173755

GENETIC DIVERSITY ANALYSIS AND IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH LEAF BLIGHT RESISTANCE IN TARO (*Colocasia esculenta* (L.) Schott)

by

ASWATHY ANAND A.

(2010-09-106)

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

Department of Plant Biotechnology

M.Sc. Integrated Biotechnology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2015

DECLARATION

I hereby declare that this thesis entitled "**Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.)" Schott** 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, associate ship, fellowship or other similar title, of any other university or society.

Place: Vellayani

Date: 14/01/2016

ASWATHY ANAND A.

(2010-09-106)

भाकृअनुप-केन्द्रीय कन्द फसल अनुसंधान संस्थान
(भारतीय कृषि अनुसंधान परिषद)
श्रीकार्यम, तिरुवनन्तपुरम -६९५०१७, केरल,भारत
ICAR-CENTRAL TUBER CROPS RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
Sreekariyam, Thiruvananthapuram-695 017, Kerala, India
ISO 9001:2008

CERTIFICATE

Certified that this thesis, entitled **"Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)"** is a record of research work done independently by **Ms. Aswathy Anand A (Admn no. 2010-09-106)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place: Sreekariyam
Date: 14-01-2016

Dr. A. Asha Devi
(Major Advisor, Advisory Committee)
Principal Scientist (Genetics)
Division of Crop Improvement
ICAR-Central Tuber Crops Research Institute
Sreekariyam,Thiruvananthapuram – 695 017

डॉ.आशा देवी. ए
Dr. ASHA DEVI. A
Principal Scientist (Genetics)
ICAR - Central Tuber Crops Research Institute
Sreekariyam, Thiruvananthapuram - 695 017

Phone : 91-0471-2598551 to 2598554
Director (Per.) : 91-0471-2598431
(Res.) : 91-0471 - 2441957
Admn.Officer : 91-0471-2598193
Fax : 91-0471-2590063
E-Mail: ctcritvm@yahoo.com
ctcritvm@gmail.com
Web : http://www.ctcri.org

RIGHT TO INFORMATION

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Aswathy Anand A., a candidate of the degree of **Master of Science in (Integrated) Biotechnology** with major in Biotechnology agree that the thesis entitled **"Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)"** may be submitted by Ms. Aswathy Anand A., in partial fulfillment of the requirement of the degree.

Dr. Mrs. Asha Devi A.

Chairman, Advisory Committee

Principal Scientist

Division of Crop Improvement

ICAR- Central Tuber Crops Research Institute Sreekariyam

Thiruvananthapuram-695017

Dr. B. R. Reghunath

(Member, Advisory Committee)

Professor and Head

Department of Plant Biotechnology

College of Agriculture, Vellayani

Dr. J. Sreekumar

(Member, Advisory Committee)

Senior Scientist

Section of Extension and Social Sciences

ICAR-CTCRI

Dr. Mrs. S. S. Veena

(Member, Advisory Committee)

Principal Scientist

Division of Crop Protection

ICAR-CTCRI

Dr. Mrs. Asha K. I.

(Member, Advisory Committee)

Principal Scientist

Division of Crop Improvement

ICAR-CTCRI

ACKNOWLEDGEMENT

Sometimes our light goes out but is blown into flame by another human being. Each of us owes deepest thanks to those who have rekindled this light.

I wish to express my gratitude to those who generously helped me colour the mosaic of this endeavourwith the tiles of theirknowledge, expertise and memories.

My grateful thanks to the following:

First and foremost, I would like to thank my greatest mentor of all: GOD. I know that I am here and that I am able to write all of this for a reason. I will do my best in never forgetting what a great fortune I have had in just being here, and that it comes with a lesson and a responsibility. I hope I am doing the work you have planned me to do.

Dr.Swapna Alex, our Course Director, who paved the path for carrying out the project in a very renowned institution and has remained a good teacher throughout the venture.

