significant correlation with yield. Correlation of yield and its component characters alone does not provide precise information on the contribution of these characters on yield and is inadequate to interpret the cause and effect. The path analysis confirms if the correlation of component characters and hence the analysis was carried out with characters showing significant correlation with

yield. Number of female flowers per inflorescence, fruit weight (with husk), shell and meat weight, quantity of liquid endosperm, copra content, pole to pole circumference, number of nuts per bunch, girth of palm (20 cm), and number of bunches per palm per year showed high direct effect and indicates a true relationship between yield and these characters. Improvement in yield was effective through the direct selection of these characters. Height, number of green leaves, nut weight, volume of fruit and number of female flowers one month after pollination showed negative direct effect and indirect selection of characters was effective in yield improvement.

The difference between GCV and PCV was high for all characters showing high genetic variability except plant height and it indicates low environmental influence on this character. Height of the palm, number of green leaves, leaf length, number of inflorescence at the time of observation, number of female flowers per inflorescence, shell and meat weight (without water) and copra content showed high heritability along with high genetic advance. These characters are governed by additive gene action and selection will be effective for improvement of these characters.

Standard heterosis is more acceptable compared to relative heterosis and heterobeltiosis. Height of palm showed significant negative heterosis over standard check for both hybrids and indicating dwarfness of hybrids compared to check. The nut characters of Annur x MYD revealed a significant positive heterosis over standard check (Keraganga) and are more preferable. Yield showed significant negative standard heterosis for both hybrids. It was due to non-attainment of stabilized yield.

Molecular characterization of the Annur (*interse*) and the hybrids from it was also attempted using Simple Sequence Repeat markers (SSR). DNA was isolated from all the parental palms and check cultivars. The quality and quantity of genomic DNA obtained was verified using agarose gel electrophoresis and spectrophotometer. The quantity of DNA samples ranged from 350 to 1300 ng/ μ l with good quality.

123

Screening of SSR markers for parents and check palms revealed polymorphism for the primers CAC02, CAC11, CNZ40, CnCirA9, CnCirB12, CnCirC3, CnCirC5, CnCirC7, CnCirE2, CnCirE12, CnCirF2, CnCirG11, CnCirH4 and CnCirH7. Out of 34 SSR markers, 27 were monomorphic between WCT and *interse* of Annur. Genetic similarity analysis using the SSR markers revealed the high similarity between *interse* palms of Annur and WCT. Dwarf palms *viz*. MYD and GB also showed high genetic similarity with each other. Annur x MYD was found to be superior in yield and nut characters compared to the Annur x GB, which would be due to higher genetic diversity of its parental palms.

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MORPHO-MOLECULAR CHARACTERISATION OF D x D HYBRIDS DEVELOPED USING 'ANNUR', THE DWARF ECOTYPE OF WCT COCONUT (Cocos nucifera L.)

By

ANUPRASAD T E

(2018 - 11 - 153)

Abstract of the Thesis

submitted in partial fulfilment of the requirement

for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University



DEPARTMENT OF PLANT BREEDING AND GENETICS COLLEGE OF AGRICULTURE PADANNAKKAD, KASARAGOD- 671314 KERALA, INDIA

2021

ABSTRACT

Coconut is one of the most extensively grown crop in the world, playing a significant role in the economic, cultural, and social life of over 80 tropical countries. Coconut cultivars are classified into tall (variety *typica*) and dwarf (variety *nana*) types. Short statured palms are currently receiving more attention due to various socio-economic factors. However, the poor quality of nuts for various processing aspects and high susceptibility of dwarf palms to pest and diseases makes them unsuitable for large scale cultivation. The solution for this problem is development of suitable hybrids by combining the desirable features of tall and dwarf palms.

West Coast Tall (WCT) is the most widely cultivated variety in Kerala and several ecotypes of WCT were reported. In a study initiated in 2005 at RARS Pilicode, an ecotype of WCT was detected in Annur, a place in Kannur district which showed dwarfing nature with nut qualities similar to WCT. The Annur ecotype was crossed in 2007-08 with the dwarf cultivars Malayan Yellow Dwarf (MYD) and Gangabondam (GB). The hybrid seed nuts along with *interse* seed nuts of Annur were planted at RARS Pilicode in 2009. The first flowering was recorded in 2014.

