GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS

KRISHNAVENI VIJAYAKUMAR

B.Sc.-M.Sc. (INTEGRATED) BIOTECHNOLOGY

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS

by

KRISHNAVENI VIJAYAKUMAR

(2015-09-016)

THESIS

Submitted in partial fulfilment of the

Requirement for the degree of

MASTER OF SCIENCE (INTEGRATED) IN BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

B.Sc.-M.Sc. (INTEGRATED) BIOTECHNOLOGY

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

DECLARATION

I hereby declare that this thesis entitled **"GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Place: Vellayani,

Date: 26.09.2020

KRISHNAVENI VIJAYAKUMAR
(2015-09-016)

CERTIFICATE

Certified that this thesis entitled **"GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS"** is a record of research work done by **Ms. Krishnaveni Vijayakumar (2015-09-016)** under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place:

Date:

Dr. Asha Devi, A

(Major Advisor, Advisory committee)

Principal Scientist

Division of Crop Improvement

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Krishnaveni Vijayakumar (2015-09-016),** a candidate for the degree of **Master of Science (Integrated) in Biotechnology**, agree that the thesis entitled "**GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS**" may be submitted by, Ms. Krishnaveni Vijayakumar in partial fulfillment of the requirement for the degree.

Dr. A. Asha Devi
(Major Advisor, Advisory Committee)
Principal Scientist, Division of Crop Improvement
ICAR- Central Tuber Crops Research Institute, Sreekariyam
Thiruvananthapuram- 695017

Dr. Swapna Alex
Professor
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram- 695 522

Dr. K. I. Asha
Principal Scientist
Principal Scientist
Division of Crop Improvement ICAR- Central Tuber Crops Research Institute, Sreekariyam Thiruvananthapuram- 695017

Dr. K. B. Soni
Professor and head
Department of PlantBiotechnology
College of Agriculture, Vellayani
Thiruvananthapuram-695522

ACKNOWLEDGEMENT

With boundless love and appreciation, I would like to extend my heartfelt gratitude and appreciation to the people who helped me complete this study.

I would like to express my sincere gratitude to my beloved advisor Dr. A. Asha Devi for her continuous support, patience and motivation. In addition to her support for completing the project, her advice was valuable to me in every aspect.

I express my profound gratitude to Dr. Archana Mukherjee, former Director, ICAR-CTCRI and Dr. V. Ravi, Direcor (A), ICAR-CTCRI for providing all facilities. Dr. M. N. Sheela, Head, Division of Crop Improvement, for the constant encouragement and support. I am pleased to place on records my profound gratitude to Dr. (Mrs.) Soni K. B., Professor & Head and Dr. (Mrs.) Swapna Alex, Professor, Department of Plant Biotechnology, College of Agriculture, KAU, Vellayani, to whom I am immensely grateful for their valuable and helpful suggestions during my course period and research work.

I cordially offer my gratitude to the member of the advisory committee Dr. Asha K. I., Principal Scientist, ICAR-CTCRI, Thiruvananthapuram, for her constant help, encouragement and valuable suggestions during the research work and preparation of the thesis. I take this opportunity to place on records my sincere thanks and gratitude to Dr. Vijayaraghava Kumar, Former Head, Department of Statistics, College of Agriculture, KAU, Vellayani for helping in the statistical analyses.

A few lines are too short to make a complete account of my deep and sincere thanks to Dr. Prakash Krishnan B. S., Technical Assistant, ICAR-CTCRI for his timely help and guidance. Dr. C. Mohan, Dr. N. Krishna Radhika, Dr. C. Visalakshi Chandra, Dr. Shirly Raichal Anil, Dr. K. M. Senthilkumar and all other scientists at ICAR-CTCRI, for giving practical advice and insightful comments at different stages of my work.

My acknowledgement would be incomplete if I don't mention my gratitude to my beloved seniors, U. M. Anjitha Nair, S. Krishnaraj, R. S. Arya, Aswathy Anand and all my classmates for their invaluable care, constant support, motivation and selfless help.

I wish to express my deep gratitude to all the Heads of Divisions/Departments and staff of ICAR-CTCRI, Sreekariyam and College of Agriculture, Vellayani for their uninterrupted help in providing necessary facilities and for extension of laboratory facilities for conducting the research work. I thank them for the kind cooperation extended at all stages.

I acknowledge the favor of numerous persons who, though not been individually mentioned here, but have all directly or indirectly contributed to this work. I owe a great deal to my dear friends Sasilekha, Aswani, Elsit, Krishnendhu, Akhila and Preeja who stood with me in all my happiness, difficulties and sorrow.

I am grateful to my dear parents and brother, for their love, blessing, prayers and encouragement.

Finally, I humbly thank the Almighty for showering His blessings and bestowing the wisdom, perseverance and physical ability to accomplish this work.

KRISHNAVENI VIJAYAKUMAR

LIST OF CONTENTS

Sl. No.	TITLE	Page No.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-21
3	MATERIALS AND METHODS	22-36
4	RESULTS	37-76
5	DISCUSSION	77-88
6	SUMMARY	89-91
7	REFERENCES	92-107
8	APPENDICES	108-109
9	ABSTRACT	110-112

LIST OF TABLES

Table No.	Title	Page No.
1.	List of 30 accessions of tannia selected for the study	22
2.	Descriptors for *Xanthosoma sagittifolium* morphological characterisation as per IPGRI descriptors for below ground traits	23
3.	Reagents used for DNA isolation	27
4.	ISSR primer sequence details	29
5.	Composition of PCR reaction mix for ISSR primers	30
6.	SSR primer sequence details	34
7.	Composition of PCR reaction mix for SSR primers	36
8.	Details of corm characters measured at maximum growth	39
9.	Details of cormel characters measured at maximum growth	41
10.	Shannon weaver’s index and Simpson’s diversity index	43
11.	Pearson’s correlation matrix	45
12.	Variation in the quantitative characters for 28 tannia accessions	47
13.	Details of tuber characteristics for DMRT	47
14.	Duncan’s multiple range test for significant quantitative characters	49
15.	Principal component analysis in 28 accessions of tannia based on accessions	50
16.	Principal component analysis in 28 accessions of tannia based on characters	53
17.	Details of accessions grouping under various clusters	55
18.	Yield and purity of isolated DNA	56
19.	Parameters estimated for the quantification of genetic variability using fourteen ISSR markers	67
20.	Details of accessions grouping under various clusters	69
21.	Similarity matrix based on Jaccard's coefficient for 30 tannia accessions using 14 ISSR markers	71

LIST OF FIGURES

Fig. No.	Title	Page No.
1	Cluster plot representation based on PCA based on accessions	52
2	Cluster plot representation based on PCA based on characters	54
3	Cluster dendrogram drawn using NTSys pc software package	56
4	Dendrogram of 30 tannia accessions using molecular data	70

LIST OF PLATES

Plate No.	Title	Page No.
1.	General view of *Xanthosoma sagittifolium* in the field	37
2.	A) Xa 152 and B) TTn14-6 showing elongated shape whereas C) Xa-AD/2014-15 showing cylindrical shape	38
3.	D) Xa-AD/2014-15 showing cylindrical shape E) TTn14-8 showing elliptical corm F) TTn14-5 with ovate corm and cylindrical cormel)	38
4.	G) Sreekaryam local showing elliptical corm and cormels H) Xa-12 showing elliptical corm and cormels, I) Xa-JG/2016-1 shows elliptical corm with pink coloured flesh and J) Xa-63 shows elliptical cormels	38
5.	K) Xa-JU/10-8 with dark brown exterior surface shows elliptical corm and cormels, L) TTn14-6 shows elliptical corm and elongated cormels and M) Xa-AKI/2015-9 with ovate corm and elliptical cormels	39
6.	N) Xa-152 with elliptical corm and elongated cormels and O) Xa-75 with ovate corm and cylindrical cormels	39
7.	DNA isolated using modified Dellaporta protocol	58
8.	Primer screening image	59
9.	Primer screening image	59
10.	Agarose gel profile (2%) with the primer UBC 807	60
11.	Agarose gel profile (2%) with the primer UBC 808	60
12.	Agarose gel profile (2%) with the primer UBC 809	61
13.	Agarose gel profile (2%) with the primer UBC 810	61
14.	Agarose gel profile (2%) with the primer UBC 811	62
15.	Agarose gel profile (2%) with the primer UBC 817	62
16.	Agarose gel profile (2%) with the primer UBC 818	63
17.	Agarose gel profile (2%) with the primer UBC 825	63
18.	Agarose gel profile (2%) with the primer UBC 824	64
19.	Agarose gel profile (2%) with the primer UBC 827	64
20.	Agarose gel profile (2%) with the primer UBC 834	65
21.	Agarose gel profile (2%) with the primer UBC 845	65
22.	Agarose gel profile (2%) with the primer UBC 836	66
23.	Agarose gel profile (2%) with the primer $(GA)_9AC$	66
24.	Agarose gel profile (2%) showing SSR primer screening	72
25.	Agarose gel profile (2%) with the primer Ce1 B03 at 56°C	73
26.	Agarose gel profile (2%) of SSR primers at 50°C and 52°C	73
27.	Agarose gel profile (2%) of SSR primers at51°C, 55°C, 56°C, 60°C	74
28.	Agarose gel profile (2%) of SSR primers at 61°C	74
29.	Agarose gel profile (2%) of SSR primers at 66°C	75
30.	Agarose gel profile (2%) of SSR primers at 55°C	75
31.	Agarose gel profile (2%) of SSR primers - 30 cycles	76

LIST OF APPENDICES

Sl. No.	Particulars	Page No.
1.	EXTRACTION BUFFER	108
2.	TE Buffer (10 X)	108
3.	TBE BUFFER (10X)	108
4.	CHLOROFORM: ISOAMYL ALCOHOL	109
5.	70% ETHANOL	109
6.	AGE LOADING DYE (1X)	109

LIST OF ABBREVIATIONS

%	Percentage
A_{230}	Absorbance at 230 nm wavelength
A_{260}	Absorbance at 260 nm wavelength
A_{280}	Absorbance at 280 nm wavelength
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
bp	Base pair
cm	centimetre
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTPs	Deoxy nucleotide tri phosphates
EDTA	Ethylene diamine tetra acetic acid
EST	Expressed Sequence Tag
FAOSTAT	Food and Agriculture Organization Statistical Database
g	gram
h	Hour
ha	Hectare
ICAR-CTCRI	ICAR- Central Tuber Crops Research Institute
ICAR-NBPGR	ICAR-National Bureau of Plant Genetic Resources
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter simple sequence repeat
KAU	Kerala Agricultural University

kb	Kilo bases
kg	Kilogram
L	Litre
m	Meter
M	Molar
mg	Milligram
$MgCl_2$	Magnesium Chloride
min	Minute
ml	Millilitre
mm	Millimeter
mM	Millimolar
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nm	Nanometer
^oC	Degree Celsius
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidine
RAPD	Random amplified polymorphic DNA

RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
RT-PCR	Reverse transcription-polymerase chain reaction
s	second
SAS	Statistical Analysis Software
SM	Similarity Matrix
sp.	Species
SSR	Simple Sequence Repeat
Taq	*Thermus aquaticus*
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
T_m	Melting temperature
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UV	Ultra violet
V	Volt
v/v	volume/volume
W	Watt
w/v	weight/volume
µg	Microgram
µl	Microlitre
µM	Micromolar

DEDICATED TO MY PARENTS

INTRODUCTION

1. INTRODUCTION

The Araceae family comprises 109 genera and approximately 2830 species that are widely distributed, mostly across the tropical, subtropical, and temperate regions of the Northern Hemisphere. On the basis of the variation of habitats, disposition and leaf morphology, the structure of the inflorescence and pollen, floral morphology, anatomy, and chromosome number the Araceae family is divided into several subfamilies. Economically, the genera *Alocasia*, *Colocasia*, and *Xanthosoma* of this family are important either due to their ornamental use or for the starch obtained from their corms (Sepúlveda-Nieto *et. al.,* 2017).

The genus, *Xanthosoma*, has been ascribed with 50–60 species and all cultivated varieties are currently grouped under four species: *X. sagittifolium, X. caracu, X. atrovirens,* and *X. nigrum (X. violaceum)*. Of these, the two most cultivated and economically important ones are *X. sagittifolium* and *X. nigrum*. Tannia (*Xanthosoma sagittifolium* (L.) Schott) is one of the six most important root and tuber crops in the world and is next to cassava, potato, sweet potato, yam, and taro in planted area and production.

Although the species *Xanthosoma sagittigolium* (L.) Schott is exclusively of Neotropical origin, it has been introduced in many tropical areas and is being cultivated as a food plant in several developing countries. It is known to be more protein-rich than the traditionally cultivated taro (*Colocasia esculenta* L. Schott) and probably easier to digest (Giacometti *et. al.,* 1994). Tannia's highly calorific corms are rich in carbohydrates and are nutritionally superior to other roots and tubers in terms of digestible crude protein and minerals such as calcium, magnesium, and phosphorus.

Xanthosoma sagittifolium has been overlooked in agricultural policies and research activities on root and tuber crops, despite its high productivity levels and improved storability compared with other tropical root and tuber crops. Natural flowering of tannia is rare and so its cultivation is essentially by vegetative propagation. Consequently, most cultivars do not exhibit much variability. The

lack of distinct features makes it difficult to distinguish between the morphologically similar accessions.

ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, India, has been identified as the National Active Germplasm Site (NAGS) for tuber crops. Because of the pooling of germplasm from various parts of the country, there can be duplicates in the germplasm and hence characterization is required before it can be used for potential breeding and *in vitro* conservation programs. Two main descriptors are available for characterization, the International Plant Genetic Resources Institute (IPGRI) and the National Bureau of Plant Genetic Resources (NBPGR) descriptors.

The use of ISSR primers for analyses can discern closely related individuals very well and quickly (Zietkiewicz, *et al*., 1994). Microsatellites have great potential in differentiating similar accessions due to their highly informative, multi-allelic and codominant nature, which allows individuals to be uniquely genotyped (Vieira *et al.,* 2016). SSRs have been the most widely used markers for genotyping plants over the past 20 years (Mason *et al*., 2015). The identification of polymorphic set of microsatellite markers can make an important contribution to both the evaluation of germplasm and the establishment of a core collection.

In view of the above, the present investigation entitled "Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers" was undertaken with the following objectives –

1. To analyse the variation in tannia using selected morphological traits as per IPGRI guidelines.
2. To analyse the genetic variation in tannia using the selected molecular markers (ISSR).
3. To check the cross compatibility of taro SSR markers with tannia.
4. To identify the duplicates present among the selected accessions for effective conservation and exploitation of the germplasm for breeding and improvement programmes.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Keeping in view the objectives of the current study on **"Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers"**, the available literature on morphology of tannia and its molecular aspects has been reviewed and presented herewith.

2.1 TUBER CROPS

Spread across the tropical, subtropical, and temperate regions of the Northern Hemisphere the Araceae family encompasses 109 genera and almost 2830 species. Based on the disposition, variation of habitats and leaf morphology, floral morphology, the structure of the inflorescence and pollen, chromosome number and anatomy the Araceae family is divided into several subfamilies. The family Araceae cultivated in large tropical and sub-tropical regions has been acknowledged as an underexploited root crop family with an uncertain future owing to limited demand that may lead to reduced production until it becomes a minor niche crop inspite of the genera *Alocasia, Colocasia*, and *Xanthosoma* of this family being dominant due to their ornamental use or for their consumption purposes. (Judd *et al.,* 2007). *Xanthosoma sagittifolium* is a staple food in the tropics and subtropics and one among the six most important root and tuber crops worldwide. The cormels, corms, and leaves of cocoyam are an imperative basis of carbohydrates for animal feed, human nutrition and of cash income for farmers. (Sama *et al.,* 2011)

The Araceae family has plants from five genera that are cultivated for their herbaceous leaves or starch-filled storage tissues namely tubers. Most are amongst the most shade tolerating of terrestrial sustenance crops and flourish in moist to flooded conditions and are grown in moist tropical environments where other starchy food crops fail to thrive. The edible aroids are frequently confused with

one another due to their common names being the same and also due to the low flowering frequency of most species which makes identification difficult. Some are cultivated as foods of famine while others are appreciated as staple foods. In the Caribbean, they are often the only food crops left after tropical storms.

Tannia is often grown as subsistence crops mostly among other crops in intercropping fields. Just like other tuber crops and tropical root crops, it yields and the bulk of individual eatable portions vary substantially with planting density and the period that the crop was permitted to stay in production. Primary plant growth is characterized by the progress of root system and foliage growth. The growth rate of corm and cormel hastens once the peak plant leaf area is attained, well into the production period. This lingers for about 2 or more months and when the corm or cormel production phase is complete, some plant species go dormant, while others must be harvested to avoid unwarranted amounts of sprouting and a fall in the starch content and quality of the produce (Maynard *et al.,* 2003).

2.2 TANNIA

Tannia is a crop of South (tropical) American origin domesticated by the tropical American-Indians and people of the Caribbean and is called *malanga* in Cuba and *yautia* in the Dominican Republic and Puerto Rico. *Xanthosoma* species was reportedly originated and first domesticated in tropical America, particularly Central America and the Caribbean. It has subsequently spread to South East Asia, the Pacific Islands and Africa and later spread to the South Pacific and Africa (Onwueme, 1978). The Portuguese and Spanish spread it and also familiarized it to Europe and Asia. After being introduced to Africa during the slave trade, it was grown in the Pacific islands and Asia for the reason that it is highly resistant to pests and diseases. Tannia is among the world's six most important root and tuber crops and is next to yam, cassava, taro and sweet potato in planted area and production. Tannia is one among the oldest cultivated crops in the world and it grows best in the humid tropical rainforest climates and can be found established near water sources and in moist shady regions (Manner *et al.,*

2011). It can also be found in disturbed wetlands, mesic pinelands, wet ditches, and near to freshwater swamps and springs in Florida (Langeland *et al.,* 2008). On the other hand, in the West Indies, the moist or wet, disturbed areas serve as the ideal cultivation platforms (Acevedo-Rodríguez and Strong, 2005). In India, tuber crops are cultivated primarily as monsoon-fed crops in the southern, eastern and north-eastern states. They are not only the source of livelihood to small and marginal farmers but also to tribal population in these areas. Even though tannia does best in tropical conditions, it could be cultivated over a fairly wide range of temperatures and conditions, for example in Puerto Rico, areas with mean annual temperature 24°C with maximum variations ranging from 13 to 29°C show considerable success in tannia cultivation.

While the crop is best suited to heavy rainfall regions, it can also be grown with an annual rainfall as low as 100 cm as long as this is uniformly dispersed, although an average rainfall of 140-200 cm is desirable. Tannia can be developed as an upland crop under irrigation and certain early-maturing varieties can be grown without irrigation in comparatively dry situations, such as exposed steep slopes. It can be grown on an extensive diversity of soils, except hard clays or pure sands, but for optimal yields they require a deep, well-drained, rich soil, preferably with a pH of 5.5-6.5. Unlike taro, this crop cannot tolerate water logging while it readily responds to mulching.

A variety of cultivars have been carefully chosen through the years for widely varying conditions and if an appropriate cultivar is selected, tannia can be effectively cultivated from sea level up to elevations of about 1500 m.

In Costa Rica, an arrangement has been established for furnishing cultivators with "seed" devised from virus-free cultivations (tissue culture) of stem tips grown *in vitro*. The arrangement here resulted not only in the yield being quadrupled but the exportable portion of the harvest surges from 40 to almost 80

percent, which sufficiently reimburses for the cost of sowing. Eradication of the *malanga* (tannia) virus in *Xanthosoma* cultivation is by far the most lucrative control operation. The system involves producing plantlets in public or private laboratories developing them under the distinct environments needed, which then supply them to farmers or cooperatives adept of doing so themselves. These plants offer traditional "seed", i.e. stems or whole cormels portion which are sold to planters as virus-free planting material. (Giacometti *et al*., 1994)

Portions of the central corm, with three or four buds weighing 100 to 150 gm are the most generally used planting material. They give better yields than the cormels which are also sometimes used. Otherwise, the main corm can be cut into 5 cm sections, and divided into 2 pieces across its diameter. The top of the main plant including about 5 mm of corm and the leaves cut off about 20-30 cm above the base that is, setts are also used, however leaving the newly-formed leaf at the centre of the plant.

Xanthosoma can be grown as monoculture, but are rather cultivated in crop rotation systems and often they are the first crop in shifting agricultural systems, or are intercropped with plantation crops such as cocoa, rubber, bananas and coconuts. Although planting may be throughout the year, in drier areas (100 cm rainfall) it is typically just before, or at the beginning of the rainy season.

Planting tannia in ridges is often considered advantageous. The corms or cormels are generally planted 7.5-10 cm deep, with the growth bud pointing downwards however if pieces of the main rootstock are used about 2.5 cm is left above the ground. Whereas, if setts are planted with the base, the depth would be about 10 cm. The plant needs little attention after planting apart from weeding, and occasionally earthing up if the establishment was on level ground.

The most commonly used field spacing 1 x 1 m and requires about 1 t ha^{-1}

of planting material (cormels) and these can vary according to region and conditions. Mostly in practice this ranges from almost 60 x 60 cm to 180 x 180 cm. The wider spacing can increase the yield per plant (and 150 x 150 cm has been stated to give maximum yield per hectare), but this demands increased maintenance as the leaves require more time to shade out the weed growing around the plant. These spacing distances discuss only about tannia cultivated in monoculture and there is less overall practice for mixed cultivations.

The general maturation time is considered to be mature 9-12 months after planting, even though a crop can occasionally be harvested after 6 months. Generally, higher yields are acquired with later harvesting. They are often harvested as required since the mature corms rarely deteriorate if left in the ground. The older leaves begin to yellow at maturation. The cormels detached from the main corm after the whole plant is dug up. Often the soil is dug away from the plant while the exposed cormels are separated from the parent plant which is covered up with soil and left to yield a new crop. In this way the plant may carry on to crop for several years and it typically takes six years before there is an evident deterioration in vigour and quality of the cormels. Care must be taken to avoid bruising the picked cormels during harvest, otherwise they are liable to develop rots when stored. Some regions used mechanical harvesters to pick the produce.

Dry well-ventilated conditions are ideal for effective storage of tannia for time periods of up to six months. In Cameroon, traditional storage in pits under a confined atmosphere has been found to be better than storing on trays in well-ventilated huts (Bermejo and León *et al.,* 1994).

Other names of *Xanthosoma sagittifolium* include mankani, chou caraibe, tanier, macabo, belembe, mangarito, maduma, gualusa, tannie and yautia de Anglo-saxon. Inspite of being exclusively of Neotropical origin the species

Xanthosoma sagittigolium (L.) Schott is has been introduced in many tropical areas and is being grown as a food plant in developing countries, and can be found to be found naturalized in many regions. It is widely consumed worldwide by about 400 million people, be it their leaves or corms as a staple food.

2.3 ORIGIN AND DISTRIBUTION

Xanthosoma is a native to tropical America and was cultivated in tropical Central and South America from very ancient times and only in comparatively recent times (19th century) has it spread widely throughout the tropical world. It is now cultivated in tropical America, the Caribbean, West Africa and the Pacific and to a very limited extent in some other parts of the humid tropics. The name 'new cocoyam' reflects this late introduction into areas where *Colocasia* (cocoyam) was previously established.

X. sagittifolium has been purposefully introduced to many areas to be used as a food crop and fodder, and then it has escaped from cultivated zones into natural regions where it became invasive. It is presently listed as an invasive crop in the Galápagos Islands, Florida, Micronesia, Puerto Rico, French Polynesia and Costa Rica. *X. sagittifolium* owe its survival and spreading ability to several adaptations such as the reproduction both sexually by seeds and vegetatively by corms, root suckers, and tubers and can grow in a wide variety of substrates and habitats ranging from deep shaded regions beneath the canopy of natural forests to well light sunny regions. (Acevedo-Rodríguez *et al.,* 2012).

2.4 TAXONOMY, CYTOLOGY AND MORPHOLOGY

The genus, *Xanthosoma*, has been ascribed with 50–60 species, and all cultivated varieties are currently grouped under four species: *X. sagittifolium, X. nigrum* (*X. violaceum*), *X. caracu,* and *X. atrovirens*. Among these, the two most

economically significant and cultivated ones are *X. nigrum* and *X. sagittifolium* (Vaneker and Slaats, 2013). One of the major drawbacks in application of existing information and scientific data from diverse regions of indigene is the prevailing muddle in its nomenclature and taxonomy. There are many names related to *X. sagittifolium*, which *includes Xanthosoma violaceum* Schott, *Xanthosoma mafaffa* Schott, *Xanthosoma caracu* K. Koch and C.D. Bouche*, Xanthosoma atrovirens* K. Koch and C.D. Bouche and *Xanthosoma brasiliense* (Desf.) Engl. all closely linked to *X. sagittifolium*, nevertheless considerable taxonomic misperception is present in this group of taxa. Thus, this name *Xanthosoma sagittifolium* has customarily been used to refer to the most cultivated species of *Xanthosoma* (Quero-Garcia *et al*., 2010; Bradshaw, 2010). Several common names including tannia, cocoyam, malanga, yautia, callalo and new cocoyam are used to denote to the domesticated species of *Xanthosoma*. Also *Xanthosoma sagittifolium* is most popularly known as cocoyam (Giacometti and Leon, 1994; Quero-Garcia *et al*., 2010).

The taxonomy of *Xanthosoma* (mainly considering cultivated species) is quite disordered and ill recognised (Giacometti and Leon, 1994). The plant material is not easy to preserve as herbarium specimens or too large to fit a herbarium sheet, many species were designated based on poor sketches, material of unknown origin or incomplete or heterogeneous collections, and the phenotypic flexibility is practically common in the group. Apart from this many species were originally chosen by native people before Europeans began to study them, so a lot of cultivated varieties were already established. Moreover, details marked seldom appeared vital for the taxonomy of the genus, such as colour of different parts and habit that are rarely recorded on exsicattae. All these aspects contribute to the confusion existing in the taxonomy of *Xanthosoma*. This genus has a relatively complex history. When Linnaeus applied the binomial *Arum sagittaefolium* to an edible aroid from Central and South America, he cited three different occurrences: Jamaica, Brasil and Barbados. Nonetheless, he built his species notion on available explanations accessible at his time and a few of them (including Sloane's book from 1707) were not entirely firsthand evidence, collaborating up direct

observation with the fragmentary reports available. Thus, the perception of the first known edible and useful species of *Xanthosoma* already started poorly defined and has since caused confusion. When Schott erected the genus *Xanthosoma* (1832) he indirectly based his new genus in this Linnean chimeric species. While it seems that Schott deduced the Linnean species accurately, his taxonomy were primarily based on living plants and very few later specialists tracked his concepts precisely. This lead to all later researchers interpreting the local cultivated species they could spot as *Xanthosoma sagittifolium*.

In some of the world literature, the term cocoyam is collectively used to describe taro *(Colocasia esculenta*) and tannia (*Xanthosoma sagittifolium*). Nevertheless, taro should not be confused with the related aroid tannia. The direct petiole attachment to the lamina is the main distinguishing feature between these two. In *C. esculenta,* the attachment of the petiole to the lamina is peltate, implying that the petiole is attached at some point in middle of the lamina, but not at the edge. This peltate type of leaf attachment largely differentiates *C. esculenta* from *X. sagittifolium*. The latter has a hastate leaf with the petiole attached at the edge of the lamina. The local name however, changes according to the locality.

X. sagittifolium is a herb which is glabrous and erect, acaulescent when young and growing upto 2 m tall. When mature they have an erect, thick, fleshy stem that can extend up to 1 m long, with abundant leaf scars and occasionally aerial roots. The base is ovoid, enlarged, producing lateral, elongated subterranean, edible corms or tubers. There are numerous leaves, nearly in a rosette arrangement in a distal crown in mature plants or in acaulescent plants. The leaf blades are horizontal to slightly nodding, and the posterior lobes ascending and simple. The upper surface of the leaf is dark green with light green primary and secondary veins, whereas the lower surface has a light green shade, with dark green venation, and the apex is mostly obtuse, ending in an acute point, while the base cordate with non-overlapping lobes. The lowest pair of secondary veins are surrounded by marginal tissue at their insertion with the petiole, the margins are undulate, green, petioles erect, invaginate on lower 2/3, with wavy,

straight or occasionally involute margins. Inflorescence is axillary, ascending while the peduncles grow upto 20 cm long with chartaceous spathe that is 13 - 15 cm long and 6 - 7 cm long tube with grayish green colour, oblong-ovoid shape. The blade is elliptic, concave, erect, adaxially cream to white and shortly acuminate at the apex and the spadix is somewhat shorter than the spathe. The pistillate zone is cylindrical whereas, the sterile staminate zone is conical, pinkish and the fertile staminate zone appears elongated, ellipsoid and cream coloured. Its fruit is a small, yellow berry (Langeland *et al.,* 2008; Acevedo-Rodríguez and Strong, 2005).