Dr. S.K Chakrabarthy, Director, CTCRI, who gave me insightful idea about how to execute the research work successfully.

Dr. Sheela M.N.S, Head, Division of Crop Improvement, for the constant encouragement and support.

I am extremely indebted to *my advisor Dr.Asha Devi*, my guide and mentor, for providing necessary infrastructure and resources to accomplish my research work. I warmly thank her for the valuable advice, constructive criticism and her extensive discussions around my work.

Prakash Krishnan, who laid the foundation and help me successfully build the blocks of my thesis work. He was also with me and my friends through our ups and downs and who played the role of our mentor.

Dr. Mohan C, who helped me throughout the thesis work with his constant encouragement and scientific explanations.

My sincere thanks must also go to the members of my thesis advisory and exam committee: Dr. Reghunath, our H.O.D, Dr.Sreekumar, Dr.Veena and Dr. Asha K.I. They generously gave their time to offer me valuable comments toward improving my work. In particular, Dr. Sreekumar who gave me his helpful hands in statistical analysis and interpretation and Dr. Veena who provided me the infrastructure for the proper completion of my thesis work.

Vishnu and Shiny who were enthusiastic in helping us out.

Vidhya and Aswathy who gave me the intellectual ideas for the completion of the work.

I also thank all my dear friends of IBC 10th batch who gave me the wonderful tool, the memories of the long 5 years and who have also encouraged me throughout this venture for its successful execution.

Last but not the least I would like to thank my dear mom and dad who gave me this precious life. Without their prayers and support I wouldn't be able to complete this venture successfully.

Aswathy

Aswathy Anand A.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

°C	Degree Celcius
%	Percentage
µg	Microgram
µM	Micromolar
A260	Absorbance at 260
A280	Absorbance at 280
AgNO3	Silver Nitrate
AFLP	Amplified fragment length polymorphism
APS	Ammonium persulfate
bp	Base pair
CTAB	Cetyltrimethyl ammonium bromide
CTCRI	Central Tuber Crops Research Institute
DNA	Deoxyribonucleotide
dNTPs	De oxy nucleotide tri phosphates
EDTA	Ethylene diamine tetra acetic acid
µl	Micro litre
FAOSTAT	Food and Agriculture Organization Statistical Database
g	Gram
h	Hour
et al.	And other co workers
IPC	Integral plate chamber

IPGRI	International Plant Genetic Resource Institute
ISSR	Inter simple sequence repeat
mg	Milli gram
MgCl2	Magnesium Chloride
mM	Milli molar
min	Minutes
NAGS	National Active germplasm site
NBPGR	National Bureau of Plant Genetic Resource
Ng	Nanogram
Nm	Nano meter
OD	Optical Density
pg	Pico gram
PAGE	Poly acylamide gel electrophoresis
PNG	Papua New Guinea
PIC	Polymorphic Information Content
PCR	Polymerase Chain Reaction
PVP	Poly vinyl-pyrrolidine
RAPD	Random Amplified Polymorphic DNA
RNA	Ribo nucleic acid
RNAase	Ribo nuclease
rpm	Revolution per minute
s	Seconds
SSR	Simple Sequence Repeats
STR	Short Tandem Reapeat
Taq	Thermusaquaticus

TBE	Tris-borate EDTA buffer
TE	Tris EDTA Buffer
TEMED	N,N,N',N',-tetramethylethylenediamine
TrisHCl	Tris (Hydroxy Methyl) aminomethane hydrochloride
U	Enzyme Unit
UV	Ultraviolet
W	Watt

INTRODUCTION

1. INTRODUCTION

Taro is a member of Aracacea family and is one among the oldest crop cultivated alongside most humid tropic and subtropics. It is a perennial tropical plant produced for its edible corms, leaves and petioles. Taro ranks fourteenth as the most consumed vegetables world-wide (Lebot and Aradhya, 1991). Taro add profoundly to nutrition and food security in most African countries. It is also known as potato of the humid tropics and is also important as a subsistence crop and are produced as cash crops mostly for trade in local markets of India.