The present study aims at evaluation of plant stature, yield, nut quality and other important morphological traits of Annur and the hybrids developed from it. The *interse* of Annur, Annur x GB and Annur x MYD were compared with corresponding characters of the respective parents as well as WCT, Keraganga (WCT x GB) and Kerasree (WCT x MYD). Significant reduction in height was observed for hybrids *viz*. Annur x MYD (2.251 m) and Annur x GB (2.065 m) which was on par with Annur (2.725 m) and considerably lower than the corresponding values of dwarf cultivars *viz*., MYD (5.2 m) and GB (8.885 m). Number of nuts per palm per year was on par in Annur (58.2) and Annur x MYD (55.4) while that was lower in hybrid Annur x GB (34.3). However, since the palms started flowering only in 2014, stabilization of yield is not attained. Nut weight and kernel thickness were significantly higher in Annur x MYD whereas these were on par in Annur

and Annur x GB. Copra content in Annur and both hybrids were on par. In general, Annur x MYD showed superior performance for nut characters compared to Annur x GB.

Annur palms were significantly similar to WCT for most of the characters except height, rate of leaf production, nuts per bunch, nut water content. When the yield of hybrids of Annur were compared with the standard check varieties *viz*., Keraganga (WCT x GB) and Kerasree (WCT x MYD), (101.5 and 104.25 nuts per palm per year) yield of Annur x GB and Annur x MYD were low. However, both hybrids showed nut characters similar to the respective released varieties *ie*, Annur x GB was similar to Keraganga and Annur x MYD showed similar nut characters with Kerasree except copra content (161.432 g and 193 g respectively).

Better palms from each cross were analysed and palm numbers 1, 2, 15, 19, 20 and 28 were identified as better palms from *interse* of Annur. The better performing palms of hybrid, Annur x MYD were palm numbers 43, 44, 47 and 49. Three palms from Annur x GB (palm numbers 53, 55 and 56) were identified as better palms.

Correlation analysis revealed significant positive genotypic correlation of nut yield per palm with all reproductive and yield characters and with most of vegetative and nut characters. Path coefficient analysis showed high positive direct effect on number of female flowers per inflorescence, number of bunches per palm per year, number of nuts per bunch, pole to pole circumference of nut, nut weight (with husk), shell and meat weight, quantity of liquid endosperm and copra content.

High heritability coupled with high genetic advance was exhibited by the characters height of palm, leaf length, number of green leaves, number of inflorescences at the time of observation and number of female flowers per inflorescence. This indicates that these characters are governed by additive gene action and selection based on these characters will be effective.

For both hybrids, height of the palm showed negative heterosis which indicates the dwarf nature of the hybrids. In hybrid Annur x MYD, most of nut characters

showed heterosis over mid parent (relative heterosis) and better parent (heterobeltiosis). The standard heterosis was significant when compared with Keraganga but was not significant with Kerasree.

Molecular characterization of the Annur (*interse*) and the hybrids from it was also attempted using Simple Sequence Repeat markers (SSR). Good quality genomic DNA is the prerequisite for any molecular work. DNA was isolated from all the parental palms as well as 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 350 to 1300 ng/ μ l with good quality.

Screening of SSR markers for parents and check palms revealed polymorphism for the primers CAC02, CAC11, CNZ40, CnCirA9, CnCirB12, CnCirC3, CnCirC5, CnCirC7, CnCirE2, CnCirE12, CnCirF2, CnCirG11, CnCirH4 and CnCirH7. Out of 34 SSR markers, 27 were monomorphic between WCT and *interse* of Annur. Genetic Similarity analysis using the SSR markers revealed the high similarity between of *interse* palms of Annur and WCT. Dwarf palms *viz*. MYD and GB also showed high genetic similarity with each other.