The chromosome number stated for *X. sagittifolium* is 2 n = 26 (Kuruvilla *et al.,* 1989). In tannia, surrounding the spadix, the inflorescence is an erect spathe with the basal area forming a tube. The spadix comprises of female flowers at the base, male flowers near the tip, and sterile flowers in amid, in the region compressed by the neck of the spathe. Flowers and probably pollinated by beetles, euglossine bees and flies (Gibernau, 2003).

Xanthosoma sagittifolium is best adapted to tropical rainforest and humid climates with temperatures ranging from 13°C to 30°C from sea level up to 1500 m of elevation and annual precipitations greater than 150 cm. It grows best under low-light shaded conditions and due the same cause it is commonly found growing under banana, coconut, cocoa or coffee plantations (Manner *et al.*, 2011). *Xanthosoma sagittifolium* grows in an extensive variety of soils except pure sands or hard clay. It grows finest in moist, well-drained organic soil with pH ranging from 5.5 to 6.5 and cannot tolerate waterlogged conditions. Even if leaves die back, its corms can keep developing in water-stressed environments (Langeland *et al.,* 2008).

2.5 NUTRITIONAL VALUE

In general, all plant parts (leaves, cormels, inflorescence and petioles) of tannia are fit for human consumption. The widespread distribution of this crop in

various cultures and geographical regions has led to its vastly different usage from one place to another. They are predominantly used as food in cultivated areas, and the plant parts are also used as fodder/feed and even medicine, including its use as anti-poisonous agents against scorpion, tarantula and snake bites (Boakye *et al.,* 2018).

Sefa Dedeh and Agyir-Sackey (2004) reported that the nutritional composition of *C. esculenta* and *X. sagittifolium* were that of total fat 0.28 to 0.97 g 100 g^{-1}, ash 1.56 to 2.98 g $100g^{-1}$ and crude fibre 1.11 to 3.00 g $100g^{-1}$, and had found low fat, ash and crude fibre content in *X. sagittifolium*. In an evaluation of nutritional and nutraceutical properties, mineral and microelement content in certain underutilized edible aroid corms from Assam state of India by Sarma *et al.,* 2016, the values obtained for Ca and Zn in *X. sagittifolium*, were appreciable and would be ideal for consumption for supplementing it with additional food sources that are rich in other minerals. *X. sagittifolium* (Radha kochu) and *X violaceum* (Krishna kochu) exhibited satisfactory scavenging effect and had good antioxidant activity as they had high total phenol and ascorbic acid content. Literature sources reveals that total phenolics and other natural products like vitamin C and carotenoids have been shown to possess various biological properties related to antioxidant mechanisms (Luximon-Ramma *et al*., 2003; Sarma *et al*., 2016). Yang et al., 2012 reported that the antioxidant activity of plants were majorly influenced by the total phenol content of plant extracts. Thus the antioxidant potential of *Xanthosoma* variants may be attributed to the presence of flavonoids, phenolics, ascorbic acids and other constituents present in them (Sarma *et al.,* 2016). Malnutrition is a major barrier to the growth and throughput of tropical developing economies where most staples are carbohydrate-rich but deficient in micronutrients. Tannia is suggested to have greater nutritional value over other major root and tuber staples, chiefly in terms of their protein digestibility and mineral composition (Calcium, Phosphorous and Magnesium). According to Opara (2003), *Xanthosoma sagittifolium* can generally be considered as an appreciable source of dietary energy, vitamins and proteins. It is rich in zinc,

potassium, and nicotinic acid while also being a low inhibitor of trypsin in comparison to other edible aroids. All plant parts contain erratic concentrations of an acrid factor, which seems to be carried superficially as needle-like calcium oxalate raphide crystals. Concentrations of these acridity factors contrast extensively among genera, species, and varieties and is also influenced by plant stage and growing environments. Wild varieties usually requite drastic processing procedures to make them edible whereas, the more common varieties can be consumed after cooking. Due to such reasons acridity level and fiber content are among the prominent features that govern consumer reception of varieties (Maynard *et al.,* 2003). When consumed past certain thresholds, the oxalates and other anti-nutrients present might hinder with the bioavailability of other nutrients and may be unfavourable to human wellbeing (Boakye *et al.,* 2018). Thus, most conventional cooking methods employ heat by boiling, baking, roasting, or frying, either alone or in association with other elements to make delicacies. The crude fiber contents of green cocoyam (2.14%) and purple cocoyam (2.66%) determined in an Ethiopian study (Wachamo, 2018) were lower than the minimum crude fiber content (2.80%) of two related aroids 104 (*Colocasia* and *Xanthosoma*) from Assam State of India (Sarma *et al*., 2016). This study showed that fiber contents in green- and purple- *Xanthosoma* grown in Ethiopia can be an effective source of fiber. The differences in the fibers contents could be attributed to the genotype difference.

Tannia is a feasible food commodity with higher productivity, considerable nutritional profile, and better storability compared with other roots and tuber crops and have the potential to be a sustainable food security measure. The probable health benefits may possibly also be useful in the pharmaceutical industry. Augmenting the food use of current varieties and breeding new varieties with tailored end use is the key to sustained cultivation and accessibility of tannia.

2.6 MORPHOLOGICAL CHARACTERIZATION

The principal morphological characteristics of *X. sagittifolium* are as follows: leaves with sub coriaceous textures, basal insertion of the petiole, green pseudo-stem in the basal portion with exudate being white and the presence of two collector veins (Sepúlveda-Nieto *et al.,* 2017). Wild varieties of certain plants are considered as valuable sources for improving the crop in various aspects. It has been a long-established method to use various morphological characteristics to check the similarities and variabilities between wild and cultivated varieties of crops (Mac Key, 1988). In several cases, with large number of accessions having several characters, multivariate analysis and cluster analysis has been an indispensable tool for illustrating, assessing and categorising genetic resources. The morphological characters fluctuates with growth habits and this type of assortment or cataloguing based on morphological characters caused rare phenotypes to be enhanced and maintained (Santosa *et al.,* 2002). Manzano *et al*. (2001) took morphological characters such as subterranean, leaf and inflorescence characteristics along with cytogenic and biochemical characteristics to describe the germplasm. Morphological characteristics can be used to form subgroups out of various species and varieties and these traits should be quickly identified and are easily distinguishable (Wu *et al.,* 2016). This gives them an advantage over molecular markers as molecular markers involves a lot of skill for screening and study and is not cost effective although the result is precise (Pathak *et al.,* 2014).

The morphologic and genetic characterization of *X. sagittifolium* and *C. esculenta* generated in the study by Sepúlveda-Nieto *et al.* (2017) can contribute to their appropriate identification in field and germplasm banks in places where these species are used as a food source. According to several studies, morphological parameters have been extensively used in the evaluation of various crops such as *Cola acuminata* in Cameroon by Effa *et al.* (2006) in which they listed 17 morphological traits to establish a list of minimum descriptors for characterization of one collection. Mbouobda *et al.* (2007) found that the number

of shoots per plant would not be a reliable as an index of selection to predict productivity in *X. sagittifolium*. They reported a list of five qualitative descriptors while evaluating tannia in Cameroon.

Identification of clones and elimination of duplicates have always been made easier by morphological analysis and thereby creating a more effective cultivar pool for conservation and breeding purposes. Bammite *et al.* (2018) employed standard descriptor lists that have been recommended for *Colocasia esculenta* (IPGRI, 1999) and *Xanthosoma* (IBPGR, 1989) to characterize them accordingly. In Togo, agro morphological variability was observed in taro and tannia accessions. For tannia, three morphotypes were distinguished mainly by petiole basal ring colour, the colour of the petiole, leaf blade colour, bud and cormels flesh colour. The results also suggested a selection by farmers or the effect of biotic and abiotic constraints such as rainfall irregularity, soil poverty, diseases and pests taking into account the disproportionality of groups formed. Vegetative propagation being a main propagation also backs the presence of duplicates in the collection but the observed agro morphological variability is not always synonymous to genetic diversity (Bammite *et al.,* 2018).

Opoku-Agyeman *et al*. (2004) reported 15 qualitative descriptors while evaluating *Xanthosoma* germplasms in Ghana. Among non-discriminatory qualitative traits, leaf margin color, color of cormel apex and internal color of cormels, however, showed polymorphism among tannia accessions from Ghana. They reported that 78 *Xanthosoma* accessions from 7 regions of Ghana were clustered into eight different groups, irrespective of the collection sites. The study identified morphological traits for morphological identification of tannia accessions (Opoku-Agyeman *et al*., 2004). In a study by Wachamo (2018), out of 16 qualitative traits, 9 distinguished *Xanthosoma* accessions into two groups while 7 qualitative traits did not show differences among accessions.

Solomon Fantaw *et al*. (2014) reported significant variation for all of the 16 studied quantitative traits that were studied while Villavicencio *et al*. (2016) stated that the morphological traits such as lamina width, lamina length, petiole

length, fresh weight of cormels per plant and corm size manifested significant variation among the tannia accessions. Fantaw *et al*. (2014) reported that *Xanthosoma* accessions from Ethiopia were significantly distinguished by underground traits. The analysis of 50 taro accessions from Nagaland revealed maximum contribution of underground traits such as corm girth, corm yield per plant and number of cormels towards genetic divergence (Mezhii *et al*., 2015).

The selection of superior varieties based on morphological characterisation has proved to be advantageous since conventional breeding methods for the improvement of vegetatively propagated plants is delayed (Abraham *et al.,* 2008), and this will lead to the improvement of that particular crop. On the basis of morphology, selection is done through analysing variability among the accessions (Poddar *et al.,* 2015).

2.7 MOLECULAR CHARACTERIZATION

Morphological, biochemical and molecular based genetic markers have been used for genetic diversity analysis of tannia accessions. Historical and morphological evidences has been employed to clarify the diagnostic features of *Xanthosoma* species growing in Nigeria (Mbouobda *et al*., 2007). In spite of their applicability, tannia has not yet received measured research and development effort within tropical regions (Talwana *et al.,* 2009). The usage of biochemical and molecular characterization, apart from morphological characterisation have also more imperative as they put forward more consistent and specific results. According to Ishikawa *et al*. (1989) they are employed in different systems to characterize plants and can be recognised as tags since they are positioned in loci (chromosomes) where they regulate specific traits within the organism after interaction with the environment. Molecular marker based selection relies on the markers linked to a specific trait rather than selection of a trait of interest and this has improved the efficacy of conventional breeding techniques using

morphological markers (Mwenye, 2009).

DNA markers reveal the presence or absence of a particular DNA sequence at a particular site in the genome (normally a restriction site or polymerase chain reaction (PCR) primer binding site) or polymorphism in a DNA sequence. The capability to recognize genetic variants is crucial to effective management and utilization of genetic resources (Rao, 2004). Data regarding the extent of genetic variation present among genotypes along with genetic distance between all closely related species with which hybrids could be formed serves as an aid in genetic improvement programmes (Beeching *et al*., 1993). The information on genetic diversity result from analysis and characterization which allows evaluation of genetic variability, which is an essential element in determining breeding strategies and genetic conservation plans. This can be accomplished through the characterisation of the germplasm using either morphological, biochemical or genetic markers (Mwenye, 2009; Gholiazadeh *et al.,* 2008).

Mwenye (2009) used the multiple component analyses of morphological parameters to differentiate taro and tannia genotypes in Malawi. Morphological parameters which were applied to estimate genetic parameters for yield and its components in *Xanthosoma* genotypes from Bangladesh indicated the presence of substantial variances with wide ranges of distinction among the genotypes (Paul and Bari, 2012). Those authors specified that the genotypic differences for most of the characters were oddly higher than their corresponding environmental variances. In Ethiopia, the genetic diversity of *Xanthosoma* collections was studied using multivariate analysis at Jimma Agricultural Research Center based on 16 quantitative traits (Fantaw *et al.,* 2014). The existing diversity among these genotypes offers a prospect for genetic improvement for desirable characters.

Xanthosoma species from different locations in Indonesia were characterized using the morphological parameters and isozyme markers and their results revealed that the correlation between morphological data and data from esterase and glutamate were very good (0.97 and 0.94), but there were no obvious

differences among the samples from different locations of the country (Nurmiyati *et al*., 2009). RAPD analysis of tannia has revealed that very little genetic variation exists within the USDA-ARS collections in Florida (Schnell *et al*., 1999). Whereas in Ghana, 70 *Xanthosoma* accessions analyzed with RAPD markers showed significantly higher polymorphisms (Offei *et al*., 2004). The AFLP analysis which was carried out to assess the status of species of *Caladium* versus *Xanthosoma* presented that AFLP can distinguish between the different species by their unique and different banding patterns (Loh *et al*., 2000). However AFLP fingerprinting had revealed high genetic diversity but low differentiation among Ethiopian *Xanthosoma* accessions (Wachamo 2018). RAPD analyses, - (CA) 8RY microsatellite repeat unit, chloroplast- (trnR/Q 01; trnL 03/04) and mitochondrial-specific (NAD 4.2/4.3; rps14/COB) primer pairs revealed 4 species of *Xanthosoma* among the cocoyam accessions from Jamaica (Brown and Asemota, 2009). A set of six chosen long terminal repeat (LTR) primers yielded 92% polymorphic bands across 20 cocoyam accessions (Doungous *et al*., 2015). A genetic diversity analysis of Ethiopian tannia accessions by using AFLP markers showed that three AFLP primer combinations resulted in 478 bands, of which 99.16% were polymorphic (Wachamo, 2018). The high level of polymorphism detected in this analysis suggests that the AFLP primer combinations, used in this study, were highly discriminatory, suggesting the presence of higher genetic diversity of *Xanthosoma* accessions than the formerly considered accessions in Brazil (Sepúlveda-Nieto *et al*., 2017) and in Florida (Schnell *et al*., 1999).

2.7.1 Inter simple sequence repeats (ISSR)

Inter simple sequence repeat (ISSR) procedure is a PCR centred method, which comprises of amplification of DNA fragment existing at an amplifiable length in between two identical microsatellite repeat sections oriented in opposite direction. This system uses microsatellites, typically 16–25 bp long, as primers in

a particular primer PCR reaction targeting multiple genomic loci to amplify essentially the inter- SSR sequences of different sizes. ISSRs have high reproducibility probably caused by the use of longer primers (16–25mers) as compared to RAPD primers (10- mers) which allows the following use of high annealing temperature (45– 60°C) leading to higher stringency.

ISSRs have been effectively used to estimate the magnitude of genetic diversity at inter- and intra- specific level in an extensive variety of crop species which include wheat (Nagaoka and Ogihara, 1997), rice (Joshi *et al.,* 2000), finger millet (Salimath *et al.,*1995), *Vigna* (Ajibade *et al.,* 2000), sweet potato (Huang and Sun, 2000) and Plantago (Wolff and Morgan-Richards, 1998). Benefit of ISSR-PCR above other marker techniques has been conveyed in such research by various scholars. Anchored SSR primers for example, have been proved to be more convenient and reproducible than isozymes, RFLPs and RAPDs in the diversity investigations of trifoliate orange germplasm. ISSRs were more advantageous for the analysis of diversity in the genus *Eleusine* considering quality and quantity of data output as compared to RFLP and RAPD (Salimath *et al.,* 1995).

Patel *et al.,* (2017) carried out a study with the ten different *Colletotrichum falcatum* accessions to evaluate the molecular variation among them using RAPD and ISSR markers. This study indicated the power of the selected primers for discriminating genetic diversity of the *C. falcatum* accessions.

The efficiency of the method was apparent in characterization even at the varietal level of a species. In a study conducted by Sepúlveda-Nieto *et al.,* 2017, they characterized for the first time eight ISSR markers that can be used concurrently used in the genetic analysis of *X. sagittifolium* and *C. esculenta* and other related taxa for instance *X. violaceum* and *X. undipes*, which can assist in characterization and identification. The results with the 15 ISSR markers showed that only one primer did not have any amplification products, while other primers gave high resolution profiles which were chosen for next stage. From these

amplifications, 334 loci were obtained, with an average of 41.75 loci, varying between 22 (UBC 866) and 73 (UBC 2). Of these, 321 loci (96.11%) exhibited polymorphism and the rest 13 loci (3.89%) were monomorphic which showed an average of 26.52% genetic diversity among the species.

2.7.2 Simple Sequence Repeats (SSRs)

Simple sequence repeats (SSRs) are a valuable source of genetic markers because of their abundance, high rate of polymorphism, ubiquitous distribution throughout the genome, co-dominant inheritance, high extent of allelic diversity, and ease of assay by PCR. Hence, SSRs are considered excellent molecular markers in studies of germplasm characterization, genetic diversity, and genetic mapping. Microsatellites (1 to 10 nucleotides) and minisatellites (>10 nucleotides) are subcategories of tandem repeats (TRs) that, together with the predominant interspersed repeats (or remnants of transposable elements), make up genomic repetitive regions.

Due to the fact that they are highly informative, co-dominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species, SSRs have been the most extensively used markers for genotyping plants over the past 20 years (Mason, 2015). In particular, SSRs are convenient for wild species in studies of diversity measured on the basis of genetic distance, to estimate gene flow and crossing over rates and in evolutionary studies, above all to intraspecific genetic relations.Over the past few years, SSR markers have been extensively used in genetic diversity analysis (Dirlewanger *et al.,*2002; Hasnaoui *et al.,* 2012; Shiferaw *et al.,* 2012; Emanuelli *et al.,* 2013; Ren *et al.,* 2014). SSRs were evidenced to be convenient for genetic analysis in the genus *Chrysanthemum* and its related genera (Feng *et al.,* 2016). It was also found that 44% (38 out 86) of *C. nankingense* SSRs were suitable for genetic diversity study among medicinal *C. morifolium* varieties. SSR markers identified by Cathebras *et*

al. (2014) gave variable degrees of heterozygosity, detected at stages ranging from 0.00 to 0.97 in tannia. Evaluation of genetic diversity and relationship among plant populations is the basis of selective breeding programs.

The study by Wachamo (2018) revealed that the majority (91.7%) of the SSR loci were polymorphic within 100 *Xanthosoma* accessions. PCoA, NJ tree based on Nei's genetic distance and population genetic structure analyses resulted in clusters that were associated with green and purple morphotype, which can easily be differentiated by their leaf color (green or purple). This indicated that the significant fraction of the SSR markers were between morphologically distinct cocoyams and there was high genetic diversity within populations, within distinct morphotypes. This showed that the vegetative propagation from stem underground structures, may be due to fixed SSR markers genetic structure and *Xanthosoma* grown in Ethiopia may have remained genetically unchanged with high heterozygosity (Wachamo, 2018).

In a study conducted by Ratna Kumari *et al.* (2019), the cross transferability of SSR markers was checked across nine species of pepper and 39 out of 50 primers were polymorphic with respect to the allele size amplified in *Piper nigrum.* Out of 50 SSRs used, 19 and 31 SSRs were amplified in 9 and 7 species, respectively. Thus the identified SSRs may have application in other species of the genus Piper where genome sequence is not available yet.

Studies show that as the phylogenetic distance increases, the chances of obtaining a successful amplification became thinner. Tang *et al.* (2010) established 19 *P. pubescens* GSS-SSR markers and successfully transferred them to six other *Phyllostachys* species with an average transferability of 75.3% and 66.7% polymorphism. A large set of polymorphic markers is beneficial for genome-wide association mapping, in the lack of structured populations, to identify markers for traits of interest that can be applied for marker-assisted selection (Lin *et al.,* 2014).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled **"Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers"** was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020. Details regarding experimental material and procedures used in the study are elaborated in this chapter.

3.1 GERMPLASM COLLECTION

Thirty accessions of *Xanthosoma sagittifolium* conserved in the ICAR-CTCRI repository were selected for the present study. The plants were raised in the field at a spacing of 90 x 90 cm as rows consisting of 5 plants per accession during February-December 2019 for morphological and molecular analysis, using corms as the initial planting material. Weeding and intercultural operations were carried out as per the standard procedure.

Table 1: List of 30 accessions of tannia selected for the study

Sl. No.	Sample	Accession name	Sl. No.	Sample	Accession name
1	Ta 1	Xa-SSK/2017-2	16	Ta 16	Xa-AD/2016-4
2	Ta 2	Xa-HOB-T8-2	17	Ta 17	TTn14-1
3	Ta 3	Xa-VHAK/2015-5	18	Ta 18	Xa-75
4	Ta 4	Xa-63	19	Ta 19	TTn14-5
5	Ta 5	Xa-26	20	Ta 20	Xa-12
6	Ta 6	Xa-Ju/10-8	21	Ta 23	Sreekaryam local
7	Ta 7	Xa-ADS/2014-18	22	Ta 24	TTn14-2
8	Ta 8	Xa-AD/2014-17	23	Ta 25	TTn14-8
9	Ta 9	TTn14-6	24	Ta 26	Xa-AD/2014-15
10	Ta 10	Xa-UV_3 Yerkadu	25	Ta 27	Xa-MNS/14-1
11	Ta 11	Xa-19	26	Ta 28	Xa-MTS Local

12	Ta 12	Xa-AKI/2015-9	27	Ta 29	Xa-13
13	Ta 13	TTn14-9	28	Ta 30	Xa-67
14	Ta 14	Xa-NL-6	29	Ta 31	Xa-24
15	Ta 15	Xa-152	30	Ta 32	Xa-JG/2016-1

3.2 MORPHOLOGICAL CHARACTERISTICS ANALYSIS

The morphological data was recorded by measuring, weighing and by observation. Only below ground characters were evaluated for the present study by using IPGRI descriptors at the time of harvest which was nine months from the date of planting. The traits consisted of shape of corm, corm size at maturity, exterior colour of corm, interior colour of corm, exterior surface of corm, colour of corm apex and cormel characteristics such as number of cormels produced, cormel size, shape, exterior and interior colour, exterior surface of cormel and position of cormel apex at the time of harvest which was 9 months after planting. The tubers were photographed for visual comparisons of phenotypic traits.

Quantitative characters evaluated included number of cormels produced, corm length (cm) and corm weight (g) as well as cormel length (cm) and cormel weight (g). Quantitative traits were recorded as numerical data, in units whereas, qualitative traits were recorded in the form of numerical traits as shown in table 2. All the morphological data were tabulated in excel worksheet for further statistical analysis.

Table 2: Descriptors for *Xanthosoma sagittifolium* morphological characterisation as per IPGRI descriptors for below ground traits

Serial no	**Character**	**Descriptor states**
1	Time of harvest	4-5 months - 1, 7-12 months - 2, 13-17 months - 3, >18 months - 4
2	Shape of corm	Globose-1, Ovate-2, Cylindrical-3, Elliptical-4, Mixed (state which one)-5
3	Corm size (at maturity)	Small (1-100 g) - 3, Medium (100-200 g) - 5, Large (>200 g) - 7

4	Exterior colour of corm	Light or medium brown - 1, Dark brown - 2
5	Interior colour of corm	White -1, Pink (pale red) -2, Orange -3, Yellow -4, Purple -5
6	Exterior surface of corm	Smooth – 1, Fibrous or Rough - 2
7	Colour of corm apex	White – 1, Pink/red -2, Green -3
8	Shape of cormel	Globose-1, Ovate-2, Cylindrical-3, Elliptical-4, Mixed (state which one)-5
9	Cormel size (at maturity)	Small (1-6 g) - 3, Medium (6.1-10 g) - 5, Large (>10 g) - 7
10	Exterior colour of cormel	Light or medium brown - 1, Dark brown - 2
11	Interior colour of cormel	White -1, Pink (pale red) -2, Orange -3, Yellow -4, Purple -5
12	Exterior surface of cormel	Smooth – 1, Fibrous or Rough - 2
13	Colour of cormel apex	White – 1, Pink/red -2, Green -3, Purple-5
14	Position of cormel apex	Above ground – 1, Underground - 2

3.2.1 ANALYSIS OF MORPHOLOGICAL DATA

The recorded data was subjected to various statistical analyses viz., diversity indices, correlation test, PCA and ANOVA using SAS package.

3.2.2 Diversity Indices

The percentage distribution, Shannon Weaver's diversity index and Simpson's diversity index were calculated for the various traits studied. The percentage distribution of the various traits across the 28 accessions was computed by calculating the proportion of accessions expressing a particular trait as percentage. The Shannon Weaver's diversity index (H) was calculated based on the following formula:

$$H = -\Sigma [p_i \times \ln (p_i)]$$

where,

p_i = proportion of a particular character i.e. number of individuals with a character / total number of individuals

The Simpson's diversity index (D) was calculated based on the formula given below:

$$D = 1 - \frac{\Sigma n (n-1)}{N (N-1)}$$

where,

n = number of accessions with a particular trait

N = total number of accessions

3.2.3 ANOVA

For all the quantitative morphological traits, analysis of variance was done. Mean and coefficient of variation was calculated, dividing two variances and comparing the ratio. Also Duncan's multiple range test was done for each of the traits in order to identify which of the traits contribute to the diversity among each individuals. The analysis was done using SAS (Statistical Analysis System) package.

3.2.4 Principal Component Analysis

Principal Component Analysis was done to obtain cluster plot and biplot using SAS package. Cluster plot was obtained to compare the clusters with that of the clusters in cluster dendrogram analysis. And biplot was obtained in order to determine which of the traits contribute more for the diversity of the accessions. Only 11 characters that showed variation was used for the analysis amongst the 28 accessions. Three characters which did not show any variation was not considered. Similarly, two accessions did not produce any cormels and hence was not used for the analysis. PCA was done based on accessions as well as characters.

3.2.5 Cluster Analysis

Hierarchical clustering was done and a cluster dendrogram was drawn with the help of NTSys pc software package. The aim was to assemble morphologically similar accessions in the same group and distinct accessions to separate groups. The whole analysis was based on Euclidean distance.

3.3 DNA ISOLATION PROTOCOL

For the isolation of DNA a modified Dellaporta method was followed. The material used were young leaf samples from thirty tannia accessions maintained in the ICAR-CTCRI genebank. The details of the same are as follows:

Protocol:

1 g of tissue (young leaf preferred) was weighed, quick frozen in liquid nitrogen and ground to fine powder using a mortar and pestle. Ground tissue can be stored in -80ºC either before or after grinding if needed. Tissue should not thaw before EB (Table 3) is added. The fine powder was then transferred into an Oak Ridge Tube and 15 ml extraction buffer was added and incubated at 4ºC. About 1ml of 20% SDS was added to each tube, after which it was mixed thoroughly by vigorous shaking, and then incubated in a 65°C water bath for 10 min. 5 ml of potassium acetate was added to the solution followed by vigorous shaking for proper mixing and then the tubes were incubated at 4°C for 20 min. The solution was separated by centrifugation at 12000 rpm for 20 minutes and the supernatant so obtained was transferred carefully to a new sterile tube. Isopropanol was added in equal volume, mixed by gentle inversion and incubated at -20°C for 1 hour or at 4ºC overnight.

The tubes were centrifuged at 12000 rpm for 10 minutes, the supernatant discarded and pellet dried by inverting the tubes on a tissue paper. The pellet was then dissolved in 500 µl sterile distilled water and incubated at 65°C for 15 minutes or 4°C overnight. This DNA solution was transferred to a microcentrifuge and 10 µl RNase A (10 mg ml^{-1}) added and mixed by inversion. It was incubated at 37ºC for 1 hour after which equal volume of chloroform isoamyl alcohol was

added and mixed by inversion. After centrifugation at 12000 rpm for 15 minutes the aqueous phase was collected in 1.5 ml micro centrifuge tube and added double the volume of absolute ethanol and 50 µl 3M sodium acetate (inverted and mixed gently) followed by incubation at -20°C for 1 hour or 4°C overnight. The tubes were then centrifuged at 12000 rpm for 10 minutes and the pellet so obtained was washed using 70% ethanol. After drying completely it was dissolved in 500 µl of sterile distilled water and stored at -20°C.