Taro is believed to be originated in Indo Malayan region probably in North Eastern India and Asia. In the whole world, Taro is thought to be cultivated in more than 65 countries (USDA, 2001) and is believed to be one of the earliest cultivated crop and was cultivated in India before 5000 B. C. There are two botanical varieties of taro. One is dasheen type with a large central corm having suckers, stolon and small cormels and eddoe type with smaller corm bordered by many number of cormels.

The yield of taro is approximately 5.5 t/ha on average. It is grown in an area of 51,724 ha producing 810995 t with a productivity of 15.68 ha during 2009-10. In Kerala, Taro cultivation covers about 8841 hectares and the major leading district in its area of cultivation is Kollam with 1831 hectares followed by Pathanamthitta with an area of 1144 hectares (Srinivas *et al.*, 2012). In India it is produced widely along all the agricultural states and in Kerala it is mainly cultivated in Pathanamthitta, Kollam and Alapuzha districts. Major taro varieties available in the market are Sathmukhi, Sree Rashmi, Sree Pallavi, Katyan, Ahena, Poonampet, Muktakeshi, Topi etc. Taro is cultivated in 2 more than 1.08 million hectares in the world (FAO, 2001). Consumption per person/year in homes was 29 kg in 2002, but 27.08 kg in 2008 (Nunes, 2012).

Taro production is strangled by the grasp of *Phtyophthora colocasiae* and other pests and diseases which lead to the genetic erosion of taro. Due to random changes in environmental factors, inbreeding, altered host-pollinator interactions

the genetic base is declining. Since it's important to food and cultural diversity in many regions, the alarming rate of genetic erosion has to be checked. Climatic change is yet another reason for genetic erosion and it accounts for the major threat in survival genetic resources. And furthermore the FAO, (2004), has reported that in the 20th century "some 75 percent of plant genetic diversity has been lost as farmers worldwide have left their multiple local varieties and landraces for genetically uniform, high-yielding varieties". So it's vital to study the genetic diversity of taro and conserve these varieties from further genetic erosion. Studies about genetic resources of tropical tuber crops are underway from the year 1978. The Central Tuber Crops Research Institute (CTCRI), India under the Indian Council of Agricultural Research (ICAR), Ministry of Agriculture, Department of Agricultural Research Education has included taro as a mandatory crop from its very inception in 1963 (Edison et al, 2003).

Taro germplasm conservation exists in many countries world-wide. The danger of taro leaf blight and other diseases enthused the establishment of taro genetic conservation and utilization network in 1998 initiated by IPGRI-APO. Collection and conservation of genetic resources of tropical tuber crops in India is carried out by National Bureau of Plant Genetic resources, New Delhi with its regional stations located in different agro-ecological regions and CTCRI with its regional station at Bhuwaneswar (Dwivedhi, 2013). In CTCRI, in vitro and field gene bank conservation of tuber crops is done along with its characterisation, evaluation and documentation and is known to be the National Active Germplasm Site. The Central Tuber Crops Research Institute, Trivandrum is the sole research Institute in India engaged in the genetic upgradation of all tropical tuber crops including taro. This Institute possesses the richest germplasm wealth of 424 edible genetic stocks of taro in its HQ at Trivandrum in the south and 120 in its Regional Centre at Bhubaneswar in east India (Edison *et al.*, 2003).

Taro leaf blight is a disease caused by *Phytophthora colocasiae* which is a highly infectious fungus. It is primarily a foliar pathogen, and it also affects petioles and corms. It produces dark brown flecks on the upper leaf surface. The infected

leaves by the fungus can be destroyed within 10-20 days along with a reduction in yield upto 50 % and the plants may be reduced to two or three functional leaves, compared with the 4 normal leaf growth situation (Toktok, 2006). This has led to the instigation of several breeding programs with the goal of broadening the genetic base of breeding populations in addition to selection of resistance to taro leaf blight.