The present study revealed that the genotype Annur is a dwarf ecotype of WCT with similar nut quality and hence is a very promising genotype for breeding for dwarfness. The hybridization of ecotype Annur with MYD is found to be superior in yield characters compared to the hybridization with Gangabondam. However, both hybrids inherited the short stature from Annur and are promising. Since the yield characters needs to be stabilized, evaluation based on important vegetative and reproductive characters as well as molecular fingerprinting needs to be continued inorder to identify the full potential of these dwarf hybrids.

സംക്ഷിപ്പം

ലോകത്തിൽ ഏറ്റവും വ്യാപകമായി വളരുന്ന വിളകളിൽ ഒന്നാണ് ഉയരമുള്ളവ ട്രിപ്പിക്ക), തെങ്ങ്. തെങ്ങിനങ്ങളെ കുള്ളൻ (നാനാ) എന്നിങ്ങനെ തരം തിരിക്കാം. കുള്ളൻ തെങ്ങുകൾക്ക് ഇപ്പോൾ വളരെയധികം ശ്രദ്ധ ലഭിക്കുന്നുണ്ടെങ്കിലും വർദ്ധിച്ച രോഗകീടബാധയും ഗുണമേൻമക്കുറവും ദൂഷ്യവശങ്ങളാണ്. ത്രങ്ങയുടെ ഇവയുടെ സങ്കരയിനം തെങ്ങുകളുടെ ഉത്പാദനമാണ് ഈ പ്രശ്ശങ്ങളുടെ പരിഹാരം.

പശ്ചിമതീര നെടിയ ഇനം ഫ്രവസ്റ്റ് കോസ്റ്റ് ടാൾ) ആണ് കേരളത്തിൽ കുടുതലായി കൃഷി ചെയ്യുന്ന തെങ്ങിനം. ഇവയുടെ വിവിധതരം ഇക്കോടൈപ്പുകൾ കേരളത്തിൽ കണ്ടെത്തിയിട്ടുണ്ട്. 2005 ൽ കണ്ണുർ നിന്ന് പശ്ചിമതീര ജില്ലയിലെ അന്നൂർ എന്ന സ്ഥലത്ത് നെടിയ ഇനത്തിന്റെ ഇക്കോടൈപ്പ് കണ്ടെത്തിയിരുന്നു. കുള്ളൻ 2007-08 കാലയളവിൽ അന്നൂർ ലോക്കൽ ഇനവും കുള്ളൻ തെങ്ങിനങ്ങളായ മലയൻ യെല്ലോ ഡ്വാർഫും ഗംഗാബോൻഡവുമായി പരാഗണം നടത്തി സങ്കരയിനം തെങ്ങിൻ തൈകൾ ഉത്പാദിപ്പിക്കുകയും അവ 2009 ൽ കാസർഗോഡ് പിലിക്കോട് പ്രാദേശിക കാർഷിക ള്ളെ ഗവേഷണകേന്ദ്രത്തിൽ നടുകയും ചെയ്യു.

കാർഷിക പടന്നക്കാട് കോളേജിൽ 2018-20 കാലയളവിൽ സസ്യപ്രജനന വിഭാഗത്തിന്റെ കീഴിൽ നടത്തിയ ഈ പഠനം ഊന്നൽ കൊടുത്തിരിക്കുന്നത് ഉയരം, വിളവ്, തെങ്ങുകളുടെ ത്രങ്ങയുടെ ഗുണനിലവാരം മുതലായ പ്രധാന പ്രതീകങ്ങളുടെ വിശകലനങ്ങളിലാണ്. അന്നൂർ, അന്നൂർ x ജി ബി, അന്നൂർ x എം വൈ ഡി എന്നീ തെങ്ങിനങ്ങൾ അവയുടെ മാത്യസസ്യങ്ങളുമായും കോസ്റ്റ് ടാൾ. വെസ്റ്റ് സങ്കരയിനങ്ങളായ കേരശ്രീ, കേരഗംഗ എന്നിവയുമായും താരതമ്യം ചെയ്യു. സങ്കരയിനങ്ങളായ അന്നൂർ x എം വൈ ഡി യിലും (2.251 മീ) അന്നൂർ x ജി ബി യിലും (2.065 മീ) ശ്രദ്ധേയമായ ഉയരക്കുറവ് കാണാൻ സാധിച്ചു. സ്ഥിരമായ ഉത്പാദനം എത്താത്തതിനാൽ അന്നൂർ (58.2), അന്നൂർ x എം വൈ ഡി (55.4), അന്നൂർ x ജി ബി (34.3) എന്നിവയിൽ തേങ്ങയുടെ എണ്ണം കുറവായിരുന്നു. അന്നൂരിനെയും അന്നൂർ x ജി ബി യേയും താരതമ്യം ചെയ്യുമ്പോൾ തേങ്ങയുടെ ഭാരം, കാമ്പിന്റെ വണ്ണം എന്നിവ അന്നൂർ x എം വൈ ഡി ക്കാണ് കൂടുതലായി കണ്ടത്. മൂന്നിനങ്ങളിലും കാപ്രയുടെ അളവ് ഒരുപോലെ ആയിരുന്നു. സങ്കരയിനങ്ങളിൽ ത്രങ്ങയുടെ പ്രധാന ഗുണവിശേഷങ്ങൾ താരതമ്യപ്പെടുത്തുമ്പോൾ മികച്ച പ്രകടനം അന്നൂർ x എം വൈ ഡി യായിരുന്നു കാഴ്ച വച്ചത്.