Table: 3. Reagents used for DNA isolation

EB (Extraction Buffer) components	**Required concentration**
0.5 M EDTA pH 8.0	20 mM
1 M Tris pH 8.0	100 mM
5 M NaCl	2 M
BME (beta-mercaptoethanol)	0.2%
PVP	2%
dH_2O	-

20% SDS per 100 ml: SDS 20 g in 80 ml distilled water.

3.4 ANALYSIS OF THE EXTRACTED DNA

3.4.1 Agarose gel electrophoresis

The quality and integrity of the extracted DNA was checked using 1% agarose gel electrophoresis. The gel was prepared by dissolving agarose in the required volume i.e., 1 g agarose in 100 ml of TBE buffer in a conical flask so that 1% agarose solution was obtained. This was dissolved by boiling in a microwave oven. The flask was allowed to cool down to a bearable temperature and then about 1 µl of Ethidium Bromide (EtBr) was added directly to the molten gel and gently mixed. The gel was poured into the casting tray with combs and was allowed to solidify. About 6 µl of isolated DNA sample was mixed with 2 µl of 1X loading dye and was loaded into the wells of the prepared gel. The gel was

checked using a horizontal gel electrophoresis unit run at 80 V for about 30 minutes. The run was stopped after the dye front reached $3/4^{th}$ of the gel length and then it was visualized under ultraviolet light using a gel documentation system (G : BOX, M/S Syngene).

3.4.2 Measurement of DNA quality and quantity using Nano drop spectrophotometer

The Thermo Scientific NanoDrop™ 1000 Spectrophotometer was used to measure the quality and quantity of samples with high accuracy and reproducibility.

3.4.3 Protocol for use of NanoDrop® ND-1000

The software was started by clicking on the icon. On the top left hand-side of the screen, the first panel displayed the “Nucleic Acid” button. After choosing that option the module startup panel was displayed on the screen. As the first step, the pedestals were cleaned and a water sample was used to prepare the appliance. So as to prepare a report of all readings, the “Recording” button was selected. The report can log either 12 or 32 measurements. Before doing “DNA, “RNA” or other analysis, the “blank” was set, measured and stored by placing a fresh sample of RNase free water on the pedestal and pressing “Blank” and entered “Blank” as the sample name. A straight line appeared on the screen and if this baseline is not flat then the “Blank” measurement was repeated until it was. The pedestals were cleaned in between readings using a Kim wipe. [*The measurement cycle took about 10 seconds*]

Samples were measured and stored. The information such as the sample name were recorded in the window and were displayed on the report page. The sample were cleaned from the pedestals using a Kim wipe and dried between samples. The “Show Report” button displayed all the readings associated with the current report. There were 3 options within this window as follows: Save – saves the report as a .jpg; Print – prints the report to the default printer and Exit – returns to the specific application module. The “Re-blank” option established a

new reference which is used for the calculations of all subsequent samples. The "Exit" command closed all the application modules and supporting options. The pedestals were cleaned and handled carefully after all samples were read and a measurement on a fresh replicate of the blanking solution was done to confirm that the pedestal was clean. Subsequently, the concentration of the isolated DNA samples in ng μl^{-1}, Absorbance in 260 nm, 280 nm and the ratio of absorbance at 260 nm to 280 nm were also measured and recorded for further calculations.

3.5 ISSR ANALYSIS OF TANNIA ACCESSIONS

3.5.1 PRIMER SCREENING

The following 24 ISSR primers were selected for primer screening:

Table 4: ISSR primer sequence details

CODE	PRIMER	Sequence 5'- 3'
A	UBC 807	*AGA GAG AGA GAG AGA GT*
B	UBC 808	*AGA GAG AGA GAG AGA GC*
C	UBC 809	*AGA GAG AGA GAG AGA GG*
D	UBC 810	*GAG AGA GAG AGA GAG AT*
E	UBC 811	*GAG AGA GAG AGA GAG AC*
F	UBC 815	*CTC TCT CTC TCT CTC TG*
G	UBC 817	*CAC ACA CAC ACA CAC AA*
H	UBC 818	*CAC ACA CAC ACA CAC AG*
I	UBC 824	*TCT CTC TCT CTC TCT CG*
J	UBC 825	*ACA CAC ACA CAC ACA CT*
K	UBC 827	*ACA CAC ACA CAC ACA CG*
L	UBC 834	*AGA AGA AGA GAG AGA GYT*
M	UBC 835	*AGA GAG AGA GAG AGA GYC*
N	UBC 836	*AGA GAG AGA GAG AGA CYA*
O	UBC 845	*CTC TCT CTC TCT CTC TRG*
P	UBC 851	*GTG TGT GTG TGT GTG TYG*
Q	UBC 860	*TGT GTG TGT GTG TGT GRA*

R	UBC 864	*ATG ATG ATG ATG ATG ATG*
S	$(GA)_9$ AC	*GAG AGA GAG AGA GAG AGA AC*
T	$(GA)_9$ AT	*GAG AGA GAG AGA GAG AGA AT*
U	$(ACC)_6$ Y	*ACC ACC ACC ACC ACC ACC Y*
V	UBC 867	*GGC GGC GGC GGC GGC GGC*
W	UBC873	*GAC AGA CAG ACA GAC A*
X	UBC 11	*AGA GAG AGA GAG AGA GTA*

The genomic DNA of three *Xanthosoma sagittifolium* accessions (Ta 1 - Xa- SSK/2017-2, Ta 2 – Xa-HOB-T8-2, Ta 3 - VHAK/ 2015-5) were taken initially for primer screening. The composition of the reaction mixture was as follows:

Table 5: Composition of PCR reaction mix for ISSR primers

Components	**Stock concentration**	**Required concentration**	**Volume for one Reaction (20 µl)**
Buffer with 25 mM $MgCl_2$ (Genei)	10 X	1 X	1.5 µl
$MgCl_2$ (Genei)	25 mM	0.5 mM	0.75 µl
dNTP (Genei)	25 mM	0.3 mM each	0.18 µl
Primer	10 µM	0.33 µM	0.5 µl
Template DNA	10 ng/µl	40 ng	4 µl
Taq DNA Polymerase (Genei)	5 U/µl	1 U	0.2 µl
dH_2O	-	-	7.87 µl
Total	-	-	15 µl

PCR programming for ISSR primers was carried out in a Biorad thermal-cycler with steps of initial denaturation done at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56.3°C for 1 min and

extension at 72°C for 1 min. Final extension was done at 72°C for 10 min and cooling at 4°C for ∞.

The amplified products were resolved in a 2 percent agarose gel using 100 bp ladder for checking amplification and visualized under the gel documentation system (G:BOX gel documentation M/S. Syngene).

After primer screening, 14 ISSR primers, which gave clear and reproducible polymorphic bands were chosen for analysis. PCR was performed using standardized PCR conditions and the annealing temperature which was found to be optimum for each primers. The PCR products were allowed to run in an agarose gel of 2% concentration at a voltage of 100 V for about 2 hours.

3.5.2 Agarose Gel Electrophoresis for ISSR Analysis

Gel electrophoresis was performed with 2% agarose.

3.5.3 Casting tray preparation

The casting tray which can hold a maximum of 150 ml was washed with water thoroughly to remove any remnants from the previous use. And then the tray was allowed to dry and a sticky tape was used to cover both ends of the tray so that it may hold the gel while in liquid form. Appropriate combs were selected for use in the tray which allowed loading of all the 30 accessions as well as ladders of known molecular weights. The combs were positioned in the tray suitably so that wells formed are of adequate size.

3.5.4 Gel preparation

2% of agarose was used to resolve the amplicons obtained after the PCR. For preparing a total of 150 ml gel, 3 g of agarose (M/S. Sigma Aldrich) was weighed out using a weighing balance and was directly put into a conical flask. About 150 ml of 1X TBE buffer which was prepared from a 10X TBE buffer stock solution was taken in a measuring cylinder and was poured into the conical flask. The flask was undisturbed and the gel was melted completely in a microwave oven. Using a cotton glove, the flask was taken out and allowed to

cool until the temperature became bearable and could be held with bare hands. Then, approximately 1 µl EtBr was taken out using a micropipette and was added directly into the gel. The conical flask was rotated gently and gradually in order to distribute EtBr uniformly. After proper mixing, it was poured on to the casting tray and allowed to solidify for about 30 minutes.

3.5.5 Gel loading

To begin with, the tapes were taken out and the tank was filled with1X TBE buffer up to the 3/4th mark of the tank so that the tray was completely immersed in the buffer. The PCR products were arranged in a tray according to the sample number for loading. About 4 µl of gel loading dye was added directly to the PCR tubes. Mixed well and a short spin using centrifuge was done. The comb from the set gel was removed slowly without disturbing the wells and the samples were loaded in the same order along with 100 bp DNA ladders (M/S. Thermo-Fisher).

3.5.6 Electrophoresis

The electrophoresis apparatus was connected to a power pack and the voltage was set at 120V. The movement of the tracking dye was noted. And the electrophoresis was stopped when the dye reached the bottom of the tray.

3.5.7 PCR product detection

The gel including the tray was taken out and was viewed under the ultraviolet light in a G: BOX gel documentation system (M/S. Syngene). The image was taken under appropriate exposure and saved in JPEG format for scoring.

3.6 ANALYSIS OF MOLECULAR MARKER DATA

3.6.1 Band scoring

All the images of resolved PCR products were taken. Clear and reproducible bands were taken for scoring. Binary scoring was carried out by

assigning ‘1’ for the presence of a specific band and ‘0’ for the absence of a band. The data was entered in excel and was used as an input for cluster analysis and other statistical analysis.

3.6.2 Quantification of genetic variability

As per the requirement of the studies, genetic variability can be overviewed by the estimation of genetic diversity parameters. The resulting data can be used to study marker efficiency and for comparing groups.

3.6.3 Percentage polymorphism

After scoring of bands using molecular data, bands may be present or absent in the different accessions. If a particular band is present in some accessions and absent in some others, then the band is said to be polymorphic. The percentage of polymorphism was calculated by using the formula -

No. of polymorphic bands / Total no. of bands x 100 for a particular primer.

Thus the ratio of the polymorphic bands to the total number of bands was determined.

3.6.4 Cluster Analysis

For morphological clustering of data, NTSys statistical package was used and Hierarchical clustering was done based on Euclidean distance. As a result, a dendrogram of 30 accessions was obtained which is based on morphological and molecular traits.

3.6.5 SM matrix

In order to determine the variation between two accessions, Simple matching matrix was drawn using NTSys pc software. The similarity between two accessions was represented as a number less than 1.0.

3.6.6 Dendrogram generation

NTSYS pc software was also used to generate similarity matrix as well as generating a dendrogram. The binary data were scored and statistically analyzed using NTSYS pc Ver.2.2. Pair-wise distance (similarity) matrices was computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U.S.A., Software Version 2.02 package (Rohlf, 1998). The program also generated a dendrogram, which grouped the accessions on the basis of Nei genetic distance (Nei, 1979) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis.

3.7 CROSS COMPATIBILITY CHECK OF TARO SSR PRIMERS WITH TANNIA

3.7.1 PRIMER SCREENING

The following SSR primers developed for taro were selected for primer screening for checking their cross compatibility in tannia:

Table: 6. SSR primer sequence details

CODE	PRIMER	SEQUENCE
a	Ce1 B03	F: *TTG CTT GGT GTG AAT G* R: *CTA GCT GTG TAT GCA GTG T*
b	Ce1 F04	F: *ACG AGG GAA GAG TGT AAA* R: *AGG GAA TAC AAT GGC TC*
c	Ce1 F12	F: *GAT GCC TGT CCT TAT GTT T* R: *CTT AGC GTT GTT CCC TAC*
d	Uq73-164	R: *ATG CCA ATG GAG GAT GGC AG* F: *CGT CTA GCT TAG GAC AAC ATG*
e	Uq84-207	F: *CCC ATT GGA GAG ATA GAG AGA* R: *AGG ACA AAA TAG CAT CAG CAC*
f	Uq97- 256	F: *GTA ATC TAT TCA ACC CCC CTT*

		R: *TCA ACC TTC TCC ATC AGT CC*
g	Uq110-283	F: *AGC CAC GAC ACT CAA CTA TC* R: *GCC CAG TAT ATC TTG CAT CTC C*
h	Uq132-147	F: *ACC CCG AAA AAG CCA ATG* R: *CTA TCA CTT GTT CCT CCT TCT C*
i	Uq201- 302	F: *CTA AGG AGA GGA GAT CCG AAC* R: *CAA GAC GAT GCT GAA CCA*

The genomic DNA of three *Xanthosoma sagittifolium* accessions (Ta 1 - Xa-SSK/2017-2, Ta 2 - Xa-HOB-T8-2, Ta 3 - VHAK/2015-5) were taken initially for SSR primer screening.

The cross compatibility of taro SSR primers were checked in tannia under different conditions. The PCR conditions of both taro and tannia were checked i.e.,

- Initial denaturation done at 94ºC for 5 min followed by 35 cycles of denaturation at 94ºC for 30 sec, annealing at 56.3ºC for 1 min and extension at 72ºC for 2 min. Final extension was done at 72ºC for 10 min and cooling at 4ºC for ∞.
- And initial denaturation done at 95ºC for 4 min followed by 35 cycles of denaturation at 94ºC for 30 sec, annealing at 50ºC/52ºC for 45 seconds and extension at 72ºC for 1 minute 30 seconds. Final extension was done at 72ºC for 8 min and cooling at 4ºC for ∞ respectively.

The amplification at different cycle numbers was also checked i.e., 30 and 35 cycles.

The amplification at different template DNA concentrations such as 10, 20 and 40 ng/µl were also checked.

The amplification at different temperatures were also checked i.e., 50ºC-61ºC.

Table 7: The Composition of PCR reaction mix for SSR primers was as follows:

Components	Stock concentration	Required concentration	Volume forone reaction(20 µl)
Buffer with 15mM $MgCl_2$ (Genei)	10 X	1 X	1.5 µl
$MgCl_2$ (Genei)	25 mM	1 mM	0.6 µl
dNTP (Genei)	2.5 mM	0.3 mM each	1.8 µl
Primer (F)	10 µM	0.27 µM	0.4 µl
Primer (R)	10 µM	0.27 µM	0.4 µl
Template DNA	10 ng/µl	40 ng	4 µl
Taq DNA Polymerase (Genei)	5 U/µl	1 U	0.2 µl
dH_2O	-	-	6.1 µl
Total	-	-	15 µl

The amplified products were resolved in a 2% agarose gel using 100 bp ladder to determine amplicons size and was visualized under the gel documentation system (G:BOX gel documentation M/S. Syngene).

RESULTS

4. RESULTS

The results of the study "**Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers**" carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020 are presented in this chapter.

4.1 MORPHOLOGICAL DATA ANALYSIS

Twenty-eight tannia accessions from different parts of India were used for the study (Plate 1). Underground tuber traits were recorded as per IPGRI descriptors and were recorded individually and documented. A wide range of variability was observed among the accessions for some of the underground characters studied. The photographs of the tubers are given below for comparison (Plate 2 – 6).

Plate 1. General view of *Xanthosoma sagittifolium* in the field

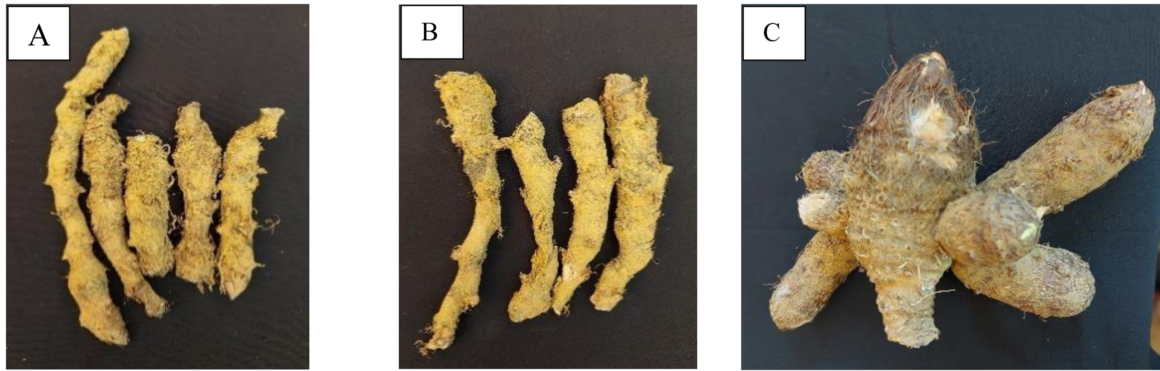

Plate 2. A) Xa 152 and B) TTn14-6 showing elongated shape whereas C) Xa-AD/2014-15 showing cylindrical shape

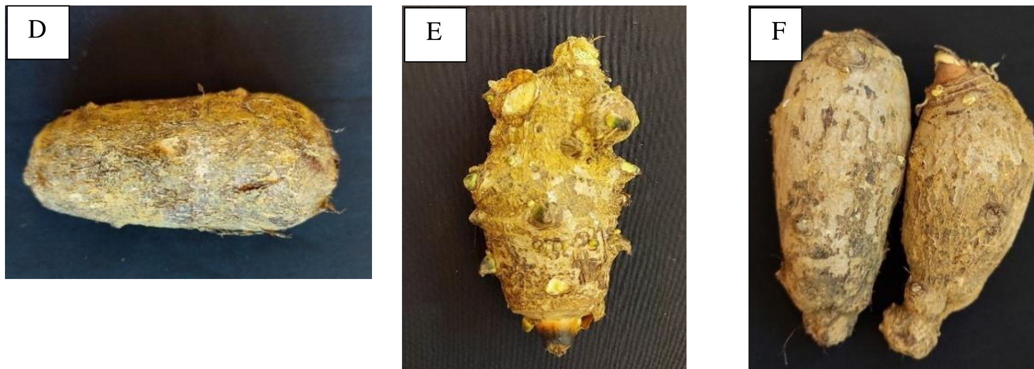

Plate 3. D) Xa-AD/2014-15 showing cylindrical shape E) TTn14-8 showing elliptical corm F) TTn14-5 with ovate corm and cylindrical cormel

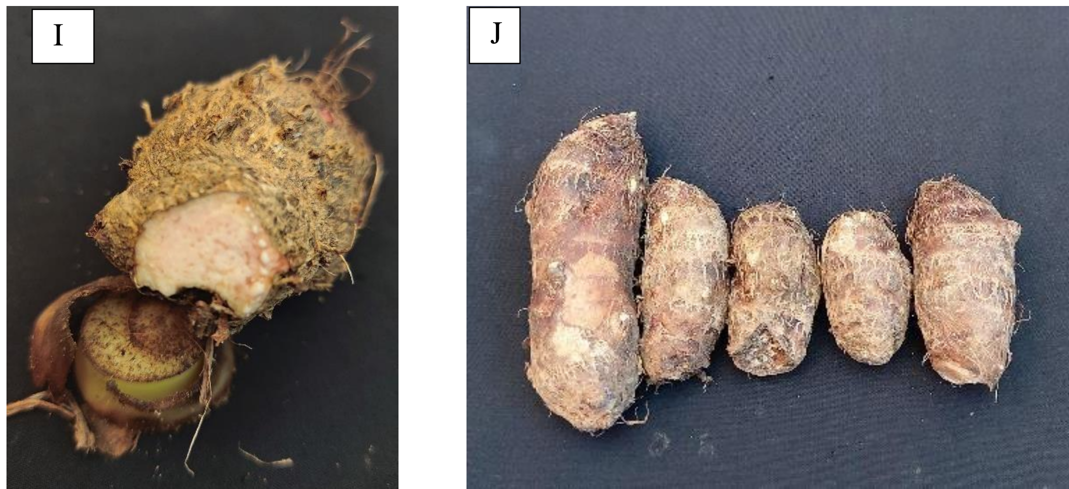

Plate 4. G) Sreekaryam local showing elliptical corm and cormels H) Xa-12 showing elliptical corm and cormels, I) Xa-JG/2016-1 shows elliptical corm with pink coloured flesh and J) Xa-63 shows elliptical cormels

Plate 5 K) Xa-JU/10-8 with dark brown exterior surface shows elliptical corm and cormels, L) TTn14-6 shows elliptical corm and elongated cormels and M) Xa-AKI/2015-9 with ovate corm and elliptical cormels

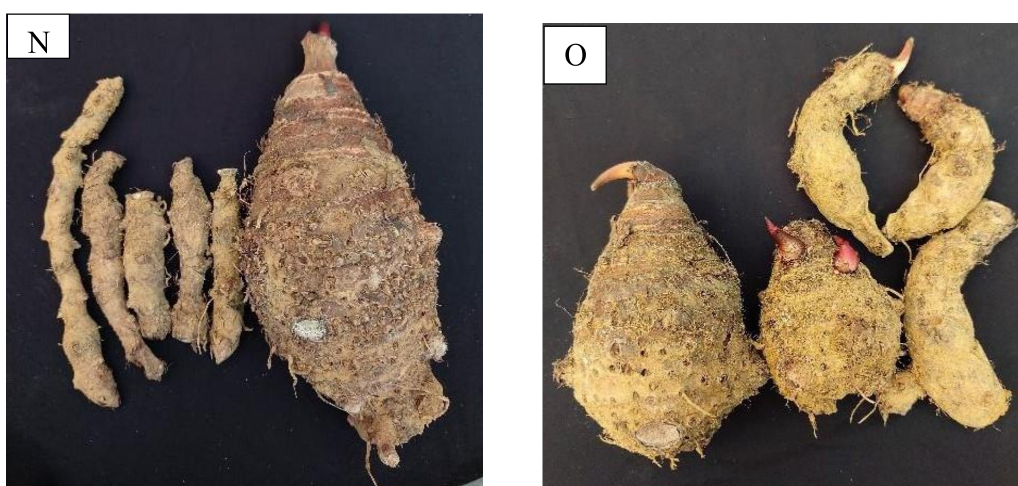

Plate 6. N) Xa-152 with elliptical corm and elongated cormels and O) Xa-75 with ovate corm and cylindrical cormels

Upon complete maturity, which was nine months after planting, the below ground tuber traits (both corm and cormel traits) of 30 tannia accessions were recorded as per IPGRI descriptors. The details are given below (Table 8-9):

Table 8: Details of corm characters measured at maximum growth

Code No.	**Accession name**	**Time of harvest (months)**	**Shape of corm**	**Corm size**	**Exterior colour of corm**	**Interior corm colour**	**Exterior surface of corm**	**Colour of corm apex**
Ta-1	Xa- SSK/2017-2	7-12	ellliptical	medium	light brown	white	fibrous	green
Ta-2	Xa- HOB-T8- 2	7-12	ovate	small	medium brown	white	rough	light pink
Ta-3	Xa-VHAK/ 2015-5	7-12	ovate	medium	medium brown	white	fibrous	green

Ta-4	Xa-63	7-12	elliptical	large	medium brown	white	rough	green
Ta-5	Xa-26	7-12	elliptical	large	medium brown	white	rough	green
Ta-6	Xa-Ju/10-8	7-12	elliptical	medium	dark brown	white	rough	green
Ta-7	Xa- ADS/2014-18	7-12	ovate	medium	medium brown	white	rough	pink
Ta-8	Xa- AD/2014-17	7-12	elliptical	medium	light brown	white	rough (small scales)	white
Ta-9	TTn 14-6	7-12	ellliptical	medium	medium brown	white laced with pink	less fibrous	pink
Ta-10	Xa- UV3 Yerkadu	7-12	elliptical	small	light brown	white	rough	pink
Ta-11	Xa-19	7-12	ellliptical	small	light brown	white	rough	light pink
Ta-12	Xa- AKI/2015-9	7-12	ovate	medium	medium brown	white	rough	pink
Ta-13	TTn 14-9	7-12	ellliptical	medium	medium brown	white	rough	pink
Ta-14	Xa-NL-6	7-12	elliptical	small	light brown	white	rough	white with pink tinge
Ta-15	Xa-152	7-12	elliptical	large	medium brown	white laced with pink	rough	pink
Ta-16	Xa- AD/2016-4	7-12	elliptical	small	light brown	white	rough	pink
Ta-17	TTn 14-1	7-12	ovoid	small	medium brown	white	rough	pink
Ta-18	Xa-75	7-12	ovate	medium	medium brown	white	rough	pink
Ta-19	TTn 14-5	7-12	ovate	small	medium brown	white	rough	pink
Ta-20	Xa- 12	7-12	elliptical	medium	light brown	white	rough	pink
Ta-23	Sreekaryam local	7-12	elliptical	small	light brown	white	fibrous	pink
Ta-24	TTn14 - 2	7-12	elliptical	small	medium brown	white	fibrous	pink
Ta-25	TTn14 - 8	7-12	elliptical	small	light brown	white	fibrous	dark green
Ta-26	Xa- AD/2014-15	7-12	elliptical	large	light brown	white	fibrous	pink
Ta-27	Xa- MNS/14-1	7-12	elliptical	medium	medium brown	white	rough	pink
Ta-28	Xa- MTS Local	7-12	elliptical	small	medium brown	white	rough	green
Ta-29	Xa -13	7-12	elliptical	small	medium brown	white	rough	pink
Ta-30	Xa -67	7-12	elliptical	small	dark brown	white	rough	green

Ta-31	Xa -24	7-12	elliptic al	small	medium brown	white	rough	green
Ta-32	Xa –JG/ 2016-1	7-12	elliptic al	small	medium brown	pink	rough	purple

Table 9: Details of cormel characters measured at maximum growth

Code No.	**Accession name**	**Time of harvest (months)**	**Shape of cormels**	**Cormel size (at maturity)**	**Exterior colour of cormels**	**Interior colour of cormels**	**Exterior surface of cormels**	**Colour of cormel apex**	**Position of cormel apex**
Ta-1	Xa-SSK/2017-2	7-12	elliptical	medium	light brown	white	fibrous	green	Under ground
Ta-2	Xa- HOB-T8- 2	7-12	cylindrica l	medium	light brown	white	rough	light pink	Under ground
Ta-3	Xa-VHAK/ 2015-5	7-12	elliptical	medium	medium brown	white	fibrous	green	Under ground
Ta-4	Xa-63	7-12	elliptical	medium	dark brown	white	rough	green	Under ground
Ta-5	Xa-26	7-12	elliptical	medium	medium brown	white	rough	green	Under ground
Ta-6	Xa-Ju/10-8	7-12	elliptical	medium	dark brown	white	rough	green	Under ground
Ta-7	Xa-ADS/2014-18	7-12	elliptical	medium	light brown	off-white	rough	pink	Under ground
Ta-8	Xa-AD/2014-17	7-12	cylindrica l	small	light brown	white	rough	white	Under ground
Ta-9	TTn 14-6	7-12	elongated	medium	light brown	white	rough	pink	Under ground
Ta-10	Xa- UV3 Yerkadu	7-12	ovate	small	light brown	white	rough	pink	Under ground
Ta-11	Xa-19	7-12	elliptical	small	light brown	white	rough	light pink	undergro und
Ta-12	Xa-AKI/2015-9	7-12	cylindrica l	medium	light brown	white	rough	pink	Under ground
Ta-13	TTn 14-9	7-12	cylindrica l	small	medium brown	white	rough	pink	Under ground
Ta-14	Xa-NL-6	7-12							
Ta-15	Xa-152	7-12	elongated	large	light brown	white	rough	pink	Under ground
Ta-16	Xa-AD/2016-4	7-12	cylindrica l	small	light brown	white	smooth	pink	Under ground
Ta-17	TTn 14-1	7-12	cylindrica l	small	medium brown	white	rough	pink	Under ground
Ta-18	Xa-75	7-12	cylindrica l	medium	light brown	white	smooth	pink	Under ground
Ta-19	TTn 14-5	7-12	elliptical	medium	light brown	white	smooth	pink	Under ground

Ta-20	Xa- 12	7-12	elliptical	medium	light brown	white	rough	pink	Under ground
Ta-23	Sreekaryam local	7-12	elliptical	medium	light brown	white	fibrous	pink	Under ground
Ta-24	TTn14 - 2	7-12	elliptical	small	medium brown	white	fibrous	pink	Under ground
Ta-25	TTn14 - 8	7-12	cylindrica l	small	medium brown	white	rough	dark green	Under ground
Ta-26	Xa- AD/2014-15	7-12	elliptical	medium	light brown	white	rough	pink	Under ground
Ta-27	Xa- MNS/14-1	7-12	cylindrica l	small	medium brown	white	rough	pink	Under ground
Ta-28	Xa- MTS Local	7-12	elliptical	small	medium brown	white	rough	green	Under ground
Ta-29	Xa -13	7-12	elliptical	medium	medium brown	white	rough	pink	Under ground
Ta-30	Xa -67	7-12	elliptical	small	dark brown	white	rough	green	Under ground
Ta-31	Xa -24	7-12							
Ta-32	Xa –JG/ 2016-1	7-12	elliptical	small	medium brown	pink	rough	purple	Under ground

4.2 Diversity indices

Based on the morphological traits recorded, the percentage distribution, Shannon's diversity index and Simpson's diversity index was computed for these traits across the twenty eight accessions of tannia are represented in Table 10. Here, two accessions (Xa-NL-6 and Xa -24) were not considered as it did not produce cormels. Time of harvest, position of cormel apex and exterior surface of corm showed no variation and remained constant for the twenty eight accessions studied. The Shannon Weaver's diversity index ranged from 0.24 to 0.98 whereas, the Simpson's diversity index ranged from 0.13 to 0.62. Size of corm and size of cormel showed the highest value for both indices.