Molecular characterization is the best way to assess variability within germplasm samples as it gives best results in a very short time with a low expend of resource and labour. Microsatellites (SSRs) are tandem repeat motifs of 1-6 bp. SSRs have affirmed to be the finest markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. It is widely used because of their polymorphism, reproducibility and co-dominant nature.

AFLP marker was used to identify geographical identification and molecular marker linked to Taro leaf blight resistance in Taro. ISSR markers on the other hand can be best used for the study because of its ease of use and a highly informative data. ISSR marker is fast, robust, and provides more informative data with less cost. It encompasses amplification of DNA segments between two identical microsatellite repeat regions directed in opposite way using primers designed from microsatellite regions. The technique uses 16-25 bp long, of di-nucleotide, trinucleotide, tetra-nucleotide or penta-nucleotide repeats for targeting multiple genomic loci. ISSR primers are used widely for studying genetic variability, MAS studies and for identification of marker linked to specific traits such as disease resistance.

In this study, we present the application of SSR technology for evaluation of the extent of genetic diversity of different cultivars of taro collected from parts of Kerala and try to identify molecular markers linked to taro leaf blight resistance using SSR and ISSR markers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Taro is an important staple food crop grown throughout many Pacific Island countries, parts of Africa, Asia and the Caribbean for its fleshy corms and nutritious leaves (Deo *et al.*, 2009). In Vanuatu, an oceanic archipelago located in south-west Pacific, taro (*Colocasia esculenta* (L.) Schott) is one of the staple crops (Sardos, 2012). Taro is one of the oldest cultivated crops grown for its edible corms and leaves (Plucknett, 1976; Kuruvilla and Singh, 1981; Coates *et al.*, 1988). It is a major root crop with wide distribution in tropics (Singh *et al*, 2008). Taro is a member of the Araceae family, which is made up of at least 100 genera and more than 1500 species (Irwin, 1998). It is grown in almost all tropical regions of the world and is a crop of considerable socio economic importance in Southeast Asia and the Pacific (Kreike, 2004). *C. esculenta* grows best in well-drained loamy soils, but it has the potential to grow in a wide variety of soils including clay, sandy, and loamy soils with pH normally ranging from 5.5 to 6.5 (Onwueme, 1999; Safo-Kantaka, 2004). About 400 million people include taro in their diets and it is the 14th most consumed vegetable worldwide (Brown, 1989; Lebot and Aradhya 1991).

It is a highly polymorphic species, cultivated from the equator to Japan (45^0N), and more than 10,000 landraces exist (Ivancic and Lebot, 2000). There are several kinds of related species viz. swamp taro (*Cyrtosperma chamissonis*), tannia (*Xanthosoma sagittifolium*), giant taro (*Alocasia macrorrhiza*) which are confused with the ordinary or true taro (*Colocasia esculenta*) prevalent in India. Tannia is the most similar species to the common taro and is characterized by relatively large, sagittate leaves, a large main corm and several cormels, which are used as food (Edison *et al*, 2003). *Colocasia* is usually cultivated by family agriculturists in traditional populations for their own consumption and for exchange for other products (Yalu *et al.*, 2009; Singh *et al.*, 2008). Taro is the second most important root staple crop after sweet potato in terms of consumption (Singh *et al.*, 2006) and is ranked the fourth root crop after sweet potato, yam and cassava in terms of its production by weight (Bourke and Vlassak, 2004). Archaeological studies indicate

its usage as early as 28,000 years ago in the Solomon Islands (Loy *et al.* 1992) and it is also cultivated and utilized in China as a food source and medicinal herb for 2000 years (Xu *et al.* 2001). Taro is being replaced in many countries by other tuber crops such as sweet potatoes and cassava largely due to constrain of pests and disease, which are becoming a limiting factor for taro production (Ivancic, 1992).