ഉയരം, ഓലയുടെ ഉദ്ദാദന നിരക്ക്, തേങ്ങാ വെള്ളം, കുലയിലെ തേങ്ങയുടെ എണ്ണം എന്നിവ ഒഴികെ ബാക്കിയുള്ള സ്വഭാവഗുണങ്ങൾ അന്നൂർ തെങ്ങുകളിലും വെസ്റ്റ് കോസ്റ്റ് ടോളിലും എല്ലാം ഒരുപോലെയാണ് കാണാൻ സാധിച്ചത്. കേരശ്രീയും കേരഗംഗയുമായി താരതമ്യം ചെയ്യുമ്പോൾ അന്നുറിന്റെ സങ്കരയിനങ്ങളിൽ വളർച്ച പൂർണമാകാത്തതിനാൽ ഉത്പാദനം കുറവായിരുന്നു. എന്നിരുന്നാലും ത്രങ്ങയുടെ സങ്കരയിനങ്ങളിലും സ്വഭാവഗുണങ്ങളിൽ രണ്ടു സങ്കരയിനങ്ങളായ കേരശ്രീ, കേരഗംഗ എന്നിവയുമായി സാദ്യശ്യം കാണാൻ സാധിച്ചു.

അന്നൂർ തെങ്ങുകളിൽ തെങ്ങ് നമ്പർ 1, 2, 15, 19 എന്നിവ മികച്ച തെങ്ങുകളായി കാണാൻ സാധിച്ചു. അന്നൂർ x എം വൈ ഡി യിൽ തെങ്ങ് നമ്പർ 43, 44, 47, 49 ഉം അന്നൂർ x ജി ബി യിൽ തെങ്ങ് നമ്പർ 53, 55, 56 എന്നിവയും മികച്ച തെങ്ങുകളായി കാണപ്പെട്ടു.

തമ്മിലുള്ള സ്വഭാവഗുണങ്ങൾ പരസൂരബന്ധം വിശകലനം ചെയ്യപ്പോൾ പ്രത്യുല്പാദന സ്വഭാവങ്ങളും, മിക്കവാറും എല്ലാ സസ്യഗുണങ്ങളും വിളവുമായി ബന്ധപ്പെട്ടിരിക്കുന്നതായി കണ്ടെത്തി. ഈ ഗുണഗണങ്ങൾ വിളവിനെ എങ്ങനെയാണ് ബാധിക്കുന്നതെന്ന് ചെയ്യപ്പോൾ പെൺ കുലയിലെ വിശകലനം പൂവുകളുടെ എണ്ണo, വലിപ്പം, ത്രങ്ങയുടെ എണ്ണം, ത്രങ്ങയുടെ ത്രങ്ങയുടെ ഭാരം, കൊപ്രയുടെ അളവ് എന്നിവ വിളവിനെ നേരിട് തേങ്ങാവെള്ളം, ബാധിക്കുന്നതായി മനസ്തിലാക്കാൻ സാധിച്ചു.