Table 10: Shannon weaver’s index and Simpson’s diversity index

Character	**Trait**	**Percentage (%)**	**Shannon Weaver's diversity index (H)**	**Simpson's diversity index (D)**
Corm characters				
Shape of corm	Globose	0	0.54	0.37
	Ovate	23		
	Cylindrical	0		
	Elliptical	77		
	Mixed (state which one)	0		
Corm size (at maturity)	small (1-100 g)	50	0.98	0.62
	Medium (100-200 g)	37		
	Large (> 200 g)	13		
Exterior colour of corm	light or medium brown	93	0.24	0.13
	dark brown	7		
Interior colour of corm	white	90	0.39	0.19
	yellow	7		
	orange	0		
	pink (pale red)	3		
	purple	0		
Exterior surface of corm	smooth	0	0.00	0.00
	fibrous or rough	100		
Colour of corm apex	white	0	0.74	0.48
	pink/red	67		
	green	30		
	purple	3		

Cormel characters				
Shape of cormels	globose	0	0.65	0.50
	ovate	4		
	cylindrical	32		
	elliptical	64		
	mixed	0		
Cormel size (at maturity)	small	43	0.82	0.55
	medium	54		
	large	4		
Exterior colour of cormels	light or medium brown	89	0.34	0.20
	dark brown	11		
Interior colour of cormels	white	89	0.34	0.20
	pink (pale red)	11		
	orange	0		
	yellow	0		
	purple	0		
Exterior surface of cormel	smooth	11	0.34	0.20
	fibrous or rough	89		
Colour of cormel apex	white	0	0.77	0.50
	pink/red	64		
	green	32		
	purple	4		
Position of cormel apex	above ground	0	0.00	0.00
	underground	100		

4.3 Pearson's correlation matrix

A correlation matrix was constructed for the different traits analysed. Positive values indicate positive correlation and the matrix reveals correlation between cormel size and cormel shape, also between exterior surface of cormel

and interior colour of corm as well as corm apex colour (Table 11). Pearson's table value (0.05) of N-1 = 0.373886.

Table 11: Pearson’s correlation matrix

	Shape corm	**corm size**	**Ext clr corm**	**Intr clr corm**	**Clr corm apex**	**shape of cormels**	**cormel size**	**exterior colour of cormels**	**interior colour of cormels**	**exterior colour of cormels**	**exterior surface of cormels**
Corm shape	1										
corm size	0.17067	1									
Ext clr corm	0.16013	0.08198	1								
Intr clr corm	0.17160	-0.07614	-0.08243	1							
Clr corm apex	0.21450	-0.04707	0.25024	0.62153	1						
shape of cormels	0.18519	0.32237	0.19571	0.2097	0.31664	1					
cormel size	-0.2933	**0.5631***	-0.28179	0.01510	-0.21570	**0.3748***	1				
exterior colour of cormels	0.2	0.26169	**0.8006***	-0.10296	0.31255	0.2444	-0.1467	1			
interior colour of cormels	0.2	0.10240	-0.0961	**0.8580***	0.31255	0.2444	0.2640	-0.12	1		
exterior colour of cormels	0.33333	0.21617	0.09617	0.10296	0.20224	0.1704	-0.0587	0.12	0.12	1	
exterior surface of cormels	0.12172	-0.10386	0.23388	**0.6015***	**0.9623***	0.3516	-0.1740	0.2921	0.2922	0.0487	1

4.4 ANOVA

Analysis of variance was performed using SAS software on the morphological data of five quantitative characters for twenty eight tannia accessions. For ANOVA, only 28 of the thirty accessions were considered as in two accessions, Ta 14 (Xa-NL-6) and Ta 30 (Xa-24), cormel formation was not there. Analysis of variance performed on the data of five quantitative characters showed significant ($P<0.01$) variation for certain characters. Based on the coefficient of variation, the degree of variability was found to be high for corm weight, number of cormels produced per plant and cormel weight (Table 12). Duncan's multiple range test (Table 13) was done to compare the mean values in ANOVA. Substantial variations was observed in certain characters for the five quantitative morphological traits. All the five characters were found to be statistically significant. For corm length, Ta 15 (Xa-152) was found to be statistically significant with 17.67 cm length, with Ta 4 (Xa-63) and Ta 5 (Xa-26) at par (14.73 and 14.57 cm, respectively). In the case of corm weight, Ta 5 (Xa-26 – 540 g) and Ta 15 (Xa-152 – 513.33 g) were found to be significant with Ta 4 (Xa-63 – 400.00 g) at par. As for the number of cormels produced per plant, Ta 7 (Xa-ADS/2014-18 - 10.67) was found significant with Ta 2 (Xa-HOB-T8-2 – 8.33) and Ta 15 (Xa-152 – 9.33), at par. For the fourth character, cormel length, Ta 1 (Xa-SSK/2017-2 – 9.73 cm) and Ta 15 (Xa-152 – 11.33 cm) were significant with Ta 3 (Xa-VHAK/2015-5 – 9.70 cm), at par. Apart from this, other accessions viz., Ta 4, Ta 5, Ta 6, Ta 7, Ta 9, Ta 18, Ta 19, Ta 20, Ta 23, Ta 26 and Ta 29 were at par and the length ranged from 7.23 (Ta 23 – Sreekaryam local) to 8.63 cm (Ta 20 – Xa-12). For cormel weight, Ta 4 (Xa-63) was found significant with other accessions viz., Ta 1, Ta 2, Ta 3, Ta 5, Ta 10, Ta 12, Ta 17, Ta 19, Ta 20, Ta 23, Ta 26, Ta 27, Ta 28, Ta 29 and Ta 32 were at par. The values ranged from 50.00 g (Ta 20 – Xa-12; Ta 27 – Xa-MNS/14-1 and Ta 32 – Xa-JG/2016-1) to 86.67 g (Ta 5 – Xa-26 and Ta 19 – TTn15-5) (Table 14).

Table 12: Variation in the quantitative characters for 28 tannia accessions

Characters	**Mean**	**Coefficient of variation**	**F value**
Corm length (cm)	9.524	23.757	**6.46***
Corm weight (g)	146.167	69.503	**5.04***
No. of cormels produced per plant	4.505	55.280	2.69
Cormel length (cm)	6.845	31.483	2.37
Cormel weight (g)	52.024	62.879	1.73
			Significant at P<0.01 level

Table 13: Details of tuber characteristics for DMRT

Code assigned	**Accession name**	**Corm length (cm)**	**Corm weight (g)**	**Number of cormels produced**	**Av. Cormel length (cm)**	**Av. Cormel wt (g)**
Ta-1	Xa- SSK/2017-2	12.4	166.7	3.7	9.7	63.3
Ta-2	Xa- HOB-T8- 2	8.5	93.3	8.3	6.6	53.3
Ta-3	Xa- VHAK/2015-5	9.3	163.3	3.7	9.7	66.7
Ta-4	Xa-63	14.7	400.0	4.0	8.0	113.3
Ta-5	Xa-26	14.6	540.0	4.7	7.6	86.7
Ta-6	Xa-Ju/10-8	11.4	200.0	2.7	8.4	40.0
Ta-7	Xa- ADS/2014-18	9.5	143.3	10.7	8.1	46.7
Ta-8	Xa- AD/2014-17	10.0	170.0	4.0	4.5	23.3
Ta-9	TTn 14-6	12.4	183.3	6.0	7.6	23.3
Ta-10	Xa- UV3	7.3	63.3	2.3	5.9	60.0

	Yerkadu					
Ta-11	Xa-19	5.3	26.7	1.5	3.6	10.0
Ta-12	Xa- AKI/2015-9	12.0	190.0	4.7	6.6	63.3
Ta-13	TTn 14-9	12.3	180.0	4.7	4.8	33.3
Ta-14	Xa-NL-6	4.0	21.7	0.0	0.0	0.0
Ta-15	Xa- 152	17.7	513.3	9.3	11.3	26.7
Ta-16	Xa- AD/2016-4	6.1	26.7	0.3	5.0	10.0
Ta-17	TTn 14-1	8.8	86.7	3.0	6.0	73.3
Ta-18	Xa-75	11.7	200.0	6.3	8.1	46.7
Ta-19	TTn 14-5	9.0	86.7	6.3	8.1	86.7
Ta-20	Xa- 12	11.4	120.0	4.3	8.6	50.0
Ta-23	Sreekaryam local	7.8	80.0	5.0	7.2	70.0
Ta-24	TTn14 - 2	7.3	66.7	5.5	5.5	36.7
Ta-25	TTn14 - 8	7.6	56.7	3.3	4.3	26.7
Ta-26	Xa- AD/2014-15	13.6	240.0	6.7	8.1	83.3
Ta-27	Xa- MNS/14-1	9.9	110.0	4.7	5.5	50.0
Ta-28	Xa- MTS Local	6.4	56.7	3.3	4.6	63.3
Ta-29	Xa -13	141.2	73.3	4.0	7.7	70.0
Ta-30	Xa -67	6.4	46.7	1.0	4.4	30.0
Ta-31	Xa -24	3.7	15.0	0.0	0.0	0.0
Ta-32	Xa –JG/2016-1	7.0	65.0	1.0	6.0	50.0

Table 14: Duncan's multiple range test for significant quantitative characters (***mean values with the same letter do not differ significantly***)

Accession	corm length (cm)	Corm weight (g)	No. of cormels produced per plant	Cormel length (cm)	Cormel weight (g)
Ta 1	12.433^{bcd}	166.67^{cd}	3.667^{cde}	**9.733^{a}**	63.33^{abc}
Ta 2	8.533^{defghi}	93.33^{cd}	8.333^{ab}	6.633^{bcd}	53.33^{abc}
Ta 3	9.300^{cdefgh}	163.33^{cd}	3.667^{cde}	9.700^{ab}	66.67^{abc}
Ta 4	14.733^{ab}	400.00^{ab}	4.000^{cde}	8.000^{abc}	**113.33^{a}**
Ta 5	14.567^{ab}	**540.00^{a}**	4.667^{bcde}	7.600^{abcd}	86.67^{ab}
Ta 6	11.433^{bcdefg}	200.00^{cd}	3.000^{de}	8.367^{abc}	40.00^{bc}
Ta 7	9.500^{cdefghi}	143.33^{cd}	**10.667^{a}**	8.100^{abc}	46.67^{bc}
Ta 8	10.000^{cdefgh}	170.00^{cd}	4.000^{cde}	4.467^{cd}	23.33^{bc}
Ta 9	12.367^{bcd}	183.33^{cd}	6.000^{abcde}	7.600^{abcd}	23.33^{bc}
Ta 10	7.333^{gfhij}	63.33^{cd}	2.333^{de}	5.900^{bcd}	60.00^{abc}
Ta 11	5.333^{ij}	26.67^{cd}	1.667^{de}	3.600^{d}	10.00^{c}
Ta 12	11.967^{bcde}	190.00^{cd}	4.667^{bcde}	6.633^{bcd}	63.33^{abc}
Ta 13	12.333^{bcd}	180.00^{cd}	4.667^{bcde}	4.767^{cd}	33.33^{bc}
Ta 14	4.000^{j}	21.67^{d}	-	-	-
Ta 15	**17.667^{a}**	**513.33^{a}**	9.333^{ab}	**11.333^{a}**	26.67^{bc}
Ta 16	6.100^{hij}	26.67^{d}	1.000^{e}	5.000^{cd}	10.00^{c}
Ta 17	8.767^{defghi}	86.67^{cd}	3.000^{de}	6.000^{bcd}	73.33^{abc}
Ta 18	11.667^{bcdef}	200.00^{cd}	6.333^{abcd}	8.067^{abc}	46.67^{bc}
Ta 19	9.000^{defghi}	86.67^{cd}	6.333^{abcd}	8.100^{abc}	86.67^{ab}
Ta 20	11.367^{bcdefg}	120.00^{cd}	4.333^{cde}	8.633^{abc}	50.00^{abc}
Ta 23	7.767e^{fghij}	80.00^{cd}	5.000^{bcde}	7.233^{abcd}	70.00^{abc}
Ta 24	7.267^{ghijk}	66.67^{cd}	5.467^{bcde}	5.467^{bcd}	36.67^{bc}
Ta 25	7.600^{efghij}	56.67^{cd}	3.333^{de}	4.333^{cd}	26.67^{bc}
Ta 26	13.600^{bc}	240.00^{bc}	6.667^{abcd}	8.133^{abc}	83.33^{ab}

Ta 27	9.933^{cdefg}	110.00^{cd}	4.667^{bcde}	5.567^{bcd}	50.00^{abc}
Ta 28	6.367^{hij}	56.67^{cd}	3.333^{de}	4.600^{cd}	63.33^{abc}
Ta 29	7.667^{efghij}	73.33^{cd}	4.000^{cde}	7.733^{abcd}	70.00^{abc}
Ta 30	6.433^{hij}	46.67^{cd}	1.000^{e}	4.400^{cd}	30.00^{bc}
Ta 31	3.667^{j}	15.00^{d}	-	-	-
Ta 32	7.033^{hij}	65.00	1.000^{e}	5.967^{bcd}	50.00^{abc}

4.5 Principal Component Analysis

The PCA was done using SAS software both on the basis of accessions and characters for twenty eight of the thirty tannia accessions studied. For PCA analysis only 28 of the thirty accessions were considered as in two accessions, Ta 14 (Xa-NL-6) and Ta 30 (Xa-24), cormel formation was not there.

PCA based on accessions

The first four components with Eigen values greater than one accounted for 78.40 per cent of the total variation (Table 15, Fig. 1). Component 1 was responsible for 29.79% of the total variation. Component 2 was responsible for 19.61% of the total variation while component 3 was responsible for 17.53% of the variation. Component four contributed 11.47% bringing the total to 78.40%. Here, the maximum variation was explained by the 1st component. Ta 6 (Xa- Ju/10-8), Ta 30 (Xa-67), Ta 15 (Xa-152), and Ta 32 (Xa-JG 2016-1) can be seen as accessions showing maximum variability.

Table 15: Principal component analysis in 28 accessions of tannia based on accessions

Sample	**Accessions number**	**PC 1**	**PC 2**	**PC 3**	**PC 4**
Ta-1	Xa-SSK/2017-2	0.82044	0.24929	0.54990	-0.03861
Ta-2	Xa-HOB-T8- 2	-1.68308	0.46166	-0.56346	-0.67308
Ta-3	Xa-VHAK/ 2015-5	0.30693	0.54707	0.40915	-1.46634

Ta-4	Xa-63	1.66688	-1.30873	2.06515	-0.49857
Ta-5	Xa-26	0.90803	0.28213	1.39192	0.03989
Ta-6	Xa-Ju/10-8	2.49645	-3.94217	0.70561	-0.66083
Ta-7	Xa-ADS/2014-18	-1.08824	0.58601	0.95599	-0.87584
Ta-8	Xa-AD/2014-17	-0.98537	-0.43601	-0.49240	1.31329
Ta-9	TTn 14-6	1.09236	2.26896	0.92427	0.97142
Ta-10	Xa-UV$_3$ Yerkadu	-1.58021	-0.56036	-2.01184	1.51605
Ta-11	Xa-19	-0.56571	-0.37735	-0.65699	0.95354
Ta-12	Xa-AKI/2015-9	-1.59549	0.49451	0.27856	-0.59458
Ta-13	TTn 14-9	-0.98537	-0.43601	-0.49240	1.31329
Ta-15	Xa-152	1.08334	2.93454	2.67799	0.56978
Ta-16	Xa-AD/2016-4	-1.62074	-0.26258	-1.90217	-0.62290
Ta-17	TTn 14-1	-1.58647	-0.17107	-1.47516	-0.19294
Ta-18	Xa-75	-2.14327	0.70078	-0.28919	-2.45227
Ta-19	TTn 14-5	-1.04338	0.75379	-0.74033	-3.20465
Ta-20	Xa-12	-0.57473	0.28823	1.09673	0.55190
Ta-23	Sreekaryam local	-0.66232	0.25538	0.25471	0.47340
Ta-24	TTn14-2	-0.56571	-0.37735	-0.65699	0.95354
Ta-25	TTn14-8	0.32221	-0.50779	-1.88125	0.64429
Ta-26	Xa-AD/2014-15	-0.48714	0.32107	1.93875	0.63039
Ta-27	Xa-MNS/14-1	-0.98537	-0.43601	-0.49240	1.31329
Ta-28	Xa-MTS Local	0.82945	-0.41629	-1.20383	0.36303
Ta-29	Xa-13	-0.48714	0.32107	1.93875	0.63039
Ta-30	Xa-67	2.49645	-3.94217	0.70561	-0.66083
Ta-32	Xa-JG 2016-1	6.61722	2.70938	-3.03470	-0.29608
Eigen values		**3.276**	**2.157**	**1.928**	**1.262**
Percent variation		**1.119**	**0.230**	**0.666**	**0.484**
Cumulative percentage		**0.298**	**0.494**	**0.670**	**0.784**

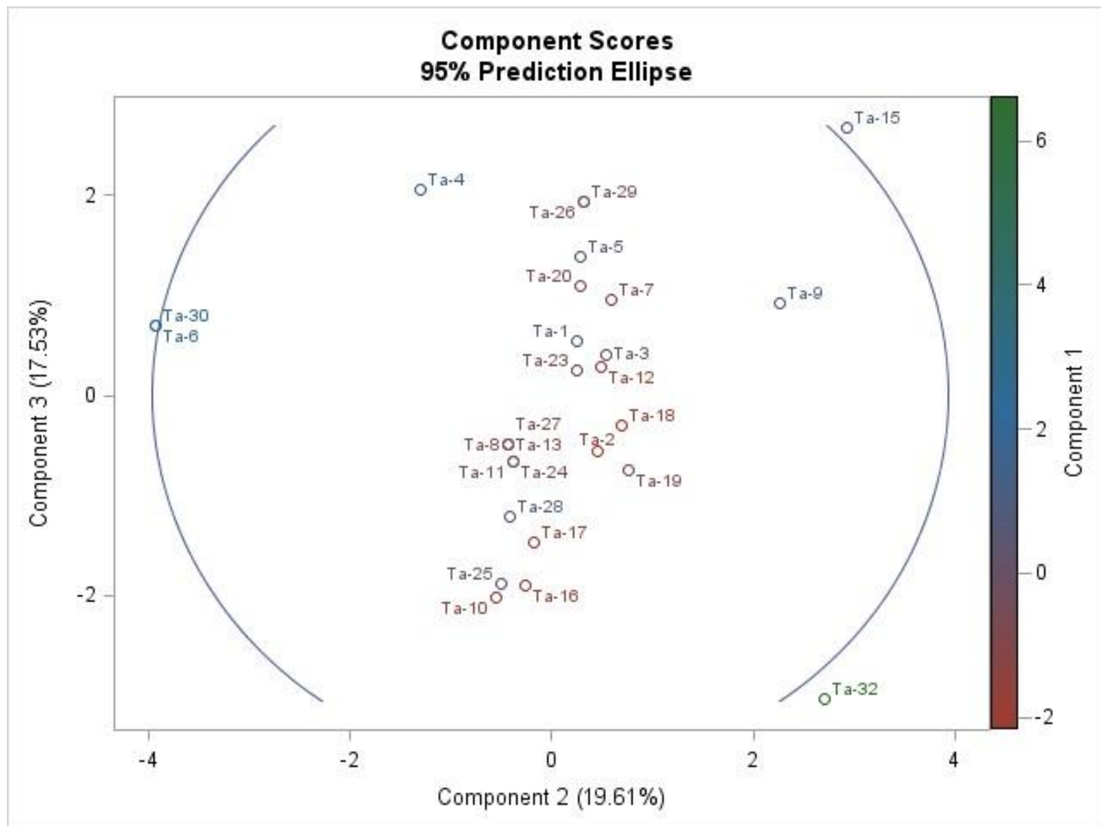

Fig. 1: Cluster plot representation based on PCA based on accessions

Principal component analysis based on characters

The first two components with Eigen values greater than one accounted for 92.04 per cent of the total variation (Table 16, Fig. 2). In this, component 1 was responsible for 85.04% of the total variation, whereas, component 2 was responsible for 7.00% of the total. Here, it was seen that the maximum variation was explained by the 1st component. These two components account for a total of 92.04% variability among the characters recorded. Characters 1 (shape of corm), 2 (corm size) 6 (shape of the cormel) and 7 (cormel size) were found to be showing the maximum variability.

Table 16: Principal component analysis in 28 accessions of tannia based on characters

Character	**PC1**	**PC 2**
Shape of corm	2.65202	2.99228
Corm size	6.20501	-0.20234
Exterior colour of corm	-4.13003	-0.19414
Interior corm colour	-4.03387	-0.49400
Colour of corm apex	-0.25926	0.45603
Shape of cormels	3.35910	-0.30486
Cormel size (at maturity)	6.02051	-1.83506
Exterior colour of cormels	-4.00462	-0.17797
Interior colour of cormels	-4.03387	-0.49400
Exterior colour of cormels	-1.66937	-0.02368
Exterior surface of cormels	-0.10560	0.27774
Eigen values	**16.157**	**1.330**
Percent variation	**14.827**	**0.668**
Cumulative percentage	**0.850**	**0.920**

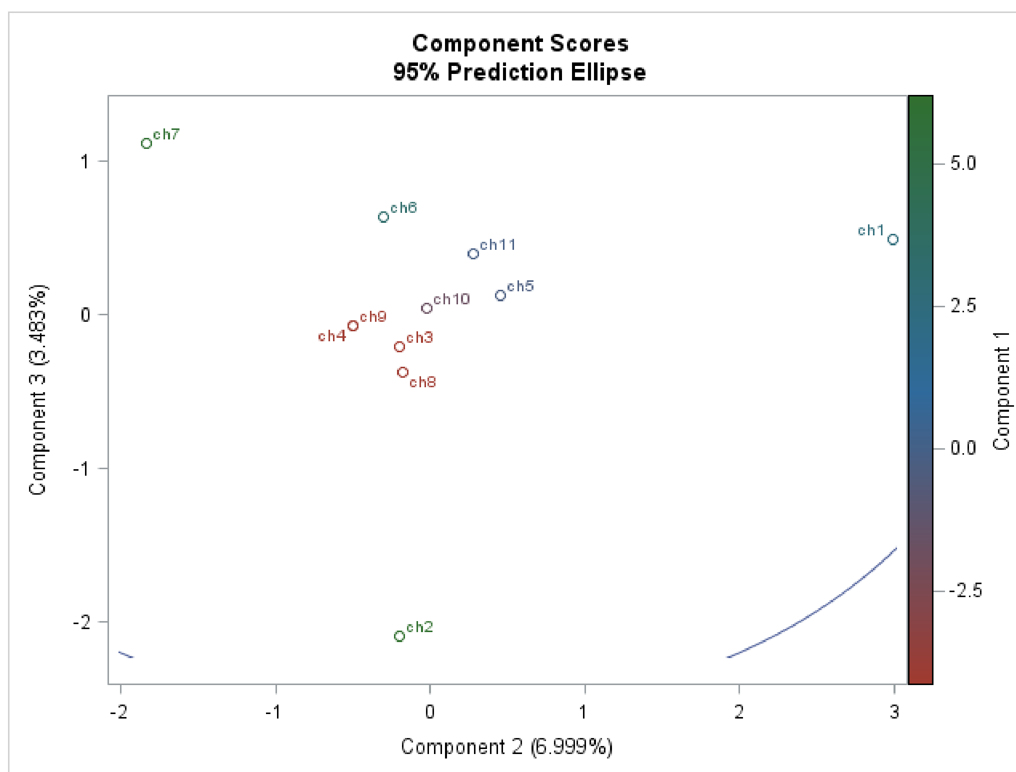

Fig. 2: Cluster plot representation based on PCA based on characters

4.6. Cluster analysis

Hierarchical clustering was done and a cluster dendrogram was drawn with the help of NTSys pc software. Morphologically similar accessions were assembled in the same group and distinct accessions were grouped separately. Cluster dendrogram resulted in four major clusters and one outlier (Fig. 3). Cluster I had four sub clusters, Ia, Ib, Ic and Id comprising seven, five, six and five accesions, respectively, Sub cluster 1a comprised of one set of duplicates viz., Ta 26 (Xa-AD/2014-15) and Ta 29 (Xa-13), while sub cluster 1c comprised of one set of duplicates viz., Ta 11 (Xa-19) and Ta 24 (TTn14-2). Sub cluster 1d on the other hand showed a set of duplicates comprising three accessions, Ta 8 (Xa- AD/2014-17), Ta 13 (TTn14-9) and Ta 27 (Xa-MNS/14-1). Cluster II, III and IV consisted to two accessions each, while Xa-JG 2016-1 remained an outlier (Table 17). Cluster 2 grouped the two accessions, TTn-14-6 and Xa-152 which produced elongated pencil like cormels which were unmarketable (Table 17). In the case of

Xa-JG/2016-1, the flesh colour was pinkish while in all the other accessions it was white. The rest of the accessions showed variability in tuber shape, size and colour as well as colour of corm apex.

Table 17: Details of accessions grouping under various clusters

Cluster		**No. of accessions**	**Accessions**
1	a	7	Xa-SSK/2017-2, Xa-VHAK/2015-5, Xa-63, Xa-26, Xa-12, Xa-AD/2014-15, Xa-13
	b	5	Xa-HOB-T8-2, Xa-AKI/2015-9, Xa-ADS/2014-18, Xa-75, TTn14-5
	c	6	Xa-UV$_3$Yerkadu, Xa-19, TTn14-2, Sreekaryam local, TTn14-8, Xa-MTS local
	d	5	Xa-AD/2014-17, TTn14-9, Xa-MNS/14-1, Xa-AD/2016-4, TTn14-1
2		2	TTn-14-6, Xa-152
3		2	Xa-Ju/10-8, Xa-67
4		2	Xa-NL-6, Xa-24
Outlier		1	Xa-JG/2016-1

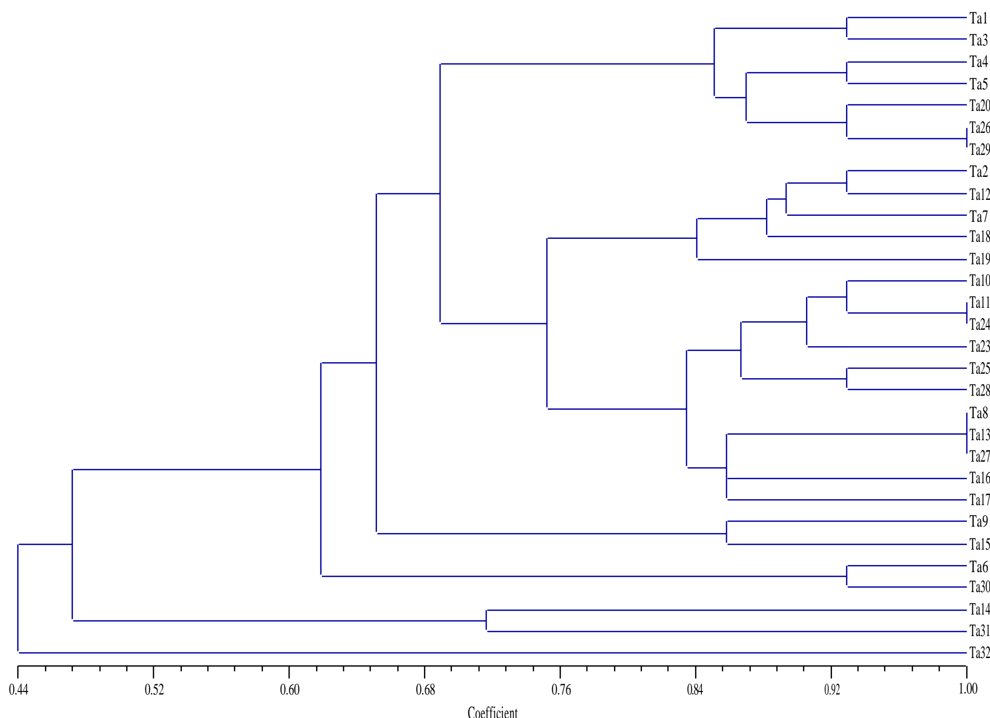

Fig. 3: Cluster dendrogram drawn using NTSys pc software package

4.7 DNA ISOLATION PROTOCOL

DNA was isolated from all 30 tannia accessions by employing a slightly modified version of Dellaporta protocol (Plate 7). This method gave good quality of DNA and revealed good absorbance values with purity ranging from 1.06 (TTn 14-5) to 2.34 (Xa-HOB-T8-2). The spectrophotometry readings of DNA samples extracted from these accessions are listed in the table 18.