Research to define the genetic diversity of taro has been largely based upon cytology (Yen and Wheeler, 1968; Kuruvilla and Singh, 1981; Coates *et al.*, 1988; Gunman and Dongxiao, 1990) and morphology (Tanimoto and Matsumoto, 1986). These studies showed that taro could be separated into either diploid or triploid types with x = 14 as the basic number of chromosomes. Chromosome numbers reported for taro from various regions include 2n = 22, 26, 28, 38 and 42 (Onwueme, 1978). The most commonly reported chromosome numbers are: diploids 2n = 28 and triploids 3n = 42 (Kuruvilla and Singh, 1981; Wang, 1983; Lebot and Aradhya, 1991; Lee, 1999). Furthermore, plants with 3n = 42 were referred to as *alowane* (male, large plant) and those of 2n = 28 were referred to as *alokine* (female, short plant) by Solomon Island farmers (Jackson *et al.*, 1977; Wang, 1983). Cultivated taro is mostly diploid (2n = 2x = 28), although some triploid forms are also found (2n = 3x = 42) (Caillon, 2006). Both diploids and triploids occur in all the regions of India, but the diploids predominate in South India over the triploids while the triploids convincingly out-numbered the diploids in the north (Sreekumari and Mathew, 1991). Polyploids in general have larger dimensions and greater adaptability which apparently enable them to thrive better in a wide range of higher latitudinal and altitudinal zones (Zeven, 1980).

There are two botanical varieties characterised by their corm shape and described as var. *esculenta* (dasheen type) and var. *antiquorum* (eddoe type). It has been suggested that of the two varieties, *C. esculenta* var. *esculenta* is diploid and var. *antiquorum* is triploid (Kuruvilla and Singh 1981; Irwin *et al.*, 1998). It is generally accepted that the majority of triploids are of Asian origin (Matthews, 1990). One third cultivated form expresses intermediate traits between the dasheen and eddoe types and is believed to be hybrids (Lebot *et al.* 2000). It has been

suggested that the eddoe type of taro was developed and selected from cultivated taro in China and Japan several centuries ago, and it was later introduced to the West Indies and other parts of the world (Purseglove, 1972).

The common names of taro cultivars in different parts of the country are arvi (Hindi), chempu (Malayalam), seppan kizhangu (Tamil), kachchi (Kannada), chamandupa (Telugu), alu (Marathi), and Kachu (Bengal) (Edison *et al*, 2003).

In many countries like Brazil, taro is grown as a non-conventional vegetable and maintaining the taro cultivars is difficult, especially because there are no studies on their cultivation and no encouragement to use these non-conventional vegetables (Diegues and Arruda, 2001). The need for developing a germplasm reservoir core arose because of limited resources available and complexities involved in maintaining and preserving the extensive taro collection. Although it has been emphasized elsewhere that the core collection is for enhancing use only and not for conservation purpose (Johnson and Hodgkins, 1999), it was felt that, under highly resource limited conditions, it is better to maintain a core (rationalized on the basis of genetic diversity contained in the larger collection) than losing the entire collection (Singh, 2008). The International Plant Genetic Resources Institute (IPGRI), formerly the International Board for Plant Genetic Resources (IBPGR), funded several collection missions in the 1980s.

2.1. IMPORTANCE OF TARO

Taro is one of the few major staple foods where both the leaf and underground parts are important in the human diet (Lee, 1999).The development of taro silage and its use as animal feed especially for swine, the potential of taro alcohol as a fuel for remote islands and the potential of taro starch as a raw material in cosmetic and plastic manufacture are all explored (Griffin, 1982). In Pacific Island countries such as Fiji and parts of Africa, taro is a staple food crop (Lebot and Aradhya, 1991; Opara, 2001). In the Solomon Islands, about 10% of people's dietary calories come from taro and 30% from other tubers (Deo *et al.*, 2009). In Samoa, prior to a devastating spread of taro leaf blight (TLB), virtually all the