ഉയരം, ഓലകളുടെ നീളം, പച്ച ഓലകളുടെ എണ്ണം, പൂങ്കുലകളുടെ എണ്ണം, പെൺപൂക്കളുടെ എണ്ണം എന്നിവ ഉയർന്ന ജനിതക മുന്നേറ്റത്തോടൊപ്പം ഉയർന്ന പൈത്യക ക്ഷമതയും പ്രദർശിപ്പിച്ചു. ഈ പ്രതീകങ്ങളെ നിയന്ത്രിക്കുന്നത് അഡിറ്റീവ് ജീൻ പ്രവർത്തനമാണെന്നും ഈ പ്രതീകങ്ങളെ അടിസ്ഥാനമാക്കിയുള്ള തിരഞ്ഞെടുപ്പ് ഫലപ്രദമാകുമെന്നും ഇത് സൂചിപ്പിക്കുന്നു.

രണ്ട് സങ്കരയിനങ്ങളിലും ഉയരത്തിൽ ഗണ്യമായ കുറവ് കാണിച്ചു. ഇത് സങ്കരയിനങ്ങളുടെ കുള്ളൻ സ്വഭാവത്തെ സൂചിപ്പിക്കുന്നു. അന്നൂർ x എം വൈ ഡി യിൽ തേങ്ങയുടെ ഗുണങ്ങൾ സങ്കര സ്വഭാവം പ്രകടിപ്പിച്ചു. തെങ്ങിനങ്ങളുടെ ജനിതകപരമായ മൂല്യനിർണായത്തിനായി ഡി എൻ എ അടിസ്ഥാനമാക്കിയുള്ള സിംപിൾ സീക്വൻസ് റിപീറ്റ് മാർക്കറുകളാണ് ഉപയോഗിച്ചത്. മാത്യതെങ്ങുകളിൽ നിന്നും കേരശ്രീ, കേരഗംഗ എന്നിവയിൽ നിന്നും ഡി എൻ എ എടുത്ത് പരിശോധിച്ചപ്പോൾ നല്ല ഗുണനിലവാരമുള്ള 350 മുതൽ 1300 നാനോഗ്രാം/മൈക്രോലിറ്റർ ഡി എൻ എ ലഭിക്കുകയുണ്ടായി.

34 എണ്ണം മാർക്കറുകളിൽ 14 എണ്ണം (CAC02, CAC11, CNZ40, CnCirA9, CnCirB12, CnCirC3, CnCirC5, CnCirC7, CnCirE2, CnCirE12, CnCirF2, CnCirG11, CnCirH4, CnCirH7) മാർക്കറുകൾ ഉപയോഗിച്ചപ്പോൾ മാത്യസസ്യങ്ങൾ തമ്മിലുള്ള ജനിതകപരമായ വുതുാസം കണ്ടെത്താൻ സാധിച്ചു. അന്നൂറും വെസ്റ്റ് കോസ്റ്റ് ടോളും തമ്മിൽ ജനിതകപരമായി ഒരുപോലെ ജനിതകസാദൃശ്യ വിശകലനങ്ങളിലുടെ അണെന്ന് കാണിച്ചു. ഈ അന്നുറിനും വെസ്റ്റ് കോസ്റ്റ് ടോളിനും വളരെയധികം സാദ7ശ7ം ഉണ്ടെന്നും അന്നൂർ വെസ്റ്റ് കോസ്റ്റ് ടോളിന്റെ കുള്ളൻ ഇക്കോടൈപ്പ് സങ്കരയിനങ്ങളിലേക്ക് കുള്ളൻ ആണെന്നും ഈ സ്വഭാവം കാണാൻ പകർന്നുനൽകുന്നുണ്ടെന്നും സാധിച്ചു. പുർണ്ണവളർച്ച എത്തിയശേഷം സ്ഥിരമായ ഉത്പാദനം എത്തിക്കഴിഞ്ഞാൽ ഈ പഠനം തുടരുകയും അവയുടെ മുഴുവൻ സാധ്യതകൾ തിരിച്ചറിയാവുന്നതുമാണ്.