Table 18: Yield and purity of DNA isolated

Sample	**Accession name**	**Concentration**	**OD value A_{260}/A_{280}**
Ta 1	Xa- SSK/2017-2	820.258	2.30
Ta 2	Xa- HOB-T8- 2	668.453	2.34
Ta 3	Xa-VHAK/ 2015-5	2774.921	2.13
Ta 4	Xa-63	1167.709	2.19
Ta 5	Xa-26	1926.193	2.22

Ta 6	Xa-Ju/10-8	1413.508	2.13
Ta 7	Xa- ADS/2014-18	711.079	2.14
Ta 8	Xa- AD/2014-17	908.637	2.25
Ta 9	TTn 14-6	687.002	2.34
Ta 10	Xa- UV3 Yerkadu	764.410	2.06
Ta 11	Xa-19	677.580	2.24
Ta 12	Xa- AKI/2015-9	969.058	2.24
Ta 13	TTn 14-9	907.885	2.26
Ta 14	Xa-NL-6	663.440	2.04
Ta 15	Xa-152	625.825	2.27
Ta 16	Xa- AD/2016-4	797.384	2.28
Ta 17	TTn 14-1	780.047	2.06
Ta 18	Xa-75	552.695	1.52
Ta 19	TTn 14-5	658.933	1.06
Ta 20	Xa- 12	795.252	2.21
Ta 23	Sreekaryam local	820.283	2.13
Ta 24	TTn14 - 2	806.619	2.16
Ta 25	TTn14 - 8	906.465	2.04
Ta 26	Xa- AD/2014-15	1261.035	2.13
Ta 27	Xa- MNS/14-1	383.335	2.17
Ta 28	Xa- MTS Local	1180.901	2.08
Ta 29	Xa -13	750.100	2.28
Ta 30	Xa -67	811.874	2.12
Ta 31	Xa -24	1067.152	2.04
Ta 32	Xa –JG /2016-1	778.322	2.18

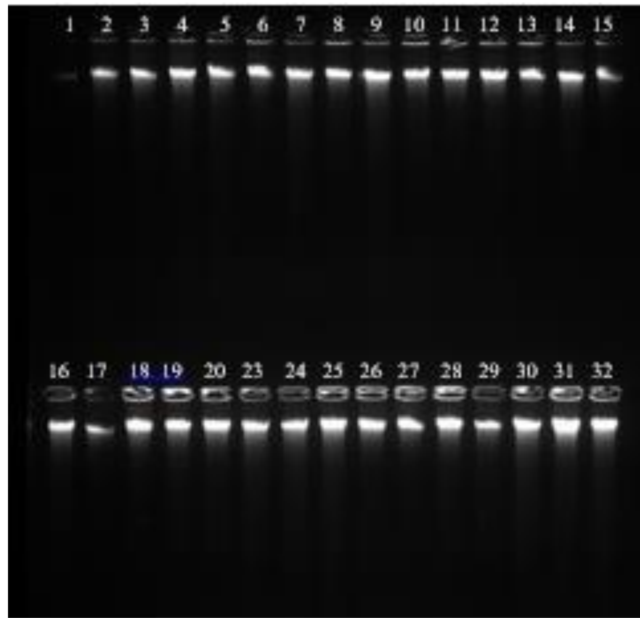

Plate 7. DNA isolated using modified Dellaporta protocol

Ta 1: Xa- SSK/2017-2; Ta 2: Xa- HOB-T8- 2; Ta 3: VHAK/ 2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Ju/10-8; Ta 7: Xa- ADS/2014-18; Ta 8: Xa- AD/2014-17;Ta 9: TTn 14-6; Ta 10: Xa- UV3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9;Ta 13: TTn 14-9; Ta 14: Xa-NL-6; Ta 15: 152; Ta 16: Xa- AD/2016-4; Ta 17 : TTn 14-1; Ta 18: Xa-75; Ta 19: TTn 14-5; Ta 20: Xa- 12; Ta 23: Sreekaryam local; Ta 24: TTn14 – 2; Ta 25: TTn14 – 8; Ta 26: Xa- AD/2014-15; Ta 27: Xa- MNS/14-1; Ta 28: XA- MTS Local; Ta 29: Xa -13; Ta 30: Xa -67; Ta 31: Xa -24; Ta 32: Xa –JG 2016-1

4.8 Primer screening

A total of 24 ISSR primers were taken for determining its amplifying capability. DNA samples of three accessions (Ta 1: Xa- SSK/2017-2; Ta 2: Xa-HOB-T8-2 and Ta 3: Xa-VHAK/2015-5) were taken for screening purpose. Plates 8 and 9 show the gel profile of screening the total primers. A total of 9 primers were eliminated from further analysis as they gave unclear or no bands. The primers which were eliminated were: UBC 815, UBC 835, UBC 851, UBC 860, UBC 864, UBC 873, UBC 11, and $(ACC)_6Y$.

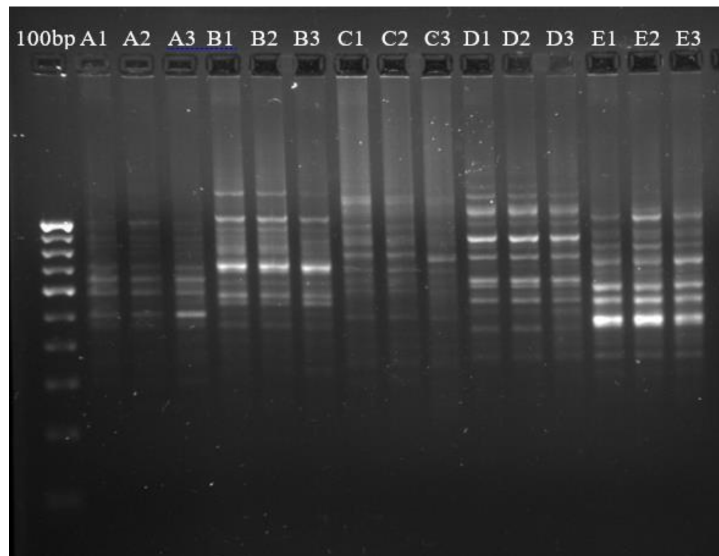

Plate 8. Primer screening image

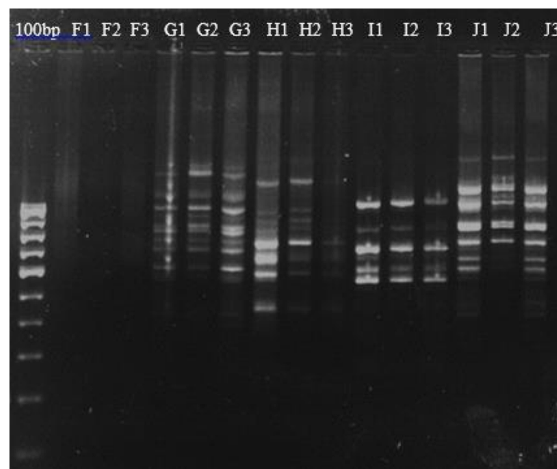

Plate 9. Primer screening image

4.9 ISSR analysis of tannia accessions

After standardizing the PCR conditions using the selected primers and their respective annealing temperatures, the amplicons were resolved in 2% agarose gel and the bands obtained for primers are shown in Plate 10 – 23.

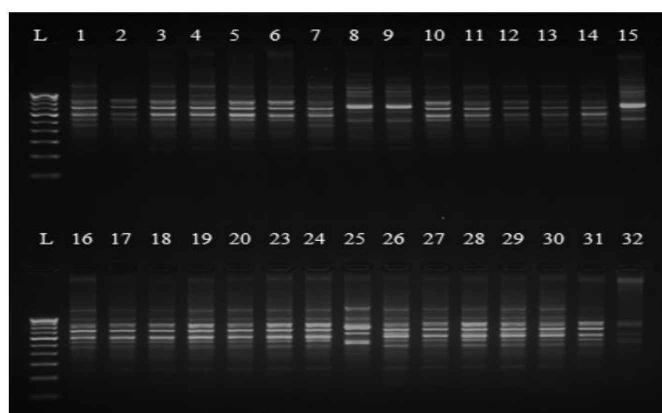

Plate 10. Agarose gel profile (2%) with the primer UBC 807

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

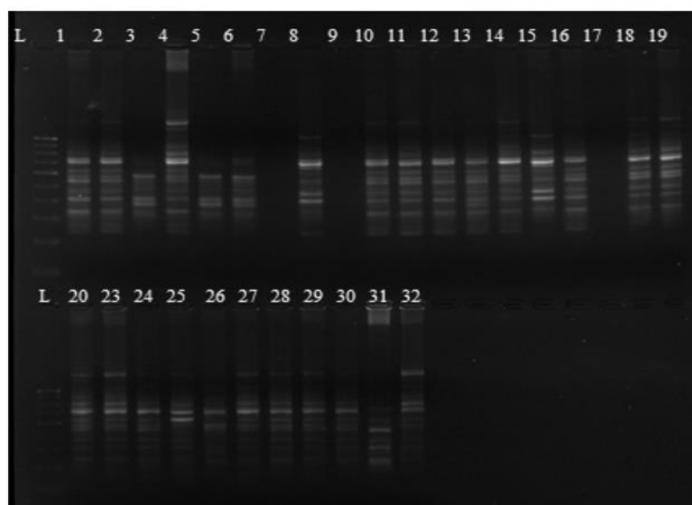

Plate 11. Agarose gel profile (2%) with the primer UBC 808

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa -13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

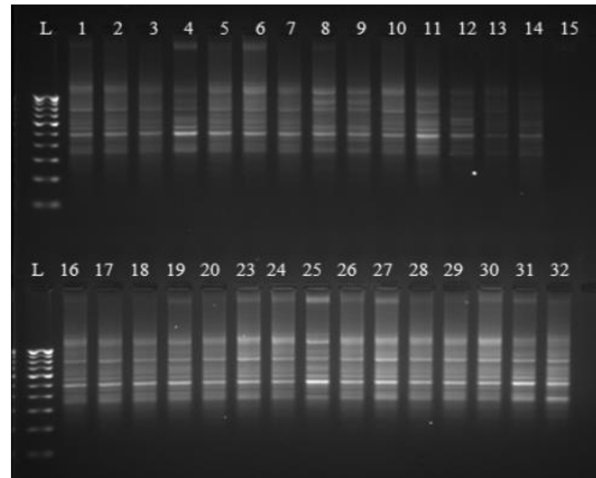

Plate 12. Agarose gel profile (2%) with the primer UBC 809

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

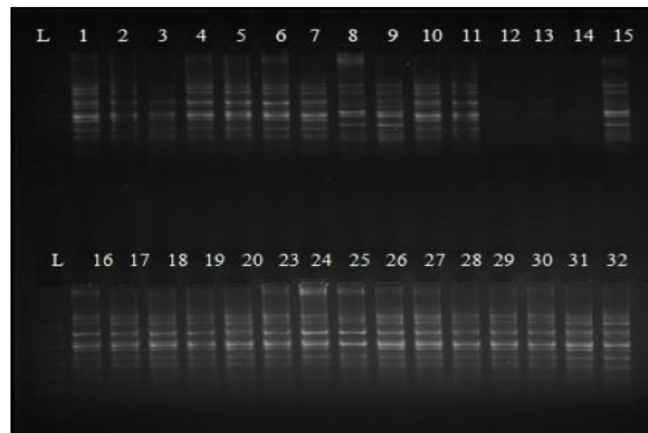

Plate 13. Agarose gel profile (2%) with the primer UBC 810

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa -13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

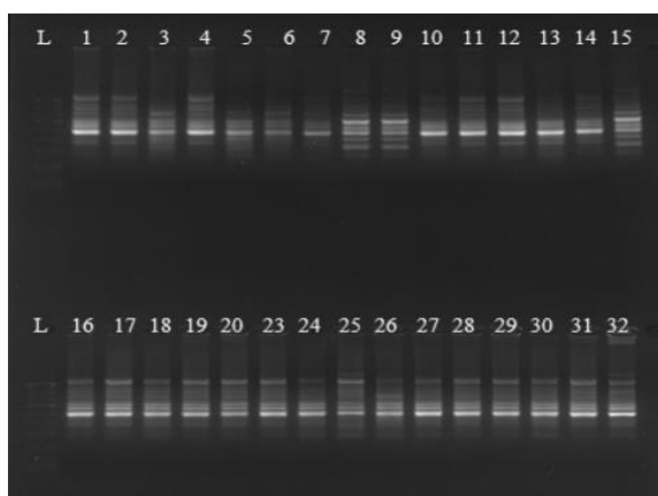

Plate 14. Agarose gel profile (2%) with the primer UBC 811

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

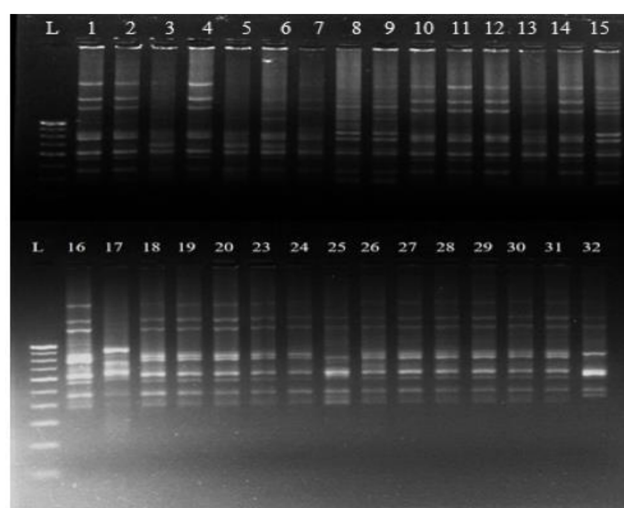

Plate 15. Agarose gel profile (2%) with the primer UBC 817

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

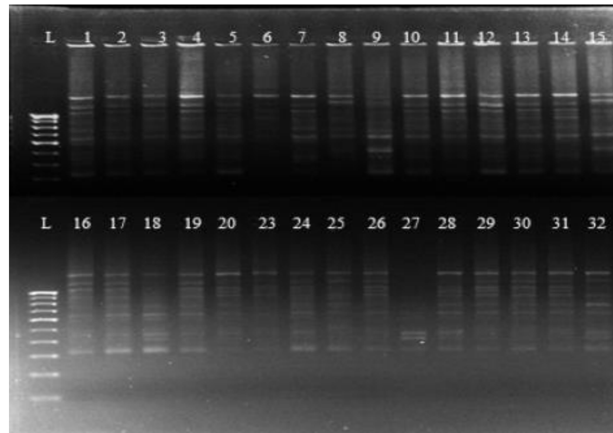

Plate 16. Agarose gel profile (2%) with the primer UBC 818

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa -13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

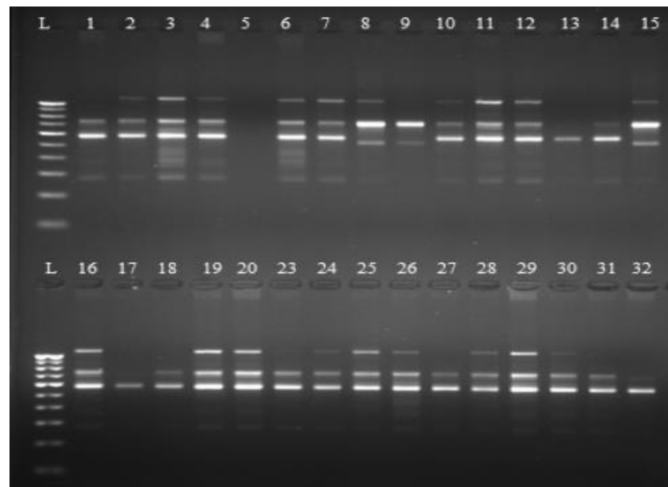

Plate 17. Agarose gel profile (2%) with the primer UBC 824

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

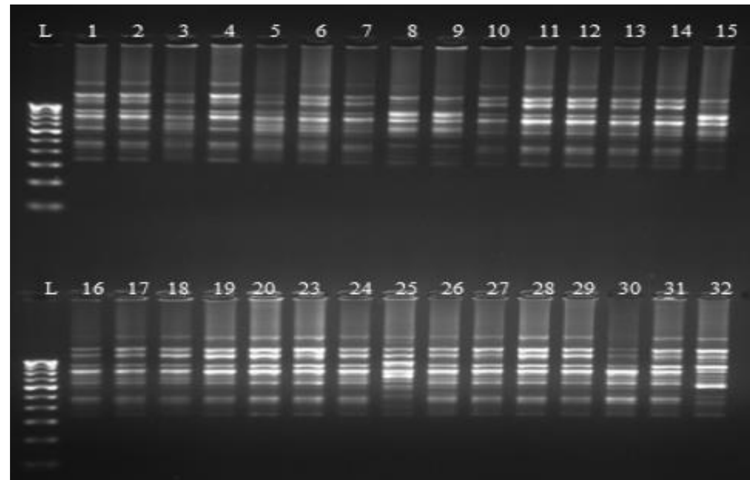

Plate 18. Agarose gel profile (2%) with the primer UBC 825

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa- MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa– JG/2016-1

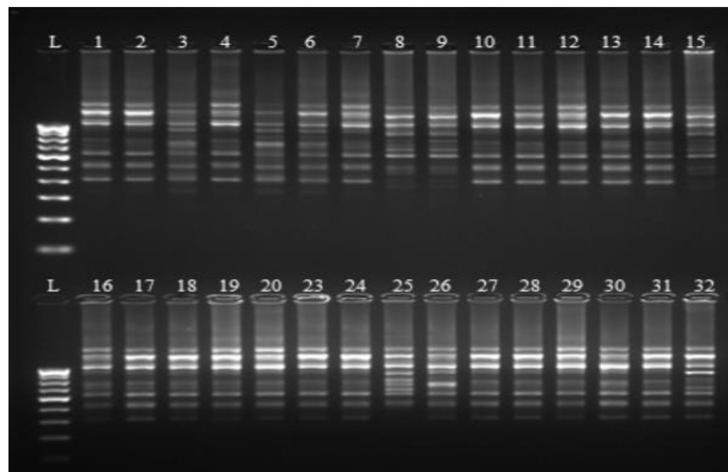

Plate 19. Agarose gel profile (2%) with the primer UBC 827

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa -13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

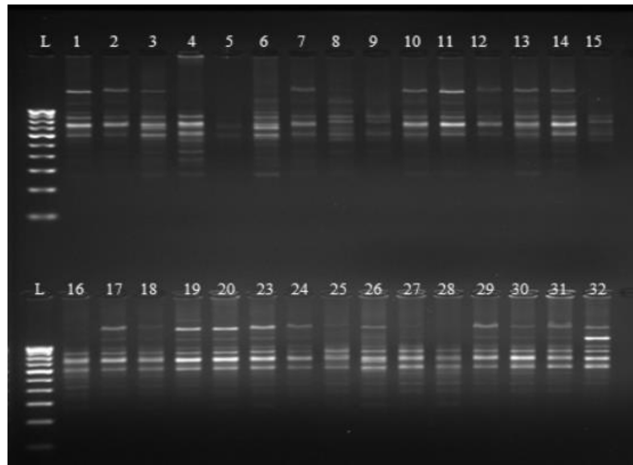

Plate 20. Agarose gel profile (2%) with the primer UBC 834

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

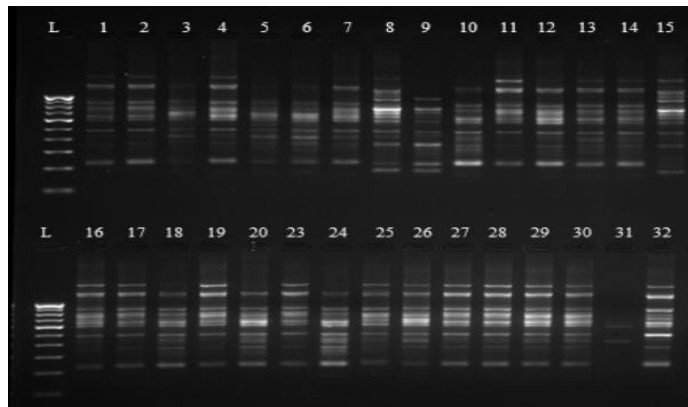

Plate 21. Agarose gel profile (2%) with the primer UBC 836

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa-AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

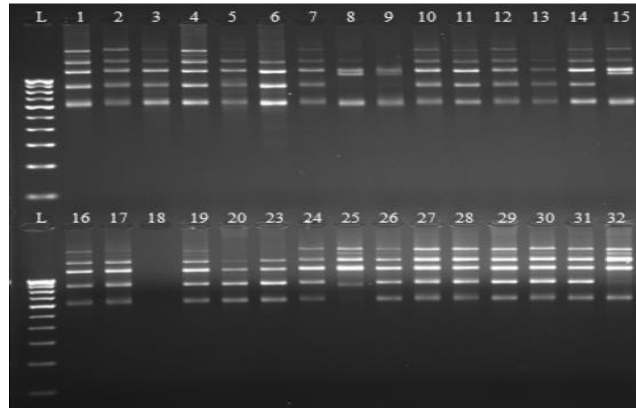

Plate 22. Agarose gel profile (2%) with the primer UBC 845

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa-AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

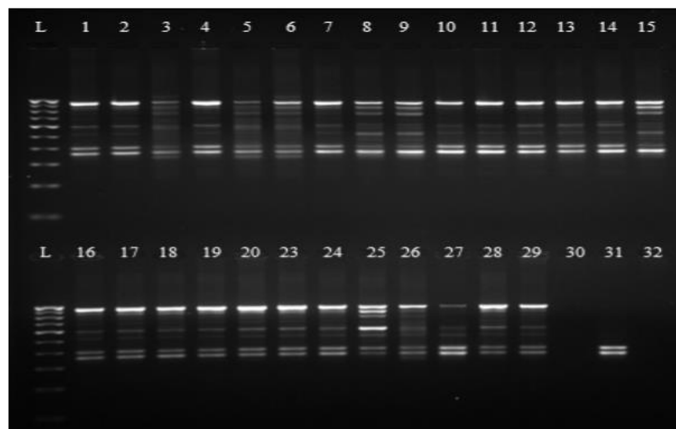

Plate 23. Agarose gel profile (2%) with the primer (GA)$_9$AC

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV$_3$ Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa-12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa-AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa-13; Ta 30: Xa-67; Ta 31: Xa-24; Ta 32: Xa–JG/2016-1

4.10 ANALYSIS OF MOLECULAR MARKER DATA USING ISSR MARKERS

4.10.1 Quantification of genetic variability

The various parameters estimated for the quantification of genetic variability using twenty-eight ISSR markers is shown in table 19. The primers showed 91.64% polymorphism and the number of bands ranged from 7 to 19 of which, UBC 808, UBC 810, UBC 818 and UBC 824 showed 100% polymorphism. The percentage polymorphism ranged from 81.82 to 100 percentage.

Table 19: Parameters estimated for the quantification of genetic variability using fourteen ISSR markers

Primer used	**Annealing temperature (° C)**	**No. of bands**	**Number of polymorphic bands**	**% polymorphism**
UBC 807	56.3	12	11	91.67
UBC 808	56.3	19	19	100.00
UBC 809	56.3	10	9	90
UBC 810	56.3	15	15	100
UBC 811	56.3	11	9	81.82
UBC 817	56.3	11	10	90.91
UBC 818	56.3	12	12	100.00
UBC 824	56.3	9	9	100.00
UBC 825	56.3	11	9	81.82
UBC 827	56.3	12	10	83.33
UBC 834	56.3	11	10	90.91
UBC 836	56.3	18	17	94.44
UBC 845	56.3	7	6	85.71
$(GA)_9AC$	56.3	13	12	92.31
Mean				91.64

4.10.2 Simple Matrix Diversity Index

The similarity matrix between the accessions was obtained by determining the Jaccard's coefficient. Similarity matrix based on Jaccard's coefficient for 30 tannia accessions using 14 ISSR markers is given in table 21. Here, two sets were found having 100% similarity viz., Ta 13 (TTn14-9) and Ta 27 (Xa-MNS/14-1) as well as Ta 19 (TTn14-5) and Ta 28 (Xa-MTS-local) (Table 20).

4.10.3 Cluster analysis

Cluster analysis was done based on Euclidean distance (Hierarchical clustering), in which the thirty accessions were grouped into four major clusters shown as in the dendrogram (Fig. 4). Maximum number of accessions was included in cluster I and it contained 22. Cluster II comprised two whereas, cluster III and IV comprised 3 each (Table 21). The biggest cluster, I had two sub cluster Ia and Ib along with three divergent lines viz., Ta 17 (TTn14-1), Ta 31 (Xa-24) and Ta 7 (Xa-AD/2014-18). Sub cluster Ia comprised one divergent line Ta 4 (Xa- 63). Apart from this, sub cluster Ib comprised two duplicates Ta 13 (TTn14-9) and Ta 27 (Xa-MNS/14-1) as well as Ta 19 (TTn14-5) and Ta 28 (Xa-MTS-local) which showed 100% similarity. Cluster II comprised two accessions, Ta 25 (TTn14-8) and Ta 32 (Xa-JG/2016-1), while cluster III comprised Ta 3 (Xa- VHAK/2015-5), Ta 5 (Xa-26) and Ta 6 (Xa-Ju/10-8). Cluster IV also comprised three accessions, Ta 8 (Xa-AD/2014-17), Ta 9 (TTn14-6) and Ta 15 (Xa-152). Both TTn14-6 and Xa-152 produced elongated pencil like cormels, whereas, Xa- AD/2014-17 produced normal cylindrical cormels. Cluster II had two accessions collected from the North east region pooling together.