population's dietary intake from tubers (one-fifth of the overall diet) came from taro. Taro flour and other products are used extensively for infant formulae in the United States and have formed an important constituent of proprietary canned baby foods (Lee, 1999). Young taro leaves are used as a main vegetable throughout Melanesia and Polynesia where they are usually boiled or covered with coconut cream, wrapped in banana or breadfruit leaves and cooked on hot stones (Deo *et al.*, 2009). It has attained considerable economic importance as a fresh crop in many large islands in the region such as Samoa, Fiji and others (Hanson and Imamuddin, 1983). The petioles are used for preparation of soup (Sastrapradja and Hambali, 1979). It is a significant component of the food consumed by traditional communities; however, the crops are not organized into formal production chains as are conventional vegetables such as potatoes, tomatoes, cabbage and lettuce (Diegues, 2001; Madeira *et al.*, 2008; FAO, 2010).

2.2. ORIGIN AND DISTRIBUTION

Taro [*Colocasia esculenta* (L.) Schott, Family Araceae] is an edible aroid distributed in the humid tropics and subtropics (Lakhanpaul, 2003). It was mentioned in Chinese books as early as 100 B.C. and was being grown in Egypt in the beginning of the Christian era (Whitney *et al.*, 1939; Plucknett *et al.*, 1970). The origins of wild taros and their domesticated relatives and the directions of dispersal remain debatable (Matthews and Terauchi, 1994 cited by Yen, 1995). The centre of origin of taro is generally believed to be between Myanmar and Bangladesh (Plucknett, 1976). Taro is thought to have originated in North Eastern India and Asia (Kuruvilla and Singh, 1981; Hanson and Imamuddin, 1983; Ivancic, 1992) and was gradually spread worldwide by settlers. It is thought to have originated in the Indo-Malayan region, probably in eastern India and Bangladesh, and spread eastward into Southeast Asia, eastern Asia, and the Pacific Islands; westward to Egypt and the eastern Mediterranean; and finally southward and westward from there into Africa, where it moved into the Carribbean and the Americas (Chang, 1958; Yen and Wheeler, 1968). It generally grows in humid areas (2,000 mm

rainfalls) and is cultivated for its starchy corm (Sardos, 2012). As such, it is now cultivated in more than 65 countries worldwide (USDA, 2001).

In Papua New Guinea (PNG), taro is the second most important root crop staple after sweet potato and it is a dietary staple from the coastal lowlands up to an altitude of 2,200 m in the Oksapmin-Telefomin area (Singh, 2008). In Brazil, taro has a limited distribution in the mid-western and south-eastern regions. The wild *Colocasia* species show a greatest diversity in the range from northeast India to southern China, within the Himalayan region of mainland South-East Asia (Ahmed *et al.* 2013; Hunt *et al.*, 2013; Matthews, 1991; Quero-Garcia *et al.*, 2004). Taro cultivation at Kuk in the Western Highlands Province, for example, has been dated to be 9,000 B.C. (Golson 1977). It has been suggested that it was the principal crop in the highlands prior to the introduction of sweet potato (*Ipomoea batatas*) 300-500 years ago (Clarke, 1977; Bayliss-Smith, 1982, 1985). With a long history of association with human society, taro has emerged as an extrinsic symbol in traditional beliefs and rituals among many ethnic groups (Barrau, 1965; Panoff, 1972; Barth, 1975; Morren and Hyndman, 1987).

2.3. HABITAT AND BOTANY

C. esculenta can be found growing mainly in moist forests and wet areas in riparian habitats, riverbanks, along streams, marshes, and canals (Safo-Kantaka, 2004; Acevedo-Rodríguez and Strong, 2005; Langeland *et al.*, 2008). It can also be found in secondary forests, roadsides, and disturbed areas near to abandoned crop fields (Wagner *et al.*, 1999; Acevedo-Rodríguez and Strong, 2005). It is listed among the 200 most invasive plants in the region of Queensland and is also a problem in the coastal districts of New South Wales and along the waterways in Western Australia (Queensland Department of Primary Industries and Fisheries, 2011). Taro is a herbaceous plant which grows to a height of 1-2 m. The plant consists of a central corm (lying just below the soil surface) from which leaves grow upwards, roots grown downwards, while cormels, daughter corms and runners

(stolons) grow laterally. The root system is fibrous and lies mainly in the top one meter of soil. Cormels, daughter corms and runners grow laterally.