Table 20: Details of accessions grouping under various clusters

Cluster		No. of accessions	Accessions
1	a	7	Ta 1 (Xa-SSK/2017-2), Ta 2 (Xa- HOB-T8- 2) , Ta 10 (Xa-UV3 Yerkadu), Ta 11 (Xa-19), Ta 12 (Xa- AKI/2015-9), Ta 14 (Xa-NL-6)
			Divergent line- Ta 4 (Xa-63)
	b	15	Ta 13 (TTn 14-9), Ta 27 (Xa- MNS/14-1) ,Ta 19 (TTn 14-5) Ta 28 (Xa- MTS Local), Ta 26 (Xa- AD/2014-15), Ta 29 (Xa -13), Ta 30 (Xa -67), Ta 16 (Xa- AD/2016-4), Ta 18 (Xa-75), Ta 24 (TTn14-2), Ta 20 (Xa-12), Ta 23 (Sreekaryam local)
			Divergent lines - Ta 17 (TTn 14-1), Ta 31 (Xa -24), Ta 7 (Xa-ADS/2014-18)
2		2	Ta 25 (TTn14-8) , Ta 32 (Xa-JG/2016-1)
3		3	Ta 3 (Xa-VHAK/2015-5), Ta 5 (Xa-26) , Ta 6 (Xa-Ju/10-8)
4		3	Ta 8 (Xa-AD/2014-17), Ta 9 (TTn14-6) , Ta 15 (Xa-152)

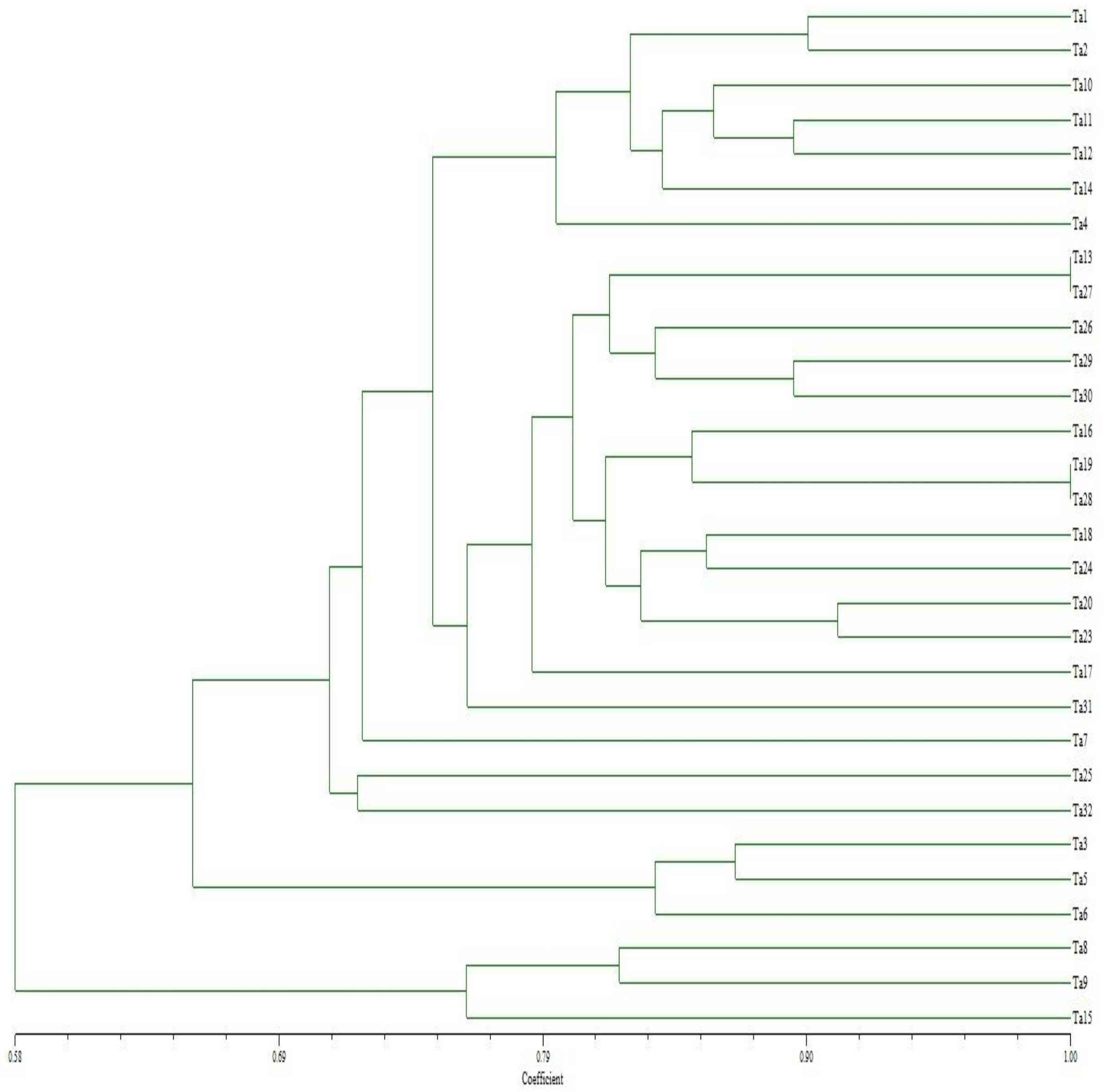

Fig. 4: Dendrogram of 30 tannia accessions using molecular data

Table 21: Similarity matrix based on Jaccard's coefficient for 30 tannia accessions using 14 ISSR markers

	Ta1	Ta2	Ta3	Ta4	Ta5	Ta6	Ta7	Ta8	Ta9	Ta10	Ta11	Ta12	Ta13	Ta14	Ta15	Ta16	Ta17	Ta18	Ta19	Ta20	Ta23	Ta24	Ta25	Ta26	Ta27	Ta28	Ta29	Ta30	Ta31	Ta32
Ta1	1																													
Ta2	0.9	1																												
Ta3	0.66	0.71	1																											
Ta4	0.81	0.81	0.62	1																										
Ta5	0.66	0.66	0.87	0.62	1																									
Ta6	0.72	0.69	0.81	0.68	0.86	1																								
Ta7	0.75	0.75	0.71	0.71	0.73	0.71	1																							
Ta8	0.54	0.59	0.53	0.55	0.51	0.58	0.58	1																						
Ta9	0.5	0.54	0.57	0.47	0.58	0.59	0.64	0.82	1																					
Ta10	0.84	0.84	0.69	0.8	0.69	0.75	0.77	0.59	0.58	1																				
Ta11	0.84	0.82	0.66	0.79	0.69	0.73	0.77	0.6	0.53	0.87	1																			
Ta12	0.8	0.82	0.63	0.81	0.66	0.71	0.75	0.58	0.54	0.85	0.89	1																		
Ta13	0.76	0.72	0.56	0.73	0.58	0.65	0.69	0.59	0.58	0.76	0.84	0.78	1																	
Ta14	0.84	0.8	0.61	0.76	0.63	0.7	0.73	0.56	0.5	0.81	0.87	0.83	0.76	1																
Ta15	0.56	0.61	0.56	0.55	0.55	0.58	0.57	0.79	0.73	0.61	0.58	0.57	0.57	0.6	1															
Ta16	0.8	0.79	0.61	0.8	0.62	0.69	0.7	0.62	0.6	0.8	0.81	0.78	0.8	0.73	0.61	1														
Ta17	0.75	0.71	0.65	0.66	0.66	0.65	0.74	0.6	0.66	0.71	0.76	0.73	0.74	0.7	0.55	0.77	1													
Ta18	0.75	0.72	0.62	0.68	0.65	0.69	0.7	0.61	0.66	0.77	0.76	0.76	0.82	0.72	0.6	0.82	0.82	1												
Ta19	0.78	0.72	0.57	0.75	0.57	0.62	0.64	0.58	0.53	0.73	0.81	0.79	0.82	0.73	0.56	0.85	0.77	0.8	1											
Ta20	0.76	0.71	0.59	0.69	0.63	0.69	0.73	0.57	0.59	0.75	0.79	0.79	0.79	0.72	0.55	0.79	0.78	0.82	0.83	1										
Ta23	0.79	0.75	0.62	0.71	0.65	0.69	0.74	0.62	0.61	0.76	0.8	0.76	0.77	0.75	0.58	0.82	0.83	0.84	0.84	0.91	1									
Ta24	0.76	0.73	0.67	0.7	0.71	0.73	0.77	0.62	0.66	0.76	0.76	0.77	0.77	0.71	0.61	0.81	0.8	0.86	0.8	0.82	0.84	1								
Ta25	0.67	0.66	0.64	0.66	0.65	0.65	0.71	0.68	0.68	0.69	0.71	0.68	0.69	0.64	0.64	0.77	0.73	0.75	0.74	0.71	0.75	0.77	1							
Ta26	0.71	0.68	0.66	0.67	0.69	0.74	0.73	0.61	0.64	0.7	0.75	0.72	0.79	0.69	0.62	0.79	0.8	0.78	0.78	0.82	0.81	0.83	0.82	1						
Ta27	0.76	0.72	0.56	0.73	0.58	0.65	0.69	0.59	0.58	0.76	0.84	0.78	1	0.76	0.57	0.8	0.74	0.82	0.82	0.79	0.77	0.77	0.69	0.79	1					
Ta28	0.78	0.72	0.57	0.75	0.57	0.62	0.64	0.58	0.53	0.73	0.81	0.79	0.82	0.73	0.56	0.85	0.77	0.8	1	0.83	0.84	0.8	0.74	0.78	0.82	1				
Ta29	0.76	0.74	0.64	0.73	0.63	0.7	0.75	0.6	0.6	0.77	0.84	0.77	0.84	0.75	0.57	0.82	0.8	0.79	0.86	0.85	0.85	0.83	0.76	0.84	0.84	0.86	1			
Ta30	0.73	0.69	0.62	0.68	0.61	0.67	0.68	0.61	0.58	0.7	0.77	0.73	0.82	0.73	0.57	0.76	0.8	0.75	0.82	0.77	0.77	0.8	0.74	0.83	0.82	0.82	0.89	1		
Ta31	0.77	0.7	0.65	0.68	0.64	0.68	0.68	0.52	0.57	0.75	0.75	0.73	0.78	0.72	0.54	0.73	0.77	0.72	0.76	0.74	0.75	0.76	0.67	0.73	0.78	0.76	0.79	0.8	1	
Ta32	0.65	0.63	0.6	0.6	0.57	0.6	0.65	0.58	0.56	0.63	0.66	0.62	0.75	0.66	0.54	0.66	0.74	0.7	0.73	0.68	0.73	0.72	0.72	0.73	0.75	0.73	0.75	0.77	0.73	1

4.11 CROSS COMPATIBILITY CHECK OF TARO SSR PRIMERS WITH TANNIA

Nine taro SSR primers belonging to the common Ce and uq series were tried in three tannia accessions initially to check for cross compatibility. Ce1 B03, Ce1 F04, Ce1 F12, Uq 73-164, Uq 84-207, Uq 97-256, Uq 110-283, Uq 132-147, Uq 201-302 were the primers used. The SSR primers showed multiple banding pattern which varied from the typical SSR banding patterns and unspecific bands of different sizes were obtained from all the nine SSR primers tried. The results of the primers of Ce 1 series is shown in Plate 24-25. Apart from that various temperature ranges were tried ranging from 50°C-66°C (Plate 26-30), template DNA concentration ranging from 10-40 ng (Plate 30) and different PCR cycle numbers, 30 and 35 (Plate 31).

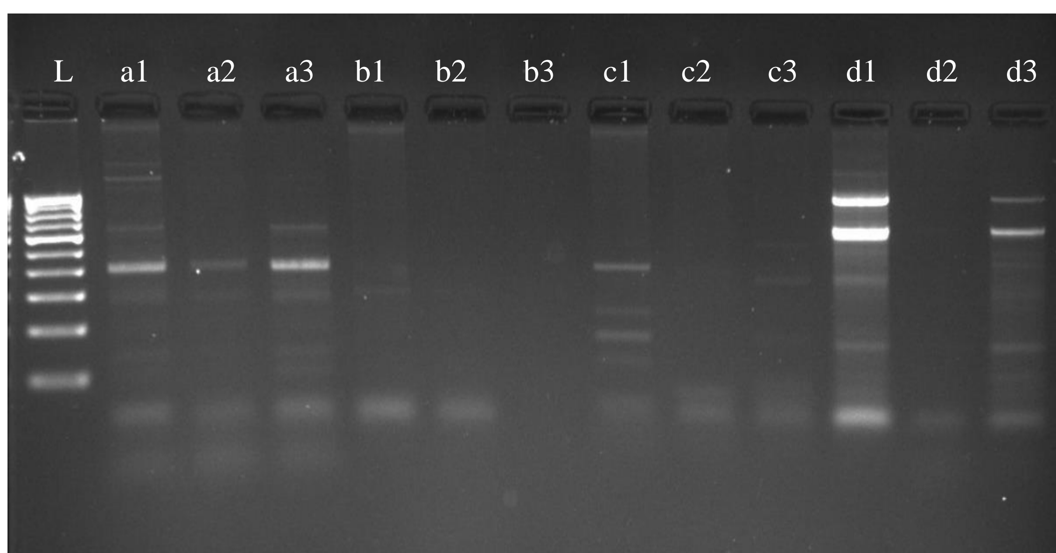

Plate 24. Agarose gel profile (2%) showing SSR primer screening

Ta 1 - Xa-SSK/2017-2; Ta 2 - Xa-HOB-T8-2; Ta 3 - VHAK/2015-5
a- Ce1 B03; b- Ce1 F04; c- Ce1 F12; d- Ce1 F12

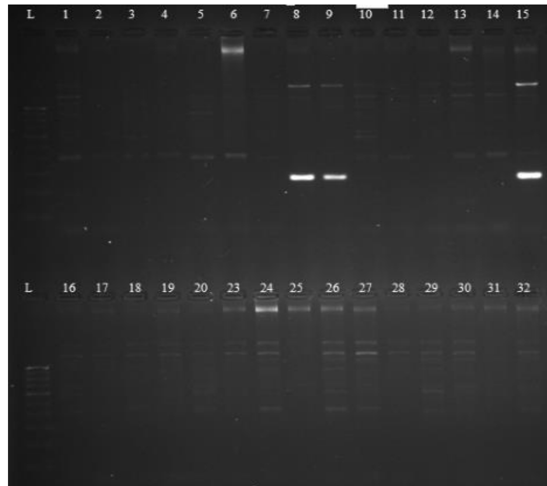

Plate 25. Agarose gel profile (2%) with the primer Ce1 B03 at 56ºC

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5; Ta 4: Xa-63; Ta 5: Xa-26; Ta 6: Xa-Ju/10-8; Ta 7: Xa-ADS/2014-18; Ta 8: Xa-AD/2014-17; Ta 9: TTn14-6; Ta 10: Xa-UV_3 Yerkadu; Ta 11: Xa-19; Ta 12: Xa-AKI/2015-9; Ta 13: TTn14-9; Ta 14: Xa-NL-6; Ta 15: Xa-152; Ta 16: Xa-AD/2016-4; Ta 17: TTn14-1; Ta 18: Xa-75; Ta 19: TTn14-5; Ta 20: Xa- 12; Ta 23: Sreekaryam local; Ta 24: TTn14–2; Ta 25: TTn14–8; Ta 26: Xa- AD/2014-15; Ta 27: Xa-MNS/14-1; Ta 28: Xa-MTS Local; Ta 29: Xa -13; Ta 30: Xa -67; Ta 31: Xa -24; Ta 32: Xa –JG/2016-1

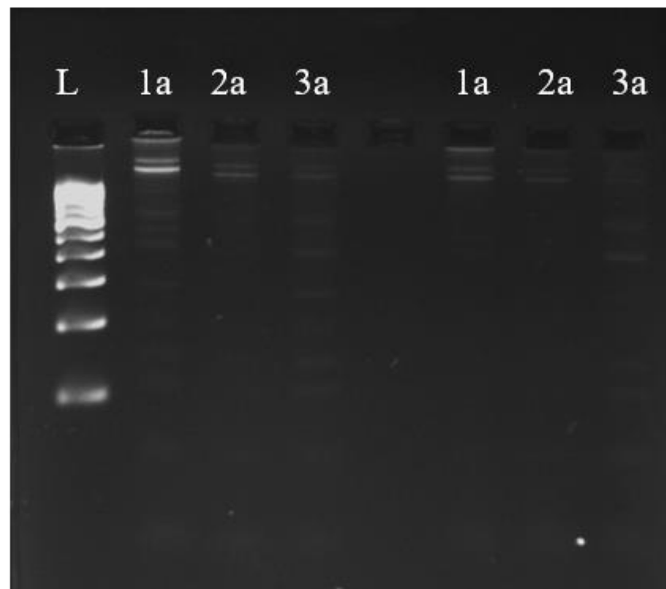

Plate 26. Agarose gel profile (2%) of SSR primers at 50ºC and 52ºC

a-Ce1 B03;

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5

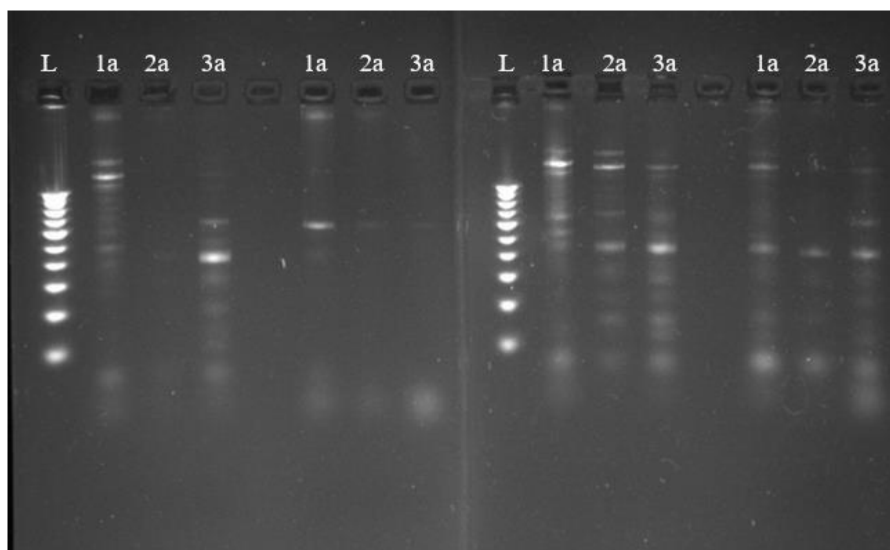

Plate 27. Agarose gel profile (2%) of SSR primers at

51 °C, 55 °C, 56 °C, 60 °C

a- Ce1 B03

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2;

Ta 3: Xa-VHAK/2015-5

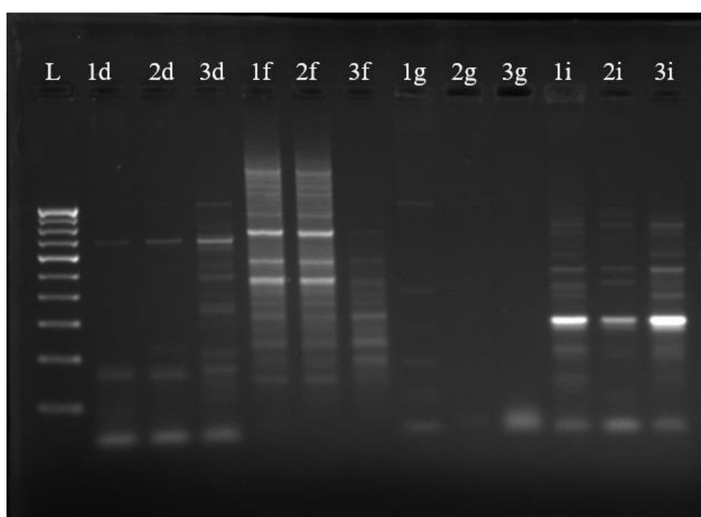

Plate 28. Agarose gel profile (2%) of SSR primers at 61°C

d- Uq 73-164; f- Uq 97-256, g- Uq 110-283, i- Uq 110-283

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5

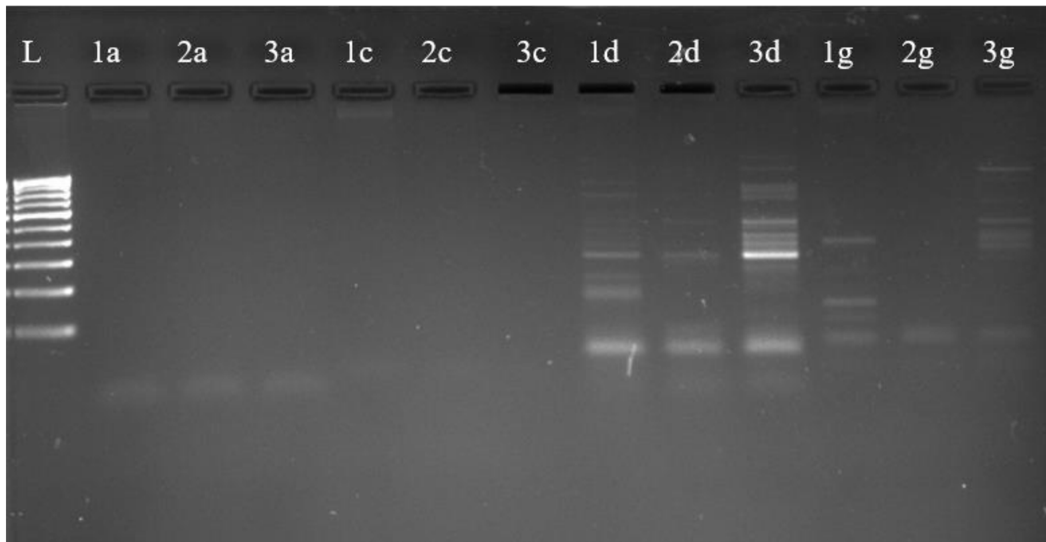

Plate 29. Agarose gel profile (2%) of SSR primers at 66ºC

a-Ce1 B03; c- Ce1 F12; d- Uq 73-164; g- Uq 110-283

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5

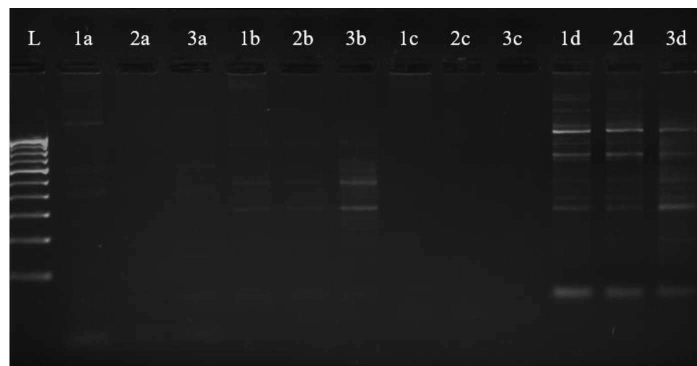

Plate 30. Agarose gel profile (2%) of SSR primers at 55ºC

20ng Template DNA used

a-Ce1 B03; b- Ce1 F04; c- Ce1 F12; d- Uq 73-164; Uq 84-207

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2; Ta 3: Xa-VHAK/2015-5

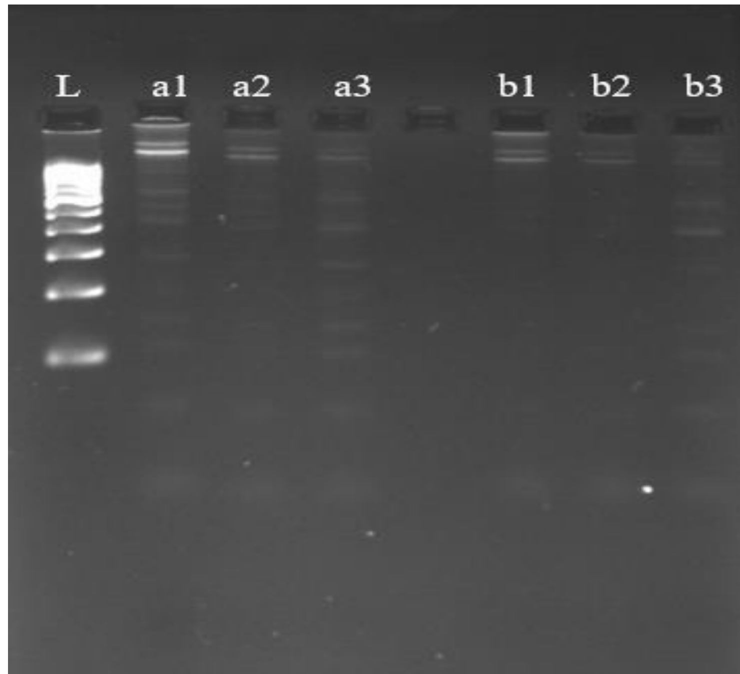

Plate 31. Agarose gel profile (2%) of SSR primers - 30 cycles

a-Ce1 B03; b- Ce1 F04;

Ta 1: Xa-SSK/2017-2; Ta 2: Xa-HOB-T8-2;

Ta 3: Xa-VHAK/2015-5

As cross compatibility was not obtained, more SSR primers needs to be tested in future, preferably developed for tannia.

DISCUSSION

5. DISCUSSION

Edible aroids play a substantial part in the livelihood of millions of rather underprivileged societies in the developing countries (Sarma *et al*., 2016). Being potential crops for income generation, nutritional enhancement and food security at domestic level, they have a superior capability to yield more energy per hectare per day and thereby provide satisfactorily even under adverse environments where other crops fail (Onwueme, 1999). Tannia (*Xanthosoma sagittifolium* (L.) Schott) is one of the edible aroids in the Araceae family and is likely to have been domesticated and cultivated in the northern part of South America from very ancient times (Giacometti and Leon, 1994; Bown, 2000) and has since been spread all over the tropical world extensively. The main areas of distribution of the crop consist of the Caribbean (Cuba, Dominican Republic, Puerto Rico, West Indies), USA (Hawaii, Florida), West Africa (Ghana, Nigeria, Togo, Cameroon) and tropical Asia (Indonesia, Malaysia, the South Pacific Islands) (Ponce, 2010).

Xanthosoma sagittifolium is a staple food in the tropics and subtropics and is one of the six most important root and tuber crops worldwide (Sama *et al.,* 2011). The morphologically less distinct species of tannia appear similar phenotypically and therefore owe to the present confusion existing in its nomenclature with various names being used synonymously (O'Hair and Maynard, 2003). Thus, the name of *Xanthosoma sagittifolium* (L.) Schott has usually been given to the most cultivated members of this genus (Giacometti and Leon 1994; Govaerts *et al.*, 2002; Lim, 2015). The taxonomy of *Xanthosoma* (mainly considering cultivated species) is quite chaotic and rather poorly recognised (Giacometti and Leon, 1994). Recent studies such as that by Bammitte *et al*. (2018) elucidate morphological characterisation among tannia accessions in Togo, West Africa. But there are few studies characterising tannia accessions in India. In this study, a wide range of variability was observed among the accessions for some of the underground characters studied. Here, along with morphological characteristics, molecular characteristics were taken for analysis in

order to analyse maximum diversity among accessions within the species. In this study, the genetic diversity among 30 tannia accessions collected from all across India was determined using morphological descriptors which were listed out by IPGRI and molecular characterization using 14 ISSR markers. Agarose gel electrophoresis was used to resolve the amplicons followed by statistical analysis.

5.1 MORPHOLOGICAL CHARACTERISATION

Morphological analysis was done analysing 14 qualitative and 5 quantitative underground tuber characters of 30 accessions of tannia in the germplasm. Characters like corm size and corm shape showed considerable variation among the accessions inspite of being morphological less distinct in appearance for above ground characters. The knowledge of the morphology of the tuber and its nutritional properties contribute to promote the crop, its use and preservation (Latorre-Vásquez *et al*., 2019). Genetic diversity can be evaluated by measuring variation in qualitative morphological traits which have their basis in genetic alterations that lead to visible differences in the phenotype (Rao, 2004). Such morphological characterization does not require expensive technology which is why they have been extensively used in evaluation of various crops. Several studies have used such characteristics to distinguish between tannia accessions previously (Wachamo, 2018; Fantaw *et al.,* 2014; Villavicencio *et al.,* 2016

5.1.1 Diversity Indices

Shannon Weaver's index and Simpson's diversity index were calculated for each underground character. Cormel size and corm size showed highest Shannon Weaver's as well as Simpson's index owing to maximum variability. The Shannon Weaver's diversity index ranged from 0.24 to 0.98 whereas, the Simpson's diversity index ranged from 0.13 to 0.62. Villavicencio *et al.* (2016) reported that the morphological traits such as fresh weight of cormels per plant and corm size exhibited significant variation among the *Xanthosoma* accessions.

Diversity analysis using the Shannon-Weaver diversity Index showed that six characteristics obtained high index values ranging from 0.72 to 0.80. These included weight of cormels per plant and corm size of maturity. This means that these morphological characteristics can already manifest the variation and diversity among the accessions and eight promising accessions were selected based on corm characters. These are in accordance with the study conducted here as traits such as corm size and cormel size are responsible for variability among the studied 30 *Xanthosoma* accessions.

5.1.2 Pearson's Correlation Matrix

In Pearson's correlation matrix, positive values indicated positive correlation and the matrix revealed correlation between cormel size and cormel shape, also between exterior surface of cormel and interior colour of corm as well as corm apex colour. Mbouobda *et al.* (2007) used Pearson's correlation matrix to reveal significant levels of correlation between the number of cormels per plant and their weight. In addition, a significant correlation ($p<0.01$) was noted between the weight of corm and the weight of cormels on the one hand and between the weight of corm and the number of cormels on the other hand. While studying the correlation between parameters controlling productivity in taro showed that the high correlation between the weight of corm and that of cormels could serve as a criterion for selection of productivity.

5.1.3 ANOVA

Duncan's multiple range test ($\alpha= 0.05$) was performed on the basis of quantitative traits in this study and thus the accessions was differentiated based on grouping of mean values. An accession with a particular letter differs from all other accessions which lacks that specific letter representing a trait. Xa-152 showed maximum value in corm length whereas Xa-26 showed maximum in corm weight, Xa- ADS/2014-18 showed the highest number of cormels produced and Xa-63, maximum cormel weight. Wada *et al.* (2018) did a field study which differentiated two classes of qualitative traits like position of cormel apex and shape of cormels and the Analysis of variance (ANOVA) revealed significant

variation in 11 (84.6%) of the 13 studied quantitative traits. Ajah *et al*. (2016) investigated the morphological variations in the accessions of *Colocasia esculenta* and *Xanthosoma maffafa* exposed to two oilfield chemicals using ANOVA. Similarly in this study also, using ANOVA, the degree of variability was found to be high for corm weight, number of cormels produced per plant and cormel weight based on the coefficient of variation, which is three out of the five characters studied.

5.1.4 Principal Component Analysis

PCA is often carried out to construct a new set of orthogonal coordinate axes and to find out the relative significance of sorting traits (Shinwari *et al.,* 2014). Cluster plots were prepared on PCA done both based on accessions and characters. While considering accessions, the first four components with Eigen values greater than one accounted for 78.40% of the total variation. Among them, Ju/10-8, Xa-24, Xa-152 and Xa-JG/2016-1 showed maximum variability. Fantaw *et al.* (2014) also reported four PCs which explained 70.50% of the total variation present among 64 cocoyam accessions at Jimma, Ethiopia. While considering characters, three components account for a total of 95.51% variability among the considered characters. Characters such as shape of corm, corm size and cormel size showed maximum variability. Wachamo *et al.* (2018) also reported similar results in which the overall observations for the three PCs, with the Eigen values greater than one for 13 quantitative traits explained 69.20% of the observed variations among 100 tannia accessions. Mbouobda *et al.* (2007) reported a list of five qualitative descriptors while evaluating *Xanthosoma* in Cameroon. In a study by Milián Jiménez *et al.* (2018) the PCA revealed a group of accessions in correspondence with the sorting according to the coloration of the flesh cormel in *Xanthosoma* in Cuba. Whereas, Opoku-Agyeman *et al.* (2004) reported 15 qualitative descriptors while evaluating tannia germplasm in Ghana. Among non- discriminatory qualitative traits, colour of cormel apex and internal colour of cormels, showed polymorphism among tannia accessions from Ghana. These indicate that such traits, as found in this study, exhibited considerable influence on the phenotype of the accessions and can be used for selection among accessions.

5.1.5 Cluster Analysis

Hierarchical clustering was done using NTSys pc software package to obtain a cluster dendrogram that assembled the accessions into four major clusters and one outlier. Cluster I had four sub clusters, Ia, Ib, Ic and Id in which sub cluster 1a comprised of one set of duplicates viz., Xa-AD/2014-15 and Xa-13, while sub cluster 1c also comprised of one set of duplicates viz., Xa-19 and TTn14-2. Sub cluster 1d on the other hand showed a set of duplicates comprising three accessions, Xa-AD/2014-17, TTn14-9 and Xa-MNS/14-1. Cluster II, III and IV consisted to two accessions each, while Xa-JG 2016-1 remained an outlier. Cluster II grouped the two accessions, TTn-14-6 and Xa-152 which produced elongated pencil like cormels which were unmarketable. In the case of Xa- JG/2016-1, the flesh colour was pinkish while in all the other accessions it was white and was identified as the most morphologically distinct one from the dendrogram. The rest of the accessions showed variability in tuber shape, size and colour as well as colour of corm apex. In the dendrogram analysis, the number 1 or 100% in the dendrogram indicate that group members had a perfect likeness, while getting closer to the number 0 means the similarity distance is going further. So it can be inferred that accessions with similar characteristics or that appear in same cluster have common ancestry and might have originated from same regions. Nurmiyati *et al.* (2009) revealed that *Xanthosoma* type from different locations did not show specific distinct morphological, which implies that differences in location did not essentially affect plant morphology. Bammitte *et al.* (2018) classified *Xanthosoma* accessions studied based on the petiole basal ring colour, the colour of basal third of the petiole, and the colour cormels flesh and bud into three distinct groups which is in accordance with the case of Xa- JG/2016-1 in this study. Whereas, according to Opoku-Agyeman *et al*. (2004), bud colour was the main distinguishing trait of *X. mafaffa* accessions collected in Ghana. These reveal that morphological characters are not necessarily affected by origin but might be influenced by physical factors/environment where *Xanthosoma* thrives.

5.2 MOLECULAR CHARACTERISATION

5.2.1 DNA Isolation Protocol

DNA was isolated by employing the slightly modified version of Dellaporta protocol (Dellaporta *et al*., 1983). Previous studies used methods such as using DNeasy plant min kit (QIAGEN), IDT Protocol adopted from Keb- Llanes *et al*. (2002), the CTAB extraction protocol, protocol of Risterucci *et al.* (2000), Alzate-Marin *et al*. (2009) and so on for DNA extraction of *Xanthosoma* (Ogwu and Osawaru, 2015; Wachamo *et al*., 2018, Doungous *et al.,* 2015; Cathebras *et al*., 2014; Sepúlveda-Nieto *et al*., 2017). However, these CTAB- based methods are not appropriate for a large number of DNA extractions in a short period of time such as for marker-assisted selection, because it is time- consuming with chloroform extraction and alcohol precipitation to separate DNA from proteins and CTAB (Hosaka, 2004). The extraction buffer used in Dellaporta protocol includes a detergent such as SDS which disrupts the membranes, a reducing agent such as β-mercaptoethanol which helps in denaturing proteins by breaking the disulfide bonds between the cysteine residues and for removing the tannins and polyphenols present in the crude extract, a chelating agent such as EDTA which chelates the magnesium ions required for DNase activity, a buffer which is Tris at pH 8 and a salt such as sodium chloride which aids in precipitation by neutralizing the negative charges on the DNA so that the molecules can come together. Polyphenols released from the vacuoles during the cell lysis process are oxidized by cellular oxidases and undergo irreversible interactions with nucleic acids causing browning of the DNA. The presence of gelling polysaccharides prevents complete dissolution of nucleic acids and imparts a viscous constituency to the DNA making it stick to the wells during gel electrophoresis (Barnell *et al.*, 1998, Diadema *et al*., 2003, Varma *et al.,* 2007). According to these authors, residual polyphenols, polysaccharides and secondary metabolites interfere with the activity of several biological enzymes like polymerases, ligases and restriction endonucleases. Most proteins and polysaccharides can be removed by a short centrifugation as a complex with the

insoluble potassium dodecyl sulphate precipitate. Protein contaminants are removed by using chloroform isoamyl alcohol which denatures the protein present in the solution. Absolute ethanol was used for nucleic acid precipitation (Dellaporta *et al.* 1983). The salts and alcohol remnants are removed by washing with 70% alcohol. In the present study, though the data showed slight RNA contamination from the OD values, upon checking the same using agarose gel, no RNA contamination was noted. Hence, this DNA was used for further molecular studies. Unopened or just opened young leaves was preferred over others due to the low concentrations of polysaccharides, polyphenols and other secondary metabolites making it easy for DNA isolation (Dabo *et al*., 1993; Zhang and Steward, 2000). This method is easy, rapid, inexpensive and feasible for extraction of a large number of PCR-quality DNA samples without any special equipment (Hosaka, 2004).

The extracted DNA was quantified using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer which works at a much lower input range than the normal spectrophotometer, which is its major advantage. Conventional quantification using a normal UV-spectrophotometer is not only time consuming but also require larger volume of sample, manual calculation and labour, whereas nanodrop only requires a single drop of the sample, is highly automated and has lower chances of error. The yield and quality of the DNA is considered to be pure if the ratio of absorbance between the 260 nm to that at 280 nm is in the range 1.8-2.0 (Weising *et al.,* 2005). The method used here gave good quality DNA and gave good absorbance values with purity ranging from 1.06 (TTn 14-5) - 2.34 (Xa-HOB-T8-2).

5.2.2 Primer Screening For PCR

PCR technique facilitates the assembly of a large amount of a specific DNA sequence without cloning, starting with just a few molecules of the target sequence (White *et al*., 2007). Due to the limitation in the diversity studies in *Xanthosoma sagittifolium* using molecular markers, in this study ISSR primers were taken for the amplification of the isolated DNA. PCR conditions already

standardized at the Molecular Biology Lab, Division of Crop Improvement, ICAR-CTCRI was employed for PCR amplification. 40 ng/µl DNA for ISSR and 20 ng/µl DNA SSR reaction mixture was also standardized for one reaction. Higher concentrations of DNA may contain more amount of polyphenols which may interact with Taq polymerase and can hinder the amplification process. According to Ahmed *et al*. (2009), large quantity of template DNA may reduce PCR efficiency due to the presence of contaminants in DNA preparations. 2 mM $MgCl_2$ was used in the study which was found to be optimum for good amplification profile. Increased Mg^{+2} concentrations enhance Taq activity up to a certain level, above which it may act as a depressant. A total of 24 ISSR primers were screened among which 14 were selected for the study. The selected primers were UBC 807, UBC 808, UBC 809, UBC 810, UBC 811, UBC 817, UBC 818,
UBC 824, UBC 825, UBC 827, UBC 834, UBC 836, UBC 845, $(GA)_9$ AC. The optimum annealing temperature was found to be 56.3ºC.

5.3 ISSRAnalysis of Tannia Accessions

ISSR analysis involves PCR amplification of genomic DNA using a single primer that targets the repeat per se, with 1–3 bases that anchor the primer at the 3′ or 5′ end. In addition to being exempt from the obligation of obtaining flanking genomic sequence information, ISSR analysis is technically simpler than many other marker systems. ISSR is the marker of choice for evaluation of genetic diversity in cocoa (Charters and Wilkinson, 2000), gymnosperms such as Douglas fir and sugi (Tsumura *et al*., 1996) and even fungi (Hantula *et al*., 1996). In a study on white lupin it has been demonstrated that among 10 primers used any two were sufficient to distinguish all the 37 accessions studied (Gilbert *et al*., 1999). Similarly, four primers were sufficient to distinguish 34 cultivars of potato (Prevost and Wilkinson, 1999) and three primers could distinguish 16 genotypes of redcurrant (Lanham & Brennan, 1998). The use of such highly informative primers lowers the cost, time and labour for diversity analysis.

After resolving the amplicons using selected ISSR primers under the annealing temperatures determined, clear polymorphic bands were obtained for all the primers selected in this study. Some of the primers showed 100% polymorphism (UBC 808, UBC 810, UBC 818 and UBC 824). Sepúlveda-Nieto *et al.* (2017) used 15 ISSR markers (UBC 1, UBC 2, UBC 813, UBC 820, UBC 834, UBC 845, UBC 851, UBC 858, UBC 860, UBC 862, UBC 864, UBC 866, UBC 885, UBC 886 and UBC 897). That analysis by ISSR molecular markers showed low polymorphism among specimens of *X. sagittifolium*, which originated from various collection sites, suggesting that these individuals have a common origin, possibly due to vegetative propagation by producers. To date, more polymorphism has been detected with the use of ISSRs than with any other assay procedure (Gupta *et al*., 1994; Salimath *et al*., 1995; Virk *et al*., 2000). While there are abundant reports with ISSR molecular markers for *C. esculenta* (Anjitha Nair *et al*., 2018; Okpul *et al.,* 2005;James *et al.,* 2012), a smaller number were found for *X. sagittifolium* (Sepúlveda-Nieto *et al.,* 2017).

5.3.1 Quantification of genetic variability

The 14 primers used in this study showed 91.64% polymorphism and the number of bands ranged from 7 to 19 of which, UBC 808, UBC 810, UBC 818 and UBC 824 showed 100% polymorphism. A high level of polymorphism (100%) indicates the presence of high variability among the selected accessions. The total percentage polymorphism ranged from 81.82 to 100 percentage. Sepúlveda-Nieto *et al.* (2017) reported eight ISSR primers (UBC 2, UBC 834, UBC 845, UBC 851, UBC 858, UBC 860, UBC 864, UBC 866) whose analysis showed low polymorphism among specimens of *X. sagittifolium*, which originated from various collection sites, suggesting that these individuals have a common origin, possibly due to vegetative propagation. Anjitha Nair *et al.* (2018) used 14 ISSR markers in 36 taro accessions for genetic diversity analysis on the basis of taro leaf blight disease resistance/susceptibility and obtained a mean percentage polymorphism of 95.7%, heterozygosity ranging from 0.75-0.87, average number of alleles ranging between 1.94-6.13 and Polymorphic Information Content (PIC) ranging from 0.71-0.86. Similarly, James *et al.* (2012) used the 15 ISSR markers

to separate 73 Hawaiian taro varieties into five main groupings. Krishnaraj *et al.* (2016) used 15 ISSR primers for the molecular characterisation of elephant foot yam varieties in India in which the primers showed 89.21% polymorphism with number of bands ranging from 3 to 14. UBC860, UBC850, UBC827, UBC847, UBC817, UBC825 and UBC864 showed 100% polymorphism. Wang *et al.* (2017) used ISSR markers in 36 pineapple accessions for genetic diversity analysis. Thirteen ISSR primers amplified 96 bands, of which 91 (93.65%) were polymorphic. Farina Mujeeb *et al.* (2017) used 16 ISSR primers to analyse the genetic diversity of *Aegle marmelos.* Out of 86 bands, 75 were polymorphic and 11 were monomorphic and primers UBC 815, 840, 888 and 890 showed 100% polymorphism.

Alansi *et al.* (2019) showed that the analysis of the genetic polymorphism in *Ziziphus spina-christi* obtained with ISSR markers demonstrated high percentage of polymorphic loci (77.36%) and largest number of polymorphic loci (82) were found in a population from At-taif region. This implied that the sampled individuals from that region could be considered to possess a higher genetic variation as compared to the other populations, therefore some topographical features might be involved. A large amount of variability was observed in these sampled populations, exhibiting high intraspecific genetic diversity. Similarly the high polymorphism percentage in this study may also be due to such variability.

5.3.2 Cluster analysis

Cluster analysis was done based on Euclidean distance (Hierarchical clustering), in which the thirty accessions were grouped into four major clusters and the maximum number of accessions (22) was included in cluster I and it had two sub cluster Ia and Ib with three divergent lines viz., TTn14-1, Xa-24 and Xa-AD/2014-18. Sub cluster Ia comprised one divergent line Xa-63. Apart from this, sub cluster Ib comprised two duplicates TTn14-9 and Xa-MNS/14-1 as well as TTn14-5 and Xa-MTS-local which showed 100% similarity. Both TTn14-6 and Xa-152 that appeared in the same cluster produces elongated pencil like cormels, whereas, Xa-AD/2014-17 produced normal cylindrical cormels. Cluster II had two

accessions collected from the North east region pooling together. Study conducted by Solomon Fantaw *et al.* (2014) showed that most of the *Xanthosoma* accessions from different districts and villages of Ethiopia were clustered together. Similarly in Ghana, 70 cocoyam accessions collected from different geographical regions clustered together (Offei *et al.*, 2004). Whereas, Opoku-Agyeman (2004) reported that 78 cocoyam accessions from seven regions of Ghana were clustered into eight different groups, irrespective of the collection sites. A previous study has shown that widely distributed plants often have higher genetic diversity than narrowly distributed ones (Godt *et al.,* 2004). In a study by Patel *et al.* 2017, a total of 39 ISSR primers were screened across 10 *Colletotrichumfalcatum* accessions, of which 21 ISSR primers showed consistent amplification and statistics related to genetic variation were estimated using NTSYS-PC by means of Dice's coefficient. The results revealed 68.07% polymorphism and similarity coefficient ranged from 0.73 to 0.93 in ISSR analysis. Also the dendrogram generated grouped accessions into different clusters which disclosed significant level molecular variation among the selected accessions. It was also evident from PCA plots that accessions are rather dispersed with tested marker systems indicating good genetic base. It can be inferred from these studies that since *Xanthosoma* is a widely cultivated plant that has travelled from its origin it can show variability and the accessions with common origins need not be always clustered together. Divergent parents can be selected from different clusters for breeding purposes. This can be used as a technique to improve a particular trait.

5.4 CROSS COMPATIBILITY CHECK OF TARO SSR PRIMERS WITH TANNIA

Taro SSR primers showed multiple banding pattern which varies from typical SSR banding patterns and unspecific bands of different sizes were observed. Ce1 B03, Ce1 F04, Ce1 F12, Uq 73-164, Uq 84-207, Uq 97-256, Uq 110-283, Uq 132-147, Uq 201-302 were the primers used. Saha *et al.* (2017) studied the cross-species transferability of expressed gene-derived SSR markers in jute by an assessment of 74 random jute eSSR primer pairs which yielded 71.6%

success in cross-species amplifications. Cathebras *et al.* (2014) studied the cross transferability of microsatellites across related species. They studied 17 polymorphic markers and cross-amplifications were carried out to test marker transferability to *Caladium lindenii* and 16 *Xanthosoma* species and inferred that some SSR markers showed cross amplification across species. But the SSR markers specific to taro selected in the present study failed to amplify with the desired specific band sizes. However Mace and Godwin (2002) had found that microsatellite markers were not transferable across genera to *Xanthosoma*. A total of 137 alleles were detected from all microsatellite loci across the 14 *Colocasia* accessions but each of the microsatellite primers tested failed to amplify within *Xanthosoma*. Transferability and polymorphism also depended on the location of the marker, with those located in the coding region being more transferrable (Lin *et al.,* 2014). Nunes *et al.* (2012) performed a molecular characterization of taro belonging to seven regional core collections using SSR polymorphisms, in seven loci (Xuqtem55, Xuqtem73, Xuqtem84, Xuqtem88, Xuqtem91, Xuqtem97 and Xuqtem110). However, the polymorphism presented by some of the loci did not always correctly represent the common phenotypic characteristics of the cultivars. A study showed that Taq DNA polymerase slipped during microsatellite *in vitro* amplification which led to insertion or deletion of repeats in sense or antisense DNA strands. It produced amplified fragments with various lengths in gel electrophoresis showed as ‘stutter bands’. Thus, in population studies by SSRs markers replication slippage effects and stutter bands have been considered (Hosseinzadeh-Colagar *et al.,* 2016). Therefore more SSR markers have to be checked for tannia to establish an exploitable set of SSR markers.

SUMMARY

6. SUMMARY

The study entitled "**Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers**" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020. The objective of the study was to analyse the genetic variation in tannia based on morphological and molecular characteristics. Thirty accessions of *Xanthosoma* from the germplasm available at the institute were selected for characterization.

The study was divided into two phases - morphological and molecular characterization. Morphological characterization (as per IPGRI descriptors) included recording observations and analysis of the below ground qualitative characteristics of tannia and measurement of the quantitative characters. The percentage distribution of each trait was recorded as it can be used to distinguish within species differences in future studies. The corm size of the tuber was identified as the one with highest percentage distribution among the traits studied. The recorded data were analysed using various statistical tools. Cormel size (H-0.82, D- 0.55) and corm size (H-0.98 , D- 0.62) showed the highest Shannon weaver's index as well as Simpson's index, while characters like time of harvest, position of cormel apex and exterior surface of corm showed no variation and remained constant for the twenty eight accessions studied.

PCA was done using SAS software both on the basis of accessions and characters for twenty eight tannia accessions. The former resulted in identification of four accessions with maximum variability viz., Ta 6 (Xa-Ju/10-8), Ta 30 (Xa-67), Ta 15 (Xa-152), and Ta 32 (Xa-JG/2016-1) whereas the latter resulted in identification of four characters responsible for variability in the accessions viz., shape of corm, corm size, shape of the cormel and cormel size. Using ANOVA, the degree of variability was found to be high for corm weight, number of cormels produced per plant and cormel weight. Duncan's multiple range test was also performed to compare the mean values in ANOVA. All the five characters were found to be statistically significant. The software used for morphological analysis

was NTSys PC software package. Cluster dendrogram was done for the 11 traits selected and it resulted in four major clusters and one outlier. Three sets of duplicates were also identified. Cluster I had four sub clusters, Ia, Ib, Ic and Id comprising seven, five, six and five accessions, respectively, Sub cluster 1a comprised of one set of duplicates. Sub cluster 1d on the other hand showed a set of duplicates comprising three accessions. Cluster II, III and IV consisted to two accessions each, while Xa-JG/2016-1 remained as an outlier. Cluster 2 grouped the two accessions, TTn-14-6 and Xa-152 which produced elongated pencil like cormels which were unmarketable. In the case of Xa-JG/2016-1, the flesh colour was pinkish while in all the other accessions it was white.

For molecular analysis DNA isolation was done using a modified Dellaporta method which gave good quality of DNA with purity ranging from
1.06 - 2.34. A total of 25 ISSR primers were taken for the screening process, out of which, 14 primers which gave clear and reproducible bands were selected at an annealing temperature of 56.3°C. The primers selected were UBC 807, UBC 808, UBC 809, UBC 810, UBC 811, UBC 817, UBC 818, UBC 824, UBC 825, UBC 827, UBC 834, UBC 836, UBC 845 and $(GA)_9$ AC.

Similarity matrix based on Jaccard's coefficient for 30 tannia accessions using 14 ISSR markers revealed two sets of accessions with 100% similarity. The least similar accessions were identified as Ta 9 (TTn14-6) and Ta 4 (Xa-63). All the other accessions showed 50% or above similarity. The primers showed 91.64% polymorphism and the number of bands ranged from 7 to 19 of which, UBC 808, UBC 810, UBC 818 and UBC 824 showed 100% polymorphism. Primer UBC 811 showed the lowest polymorphism. A total of 171 bands were detected of which 158 were polymorphic. The percentage polymorphism ranged from 81.82 to 100 percentage. Using the molecular scoring data, Hierarchical clustering was done and the thirty accessions were divided into four major clusters with the maximum number of accessions in cluster I with 22 accessions out of thirty. Cluster II comprised of two accessions whereas, cluster III and IV comprised of three each. Cluster II comprised of Ta 25 (TTn14-8) and Ta 32 (Xa- JG/2016-1) which were collected from the North east region pooling together,

while cluster III comprised Ta 3 (Xa-VHAK/2015-5), Ta 5 (Xa-26) and Ta 6 (Xa-Ju/10-8). Both TTn14-6 and Xa-152 produces elongated pencil like cormels, whereas, Xa-AD/2014-17 produced normal cylindrical cormels. Apart from ISSR markers, nine taro SSR primers belonging to the common Ce and uq series (Ce1 B03, Ce1 F04, Ce1 F12, Uq 73-164, Uq 84-207, Uq 97-256, Uq 110-283, Uq 132-147, Uq 201-302) were tried in thirty tannia accessions to check for cross compatibility at different conditions. Conditions such as temperature ranging from 50°C-66°C, template DNA concentration ranging from 10-40 ng and different PCR cycle numbers, 30 and 35 were tested. The SSR primers showed multiple banding patterns which varied from typical SSR banding patterns and unspecific bands of different sizes were obtained from all the nine SSR primers tried. The selected taro SSR primers were found to be non-transferable to tannia. A more reliable data can be obtained by checking more SSR markers.

REFERENCE

7. REFERENCES

Abraham. Z., Latha. M., and Brinda. R. 2008. Character association studies in elephant foot yam. *J. Root Crops* 34(1): 70-72.

Acevedo-Rodriguez, P. and Strong, T.M. 2005. Monocotiledons and Gymnosperm of Puerto Rico and the Virgin Island, National Museum of Natural History. Washington, DC, USA. pp. 30–51.

Acevedo-Rodriguez, P. and Strong, T.M. 2012. Catalogue of Seed Plants of the West Indies. *Smithsonian Contributions to Botany* 98: 1-1192.

Ahmed, I., Islam, M., Mannan, A., Naeem, R. and Mirza, B. 2009. Optimization of conditions for assessment of genetic diversity in barley (*Hordeum vulgare* L.) using microsatellite markers. *Barley Genetics Newsl*. 39: 5-12.

Ajah, F. O., Osuji, J. O. and Anoliefo, G. O. 2016. Morphological variations in the accessions of *Colocasia esculenta* (L.) Schott. and*Xanthosoma maffafa* (L.) Schott. Exposed to sodium thiosulphate and graphite powder treatments in Nigeria. *Archives of Current Research International* (4): 1-10.

Ajibade, S. R., Weeden, N. F., and Chite, S.M. 2000. Inter-simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Euphytica* 111: 47–55.

Alansi, S., Tarroum, M., Al-Qurainy, F., Khan, S. and Nadeem, M. 2019. Use of ISSR markers to assess the genetic diversity in wild medicinal *Ziziphus spina-christi* (L.) Wild collected from different regions of Saudi Arabia. *Biotechnology & Biotechnological Equipment* 30(5): 942-947.

Alzate-Marin, A. L., Guidugli, M. C., Soriani, H. H., Martinez, C. A. H. and Mestriner, M. A. A. 2009 DNA mini-preparation procedure suitable for PCR/SSR and RAPD analyses in tropical forest tree species. *Braz. Arch. Biol. Technol.* 52: 1217–1224.

Anjitha Nair, U. M. 2018. Identification of molecular markers for resistance to taro leaf blight in *Colocasia esculenta* (L.) Schott. M. Sc. Thesis, Kerala Agricultural University, Thrissur, 146p.

Bammite, D., Matthews, P. J., Dagnon, D. Y., Agbogan, A., Odah, K., Dansi, A., And Tozo, K. 2018. Agro morphological characterization of taro (*Colocasia esculenta*) and yautia (*Xanthosoma mafaffa*) in Togo, West Africa. *African J. of Agri. Res.* Vol. 13 (18), pp. 934-945.

Barnell P., Blanchard A. N., Bryant J. A., Smirnoff, N., and Weir A.F. 1998. Isolation of DNA from the highly mucilaginous succulent plant *Sedum telephium*. *Plant Molecular Biology Reporter* 16: 133-138.

Beeching, J., Garada, M., Nairot, M., Haysom, H.R., Hughes, M.A., and Charrier, A. 1993. An assessment of genetic diversity within collections of cassava (*M.esculenta Crantz*) germplasm using molecular markers. Ann. Bot. 72:515–520.

Bermejo, J. E. H. and León. J. 1994. Neglected Crops: 1492 from a Different Perspective. *Plant Production and Protection Series No. 26*. FAO, Rome, Italy. p. 253-258.

Boakye, A.A., Wireko-Manu, F.D., Oduro, I., Ellis, W.O., Gudjónsdóttir, M., and Chronakis, I.S. 2018. Utilizing cocoyam (*Xanthosoma sagittifolium*) for food and nutrition security: A review. *Food Sci. Nutr.* DOI: 10.1002/fsn3.602

Bown, D. 2000. *Aroids: Plants of the Arum Family*, 2nd edition. Timber Press, Portland Oregon, USA, 392 pp.

Bradshaw, J. E. 2010. *Root and Tuber Crops: Hand Book of Plant Breeding*. Scottish Crop Research Institute, UK.

Brown, V. M. and Asemota, H. 2009. PCR-based characterization of dasheen (*Colocasia* spp.) and Cocoyam (*Xanthosoma* spp.). *J. Biotechnol. Res.* 1: 28–40.

Cathebras, C. Traore, C. Malapa, R. Risterucci, A., and Chair, H. 2014. Characterization of microsatellites in *Xanthosoma sagittifolium* (Araceae) and cross-amplification in related species. *Appl. Plant Sci.* 2(6): 129–134.

Charters, Y.M. and Wilkinson, M. J. 2000. The use of self-pollinated progenies as 'in-groups' for the genetic characterization of cocoa germplasm. *Theor Appl Genet*. 100: 160–166.

Dabo, S, M., Mitchell, E. D., and Melcher, U. 1993. A method for the isolation of nuclear DNA from cotton (*Gossypium*) leaves. *Anal. Biochem.,* 210(1): 34-38.

Dellaporta S, Wood, J and Hicks, J. B. 1983. A plant DNA minipreparation: version l. *Plant Mol Biol Rept.* 1:19-21.

Diadema K., Baumel A., Lebris M., and Affre L. 2003. Genomic DNA isolation and amplification from callus culture in succulent plants, *Carpobrotus* species (Aizoaceae)*Plant Molecular Biology Reporter* 21: 173a-173e.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M., Poizat, C., and Zanetto, A. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry *(Prunus avium* L.). *Theor. Appl. Genet.* 105: 127–138.

Doungous, O., Kalendar, R., Adiobo, A., and Schulman, A.H. 2015. Retrotransposon molecular markers resolve cocoyam (*X. sagittifolium*) and taro (*Colocasia esculenta*) by type and variety. *Euphytica* 206(2): 541–554.

Effa, O. P., Niemanak, N., and Omkolo, N. D. 2006. Morphological variability of *cola acuminata* germplasm in Cameroon. *J. Boil. Sci.* 7(1): 27-33

Emanuelli, F., Lorenzi, S., Grzeskowiak, L., Catalano, V., Stefanini, M. and Troggio, M. 2013. Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biol.* 13: 39.

Fantaw, S., Nebiyu A., and Mulualem, T. 2014. Genetic diversity of Tannia (*X. sagittifolium* (L.) Schott) genotypes using multivariate analysis at Jimma, South west Ethiopia. *Int. J. Plant Breed. Genet.* 8(4): 194–204.

Feng, S., He, R., Lu, J., Mengying, J., Shen, X., Jiang, Y., Wang, Z., and Wang, H. 2016. Development of SSR Markers and Assessment of Genetic Diversity in Medicinal *Chrysanthemum morifolium* Cultivars. *Frontiers in Genetics*7: 113.

Garcia, A.F., Benchimol, L.L., Barbosa, A.M., Geraldi, I.O., Souza Jr, C.L., and Souza, A.P. 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genet. Mol. Biol.* 27(4): 579-588.

Gholizadeh, M. and Rahimi-Mianji, G. 2004. Potential use of molecular markers in the genetic improvement of livestock. *Asian journal of animal and veterinary advances* 3(3): 120-128

Giacometti, D.C. and Leon, J. 1994. Tannia, yautia (*Xanthosoma sagittifolium*). In: *Neglected Crops 1492 from Different Perspectives*, pp. 255– 258

Gibernau, M. 2003. Pollinators and visitors of aroid inflorescences. *Aroideana* 26: 73-91.

Gilbert, J. E., Lewis, R. V., Wilkinson, M. J. and Caligari, P. D. S. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theor Appl Genet.* 98: 1125–1131.

Godt, M. J. W., Hamrick, J. L., and Meier, A. 2004 Genetic diversity in *Cymophyllus fraserianus* (Cyperaceae), a rare monotypic genus. *Genetica* 122: 207–215

Govaerts, R., Frodin, D.G., and Bogner, J. 2002. World checklist and bibliography of Araceae (and Aroraceae). Royal Botanic Gardens, UK.

Gupta, M., Chyi, Y. S., Romero-Severson, J., and Owen, J. L. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theor Appl Genet.* 89: 998–1006.

Hantula, J., Dusabenyagasani, M. and Hamelin, R. C. 1996. Random amplified microsatellites (RAMS)- a novel method for characterizing genetic variation within fungi. *Eur J for Path*. 26: 159–166.

Hasnaoui, N., Buonamici, A., Sebastiani, F., Mars, M., Zhang, D., and Vendramin, G. G. 2012. Molecular genetic diversity of *Punica granatum* L. *(pomegranate)* as revealed by microsatellite DNA markers (SSR). *Gene* 493: 105–112.

Hosaka, K. 2004. An easy, rapid, and inexpensive DNA extraction method, "one-minute DNA extraction," for PCR in potato. *Amer J of Potato Res*. 81: 17-19.

Hosseinzadeh-Colagar, A., Haghighatnia, M. J., Amiri, Z., Mohadjerani, M., and Tafrihi, M. 2016. Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: Evidence which shows replication slippage occurs in extend or nascent DNA strands. *Mol Biol Res Commun*. 5(3): 167-174.

Huang, J. C. and Sun, M. 2000. Genetic diversity and relationship of sweet potato and its wild relatives in pomoea series *batatas* as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast. *Theor. Appl. Genet*. 10: 1050-1060.

IBPGR (1989). Descriptors for *Xanthosoma*. *International Board for Plant Genetic Resources*, Rome, Italy, 30 pp.

James, S. A., Bolick, H. and Clyde, I. 2012. Genetic variability within and identification markers for Hawaiian kalo varieties (*Colocasia esculenta* (L.) Schott - Araceae) using ISSR-PCR. *Hawaii Biological Survey*, Bishop Museum, Honolulu HI 96817, USA.

Joshi, S.P., Gupta, V.S., Aggarwal, R. K., Ranjekar, P. K., and Brar,D.S. 2000. Genetic diversity and phylogenetic relationship as revealed by inter- simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theor Appl Genet*. 100: 1311–1320.

Judd, W.S., Campbell, C.S., Kellog, E.A., Stevens, P.F., and Donoghue, M.J. 2007 Plant Systematics: A Phylogenetic Approach, 3rd ed, *Sinauer Associates: Sunderland*, MA, USA. pp. 249–251.

Kantety, R.V., Zheng, X., Bennetzen, J. L., and Zehr, E. 1995. Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular Breeding* 1: 365-373.

Keb-Llanes, M., González, G., Chi-Manzanero, B. and Infante, D. 2002. A rapid and simple method for small scale DNA extraction in Agavaceae and Other tropical plants. *Plant Mol Biol Rept*. 20: 299a–299e.

Kramer, M. F. and Coen, D. M. 2004. Enzymatic amplification ofDNA by PCR: Standard procedures and optimization. In: *Current Protocols in Molecular Biology*, United States of America: John Wiley and Sons, pp. 15.1.1-15.1.14.

Krishnaraj, S. 2015.Genetic diversity analysis of elephant foot yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson]. *B.Sc.-M.Sc. (Integrated) Biotechnology Thesis*. Kerala Agricultural University, Trissur, India.

Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R. and Pandey, B. K. 1999. Potential of molecular markers in plant biotechnology. *Plant Omics Journal* 2: 141-162.

Kuruvilla, K. M., Dutt, B., and Boy, R. P. 1989. Karyomorphological investigations on aroids of North-Eastern Hills. *Journal of Cytology and Genetics* 24: 13-22.

Langeland, K. A., Cherry, H. M., McCormick, C. M., Burks, C. K. A. 2008. *Identification and Biology of Non-native Plants in Florida's Natural Areas.* Gainesville, Florida, USA: University of Florida IFAS Extension, 185p.

Lanham, P.G. and Brennan, R. M. 1998. Characterization of the genetic resources of redcurrant (*Ribes rubrum*: subg. *Ribesia*) using anchored microsatellite markers. *Theor Appl Genet.* 96: 917–921.

Latorre-Vásquez, L. I., Mejía-España, D. F., Chaves-Morillo, D. M., Córdoba-Solarte, L. V., Trejo-Escobar, D. M., and Valencia-Flórez, L. F. Morphological and Nutritional Characterization of Tubers of 24 Genotypes of Native Potato. 2019. *7th International Engineering, Sciences and Technology Conference (IESTEC)* pp. 30-35.

Lim, T.K. 2015. Edible Medicinal and Non-Medicinal Plants. Volume 9: Modified Stems, Roots, Bulbs. *Springer* 24: 67–93

Lin, Y., Lu J. Y., Wu1, M. D., Zhou, M. B., Fang, W.,Yuji I., and Tang, D. Q. 2014. Identification, cross-taxon transferability and application of full-length cDNA SSR markers in *Phyllostachys pubescens. SpringerPlus* 3: 486.

Loh, J.P., Kiew, R. Hay, A., Kee, A.L. Gan, H., and Gan Y.Y. 2000. Intergeneric and interspecific relationships in Araceae tribe Caladieae and development of molecular markers using amplified fragment length polymorphism (AFLP). *Ann. Bot.* 85: 371– 378.

Luximon-Ramma, A., Bahorun, T., and Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritan exotic fruits. *Journal of the Science of Food and Agriculture* 83(5): 496-502.

Mace, S. E., Godwin, I. D., Singh, D., Mathur, P. N., Okpul M. T., Taylor, D., Hunter, R., Kambuou, V., Rao, R., and Jackson, G. 2008. Assessment and rationalization of genetic diversity of Papua New Guinea taro (*Colocasia esculenta*) using SSR DNA fingerprinting. *Genet Resour Crop Evol.* 55: 811–822

Mac Key, J. 1988. A plant breeder's aspect on the taxonomy of cultivated plants. *Biologisches Zentralblatt*. 107: 369–379.

Manner, H.I. 2011. Farm and forestry production and marketing profile for Tannia *(Xanthosoma* spp.). In: *Specialty crops for Pacific Island Agroforestry*, (Elevitch, C.R., ed). Permanent Agriculture Resources, Hawaii, USA, pp. 1- 16.

Manzano, A. R., Rodríguez Nodals, A. A., Gutiérrez, M. I. R., Mayo, Z. F. and Castiñeiras Alfonso, L. 2001. Morphological and isoenzyme variability of taro (*Colocasia esculenta* L. Schott) germplasm in Cuba. *Biodiversity International* 126: 31-40.

Mason, A. S. 2015. SSR Genotyping. In: Batley J (Ed.). *Plant Genotyping. Springer*, New York, NY. Pp. 77-89.

Maynard, D.N. and O'Hair, S.K. 2003. Edible aroids. In: Trugo, L.C. Finglas, P.M. Belton, P. Ottaway, P.B., Bressani, R. (eds). *Encyclopedia of Food Science and Nutrition* Academic press, USA. pp. 5970-5973.

Mbouobda, H.D., Boudjeko, T., Djocgoue, P.F., Tsafack, T.J., and Omokolo, D.N. 2007. Morphological characterization and agronomical evaluation of cocoyam (*X. sagittifolium* (L.) Schott) germplasm in Cameroon. J. Biol. Sci. 7:27–33.

Mezhii, T.L. Changkija, S., and Chaturvedi, H.P. 2015. Genetic diversity analysis in indigenous edible aroids of Nagaland. *Indian Res. J. Genet. Biotechno.* 7(4): 442–447.

Milián Jiménez, M. D., Concepción, O. M. and Aguila, Y. F. 2018. Integrated Characterization of Cuban Germplasm of Cocoyam (*Xanthosoma Sagittifolium* (L.) Schott). *Journal of Plant Genetics and Crop Research* 1(1): 1-18.

Mujeeb, F., Bajpai, P., Pathak, N. and Verma, S. R. 2017. Genetic diversity analysis of medicinally important horticultural crop *Aegle marmelos* by ISSR markers. *Methods Mol Biol*. 1620: 195-211.

Mwenye, O. J., Herselman, L., Benesi, I., and Labuschagne, M. 2016. Genetic relationships in Malawian cocoyam measured by morphological and DNA markers. *Crop Sci.* 56:1189–1198.

Nagaoka, T. and Ogihara, Y. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor Apppl Genet.* 94: 597–602.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *PNAS*. 76: 5269-5273

Nunes, R. S. C., Pinhati, F. R., Golinelli, L. P., Reboucas, T. N. H., Paschoalin, V. M. F. and da Silva, J. T. 2012. Polymorphic microsatellites of analysis in cultivars of taro. *Horticul. Bras.* 30: 106-111.

Nurmiyati, N., Sugiyarto, S. and Sajidan, S. 2009. Kimpul (*Xanthosoma* spp.). Characterization based on morphological characteristic and isozymic analysis. *Nusantara Biosc.* 1(3):138–145.

Nwofia, G. E., Okwu, Q. U., and Mbah, E. U. 2019. Response of thirteen tannia accessions to variations in planting date in the humid tropics. *Open Agriculture* 4: 213–226

Obidiegwu, E.J. 2015. Towards genetic engineering in cocoyam food crop: Challenges and prospects. *Adv. Genet. Eng*. http://dx.doi.org/10.4172/2169-0111.1000121

Offei, S. K., Asante, I. K., Danquah E.Y. 2004. Genetic structure of seventy cocoyam (*Xanthosoma sagittifolium*, Linn, Schott) accessions in Ghana based on RAPD. *Hereditas* 140: 123–128

Okpul, T., Mace, E. S., Godwin, I. D., Singh D. and Wagih M. E. 2005. Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta*) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterization. *Plant Genetic Resources Newsletter* 143: 8- 16.

Onokpise, O. U., Wutoh, J. G., Ndzana, X., Tambong, J. T., Meboka, M. M., Sama, A. E., and Bruns, M. 1999. Evaluation of Macao cocoyam germplasm in Cameroon. In J. Janick (Ed.), *Perspectives on new crops and new uses*. Alexandria, VA: ASHS Press. pp. 394–396

Onokpise, O.U., Tambong, J.T., Nyochembeng, L. and Wutoh, J.G. 1992). Acclimatization and flower induction of tissue culture derived cocoyam (*Xanthosoma sagittifolium* Schott) plants. *Agronomie* 12(2):93–199.

Onwueme, I.C. 1999. *Taro Cultivation in Asia and Pacific*. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, RAP Publication, Bangkok, Thailand.

Onwueme, I.C. 1978. The tropical tuber crops, yams, cassava, sweet potato, and cocoyam. Wiley, Chichester, UK.

Opara, L. U. 2001. Edible aroids: Post-harvest operations AGST/FAO.

Opoku-Agyeman, M.O, Benneti-Lartey, S.O., and Markwei, C. 2004. Agro-morphological and sensory characterization of cocoyam (*X. sagittifolium* (L.) (Schott) germplasm in Ghana. *Ghana J. Agric. Sci*. 37(1): 23–31.

Osawaru, M.E. and Ogwu, M.C. 2015. Ethnobotany and germplasm collection of two genera of cocoyam (*Colocasia* (L.) Schott) and *Xanthosoma* (L.) Schott, (Araceae) in Edo State Nigeria. *Star J.* 3(3): 23–28.

Patel, P. Katagi, R. B., Parmar, P. R., Shah, R.and Krishnamurthy, R. 2017. Assessment of genetic diversity in *Colletotrichum falcatum* Went accessions based on RAPD and ISSR markers. *Journal of Genetic Engineering and Biotechnology* 16(1): 10.1016.

Pathak, A. K., Singh, S. P., and Tuli, R. 2014. Amplified fragment length polymorphism fingerprinting to identify genetic relatedness among lychee cultivars and markers associated with small-seeded cultivars. *J. Am. Soc. Horti. Sci.* 139(6): 657–668.

Paul, K.K. and Bari, M.A. 2012. Estimates of genetic components for yield and related traits in cocoyam. *Sci. J. Krishi Found*. 10(2):127–132.

Poddar, A., Mukherjee, A., Sreekumar, J., Abraham, K., Naskar, S. K., Unnikrishnan, M. and Arup, M. 2015. Phenotypic variability among the germplasm lines of elephant foot yam (*Amorphophallus paeoniifolius*) and taro (*Colocasia esculenta*). *Int. J. of Tropical Agric*. 33(2): 377-380.

Ponce, J.P. 2010. Cocoyam. In: Fajardo, J., Lutaladio, N., Larinde, M., Rose, C. and Barker, I. (eds). *Quality Declared Planting Material: Protocols and Standards for Vegetatively Propagated Crops*, Food and Agricultural Organization of United the United Nations, Rome, Italy. pp. 41–48.

Prevost, A. and Wilkinson, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet.* 98: 107–112.

Quero-Garcia, J., Ivancic, Q. and Lebot, V. 2010. Taro and Cocoyam. In: Bradishaw, J.E. (Ed). *Hand Book of Plant Breeding: Root and Tuber Crops*, Springer Science and Business Media, London, UK. pp. 149–172.

Ramakrishna, V., Rani, P.J., and Rao, P.R. 2006. Anti-nutritional factors during germination in Indian bean (*Dolichos lablab* L.) Seeds. *World J. Dairy and Food Sci.* 1(1): 6-11.

Rao, K.N. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* 3:136–145.

Ratna Kumari, Wankhede D. P., Bajpai, A., Maurya, A., Prasad, K., Gautam, D., Rangan, P., John, K. L., Suma, A., Kangila, V. B., and Gaikwad, A. B. 2019. *PLoS ONE* 14(12): e0226002. https://doi.org/10.1371/journal. pone.0226002

Ren, X., Jiang, H., Yan, Z., Chen, Y., Zhou, X. and Huang, L. 2014. Genetic diversity and population structure of the major peanut (*Arachis hypogaea* L.) cultivars grown in China by SSR markers. *PLoS ONE* 9: e88091.

Risterucci, A. M., Grivet L., N'goran, J. A. K., Pieretti, I., Flament, M. H. and Lanaud, C. 2000. A high density linkage map of *Theobroma cacao* L. *Theoretical and Applied Genetics* 101: 948 – 955.

Saha, D., Rana, R. S., Chakraborty, S. Datta, S. Kumar, A. A., Chakraborty, A. K. and Karmakar, P. G. 2017. Development of a set of SSR markers for genetic polymorphism detection and interspecific hybrid jute breeding. *The Crop Journal* S2214-5141(17) 30039-9.

Salimath, S. S., De Oliveira, A. C., Godwin, I. D. and Bennetzen, J. L. 1995. Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome* 38: 757-763.Sama, A.E., Hughes, H.G., Abbas, M.S., and Shahba, M.A. 2011. An efficient in vitro propagation protocol of

cocoyam (*Xanthosoma Sagittifolium* (L.) Schott. *The Sci. World J.* pp. 183- 204.

Santosa, E. Sugiyama, N., Lontoh, A. P., Sutoro, S., Hikosaka. and Kawabata, S. 2002. Cultivation of *Amorphophallus paeoniifolius* (Dennst.) Nicolson in home gardens in Java. *Jpn. J. Trop. Agric.* 46: 94-99.

Sarma, A., Burhagohain, R., Barman, R.P., Dey, S.K., Phukan, R., and Sarmah, P. 2016. Variability in nutritional content of some underutilized edible aroids found in hilly terrain of Assam State of India. *World J. Pharm. Pharmac. Sci.* 2:1398–1410.

Schnell R.J., Goenaga R. and Olana, C.T. 1999. Genetic similarities among cocoyam cultivaries based on randomily amplified polymorphic DNA (RAPD) analysis. *Sci. Hortic.* 80:267–276.

Sefa-Dedeh, S. and Kofi-Agyir, S.E. 2004. Chemical composition and the effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. *Food. Chem.* 85(4):479–487.

Sepúlveda-Nieto, M.D.P, Bonifacio-Anacleto, F., de Figueiredo, C.F. de Moraes-Filho, R.M., and Alzate-Marin, A.L. 2017. Accessible morphological and genetic markers for identification of taioba and taro, two forgotten human foods. *Horticulturae* 3:49.

Shiferaw, E., Pe, M. E., Porceddu, E., and Ponnaiah, M. 2012. Exploring the genetic diversity of Ethiopian grass pea (*Lathyrus sativus* L.) using EST-SSR markers. *Mol. Breed* 30: 789–797.

Shinwari, Z.K., Rehman, H., and Rabbani, M.A. 2014. Morphological traits based genetic diversity in Safflower (*C. tinctorius* L.). *Pak. J. Bot*. 46(4): 1389–1395.

Solomon F., Nebiyu A., and Mulualem, T. 2014. Estimate of genetic components of yield and yield related traits of tannia (*Xanthosoma sagittifolium* (L.) Schott) genotypes at Jimma, South west Ethiopia. *Afr. J. Agric. Res.* 10(1): 23-30.

Somers, D.J. 2004. Molecular marker systems and their evaluation for cereal genetics. In: Gupta, P.K. and Varshney, R. (eds). *Cereal Genomics*, Kluwer Academic Press, Netherlands. pp. 19-34.

Suja, G., John, S. K. and Sundaresan, S. 2009. Potential of tannia (*Xanthosoma sagittifolium* (L.) Schott.) for organic production. *Journal of Root Crops* 35(1): 36-40.

Tang D. Q., Lu, J. J., Fang, W., Zhang, S., and Zhou, M. B. 2010. Development, characterization and utilization of GenBank microsatellite markers in *Phyllostachys pubescens* and related species. *Mol Breed* 25:299– 311.

Talwana, H. A. L., Serem, A. K., Ndabikunze, B. K, Nandi, J. O, Tumuhimbise, R., Kaweesi, T., Chumo, E. C. and Palapala, V. 2009. Production Status and Prospects of Cocoyam (*Colocasia esculenta* (L.)Schott.) in East Africa. *Journal of Root Crops* 35(1): 98-107

Tsumura, Y., Ohba, K. and Strauss, S. H. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglasfir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theor Appl Genet*. 92: 40–45.

Vaneker, K. and Slaats, E. 2013. Mapping Edible Aroids. *Iridescent*. 2: 34-45.

Varma A., Padh H., and Shrivastava N. 2007. Plant genomic DNA isolation: an art or a science. *Biotechnology Journal* 2: 386-392.

Vieira, M.L.C., Oliveira, E.J., Padua, J.G., Zucchi, M.I., and Vencovsky, R. 2006. Origin, evolution and genome distribution of microsatellites. *Genet. Mol. Biol.* 29:294–307.

Vilchez, J., Y. Rivas, Albany, N. and Molina, M. 2009. Effect of the N6 - Benzylaminopurine on in vitro multiplication of cocoyam (*X. sagittifolium* L. Schott). *Rev. Fac. Agrono*. 26: 212–22.

Villavicencio, M.L.H., Ignacio, R.M.A., Villancio, V.T., and Garcia, J.N.M. 2016. Morphological characterization and genetic diversity assessment of Gabing San Fernando (*Xanthosoma sagittifolium* L. Schott Milet) in the Philippines. *Philip J Crop Sci.* ISSN: 0117-463.

Virk, P.S., Zhu, J., Newbury, H. J., Bryan, G. J., Jackson, M. T., and Ford-Lloyd, B.V. 2000. Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. *Euphytica* 112: 275–284.

Wachamo, E. W. 2018. Morphological and Molecular Characterization, Assessment of Nutritional Composition and Micropropagation of Cocoyam (*Xanthosoma Sagittifolium* (L.) Schott) From Ethiopia. *Ph D dissertation*. Addis Ababa University, Addis Ababa, Ethiopia.

Wada, E., Feyissa, T., Tesfaye, K., Müller, C. M. and Gemeinholzer, B. 2018. Genetic diversity of Ethiopian *Xanthosoma sagittifolium* (L.) Schott accessions assessed with AFLPs. *Genet. Resour. Crop Evol.* 65(8): 2095- 2105.

Wang, J. S., Jun-hu H., Hua-rui. C., Ye-yuan, C. and Qiao, F. 2017. Genetic Diversity in Various Accessions of Pineapple [*Ananas comosus* (L.) Merr.] Using ISSR and SSR Markers. *Biochem Genet.*20: 176–183.

Wansha, L., Yan, Z., Yang, Y. and Hu, X. 2011. Isolation and characterization 19 new microsatellite loci in taro (*Colocasia esculenta*). *Am. J. Bot*. http://doi.org.10.3732/ajb.1100067

Weising K., Nybom H., Wolff K. and Kahl G. 2005. *DNA Fingerprinting in Plants: Principles, Methods and Applications* (2nd ed), CRC Press, Boca Raton.

White, T.L., Adams, W.T. and Neale, D. B. 2007. *Forest Genetics*. CABI, USA.

Wolff, K. and Morgan-Richards, M. 1998. PCR markers distinguish Plantago major subspecies. *Theor Appl Genet*. 96: 282–286.

Wu, K., Jones, R., Dannaeberger, l., and Scolnik, P. A. 1994. Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Res* 22: 3257–3258.

Zhang, J. and Steward, J. M. 2000. Economical and rapid method for extracting cotton genomic DNA. *J. Cotton Sci.* 4(3): 193-201.

Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176–183.

APPENDICES

APPENDIX I

Extraction Buffer

Composition	Concentration
0.5 M EDTA pH 8.0	20 mM
1 M Tris pH 8.0	100 mM
5 M NaCl	2 M
β-Mercaptoethanol	0.2% (v/v)
PVP	2% (w/v)
Distilled water	

APPENDIX II

TE buffer(10 X)

Composition	Concentration
Tris HCl (pH: 8)	100 mM
EDTA	1 mM

APPE NDIX III

TBEbuffer (10 X)

Composition	Concentration
Tris base	107 g
Boricacid	55 g
0.5 M EDTA (pH 8.0)	40 m

Final volume made up to 1000ml with distilled water and autoclave before use.

APPENDIX IV

Chloroform: Isoamylalcohol

Composition	Concentration
Chloroform	24 ml
Isoamylalcohol	1 ml

Mix 24 parts of chloroform with1 part of isoamyl alcohol and store in a tightly closed container

APPENDIXV

70%ethanol

Composition	Concentration
100% ethanol	70ml
Distilled water 30ml	

APPENDIX VI

AGE loading dye (1X)

Composition	Concentration
6X Loading dye	100µl
Glycerol	100 µl
Sterile distilled water	400 µl

The loading dye is made up to 600 mL.

GENETIC DIVERSITY ANALYSIS OF *XANTHOSOMA SAGITTIFOLIUM* (L.) SCHOTT USING MOLECULAR MARKERS

By

KRISHNAVENI VIJAYAKUMAR

(2015-09-016)

ABSTRACT OF THESIS

Submitted in partial fulfilment of the

requirement for the degree of

B.Sc.-M.Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

ABSTRACT

ABSTRACT

The study entitled "Genetic diversity analysis of *Xanthosoma sagittifolium* (L.) Schott using molecular markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020. The objective of the study was to analyze the genetic variation in tannia based on morphological and molecular characteristics. The knowledge can be exploited for crop improvement programs of *Xanthosoma* and can be used to develop a core collection.

Thirty accessions were selected for the study. For morphological analysis, observations on all the below-ground characteristics of the plant viz., tuber characters were taken (using IPGRI descriptors). The recorded data were analyzed using various statistical methods such as diversity indices (percentage distribution of traits, Shannon's diversity index and Simpson's diversity index), PCA, similarity matrix, cluster analysis and ANOVA. The Shannon Weaver's diversity index ranged from 0.24 to 0.98 whereas, the Simpson's diversity index ranged from 0.13 to 0.62. The time of harvest, the position of the cormel apex and the exterior surface of the corm showed no variation and remained constant for the accessions studied. Cluster dendrogram resulted in four major clusters and one outlier. Cluster I had four sub clusters (Ia, Ib, Ic and Id) comprising seven, five, six and five accessions. Cluster II, III and IV consisted of two accessions each, while Xa-JG/2016-1, the only pink-fleshed accession in the set remained an outlier. Cluster II grouped the two accessions, TTn-14-6 and Xa-152 which produced elongated pencil-like cormels that were unmarketable. The rest of the accessions showed variability in tuber shape, size and colour as well as colour of corm apex. PCA revealed that accessions Xa-Ju/10-8, Xa-67, Xa- 152, and Xa-JG/2016-1 showed maximum variability. Based on the coefficient of variation, the degree of variability was found to be high for corm weight, number of cormels produced per plant and cormel weight.

For molecular analysis, DNA was isolated by employing a slightly modified version of Dellaporta protocol. Good quality DNA ranging from 1.06 (TTn14-5) - 2.34 (Xa-HOB-T8-2) was obtained. A total of 24 ISSR primers were taken for screening of which, 14 were selected at an annealing temperature of 56.3°C. The selected primers were UBC 807, UBC 808,UBC 809, UBC 810, UBC811, UBC817, UBC818, UBC824, UBC825, UBC827, UBC834, UBC 836, UBC 845 and $(GA)_9$ AC. The PCR products were resolved in 2% agarose and the polymorphic bands obtained were subjected to various analyses. The primers showed 91.64% polymorphism and the total number of bands ranged from 7 to 19. UBC 808, UBC 810, UBC 818 and UBC 824 showing 100% polymorphism. Cluster analysis was done based on Euclidean distance in which the thirty accessions were grouped into four major clusters with the maximum number of accessions in cluster I (22). It had two sub clusters Ia and Ib along with three divergent lines viz., TTn14-1, Xa-JG/2016-1 and Xa-AD/2014-18. One of the sub clusters comprised two duplicates TTn14-9 and Xa-MNS/14-1 as well as TTn14-5 and Xa-MTS-local which showed 100% similarity. Of the three accessions grouped under cluster IV, TTn14-6 and Xa-152 produced elongated pencil like cormels, whereas, Xa-AD/2014-17 produced normal cylindrical cormels. Cluster II had two accessions collected from the Northeast region pooling together.

Apart from ISSR markers, cross compatibility of taro SSR markers with tannia accessions was also checked using nine SSR markers (Ce1 B03, Ce1 F04, Ce1 F12, Uq73-164, Uq84-207, Uq97- 256, Uq110-283, Uq132-147 and Uq201-302) using various temperature regimes and PCR conditions. However, specific bands were not obtained and it was found to be incompatible in accordance with previous studies.

The morphological and molecular assessment aided in identification of duplicates and variants present among the germplasm collection whereas the characters which were found contributing to major variability can be used to identify good yielding varieties and eliminate undesirable ones. The data generated from this study is useful for formation of core collection, effective conservation, exploitation of the germplasm as well as breeding and improvement programmes.