The leaf is peltate; the root system is fibrous and lies mainly in the top one metre of soil. The corm is a nutrient storage organ and shares the following characteristics with food storage organs in carrot, sweet potato and manioc: abundance of periderm, food storage in large, thin-walled parenchymatous cells, poorly developed vascular bundles that are few in number, presence of latex cells, mucilage cells and ergastic substances such as druses and raphides (Miyasaka, 1979). Idioblasts (cells which contain raphides or bundles of calcium oxalate crystals) also occur in the ground tissue, and in nearly all other parts of the taro plant. The raphides are associated with acridity or itchiness of taro. The density and woodiness of the corm increase with age. Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be polymorphic (Purseglove, 1972). There are eight recognized variants within *Colocasia esculenta*, of which two are commonly cultivated (O'Sullivan *et al.*, 1995). *Colocasia esculenta* (L.) Schott var. *esculenta* which possesses a large cylindrical central corm and only few cormels; agronomically it is referred to as the 'dasheen' type of taro and *Colocasia esculenta* (L.) Schott var. *antiquorum* which has a small globular central corm with several relatively large cormels arising from the corm; agronomically this variety is referred to as the 'eddoe' type of taro (Purseglove, 1972; Lebot and Aradhya, 1991).

Taro is herbaceous, but survives from year to year by means of the corms and cormels. Root formation and rapid root growth take place immediately after planting, followed by rapid growth of the shoot. Corm formation commences at about three months after planting; cormel formation follows soon afterwards in cultivars that produce appreciable cormels. By the sixth month when shoot growth declines, the corm and cormels become the main sink and grow very rapidly.

Taro is mainly vegetatively propagated (Shaw, 1975; Strauss *et al.*, 1979), but may also reproduce sexually (Ivancic, 1992). Pollination in this species is performed by flies (Onwueme, 1999). Due to vegetative/clonal propagation, there

is almost no genetic variation within the cultivars, although somatic mutations do occur, thus increasing their vulnerability to pest and diseases or changes in climatic conditions (Ivancic, 1992) in the Pacific Islands. However, sexual hybridization of taro is well documented and techniques for pollinating and growing seedlings have been established (Wilson, 1990; Tyagi *et al.*, 2004; Abraham and Ramachandran, 1960; Jos and Vijaya Bai, 1977; Strauss *et al.*, 1979; Ghani, 1979). Sexual hybridization is one way to generate new cultivars with improved qualities (Strauss *et al.*, 1979). By sexual hybridization, the first taro variety in India having good cooking quality, round to oval cormels and long keeping quality, Sree Kiran, was released by ICAR-CTCRI in the year 2004 (Abraham *et al.*, 2006).

2.4. NUTRITION

Taro corm is an excellent source of carbohydrate, the majority being starch of which 17-28% is amylose, and the remainder is amylopectin (Oke, 1990). Taro corms and leaves are also accredited to have medicinal value in reducing tuberculoses, ulcers, pulmonary congestion and fungal infection (Misra, 2002). Taro is especially useful to people allergic to cereals and can be consumed by children who are sensitive to milk, and as such taro flour is used in infant food formulae and canned baby foods (Lee, 1999). Taro corm is low in fat and protein; however, the protein content of taro corm is slightly higher than that of yam, cassava or sweet potato. The protein is rich in some essential amino acids, but is low in isoleucine, tryptophan and methionine (Onwueme, 1978). Taro leaf contains greater amounts of vitamin B-complex than whole milk (Lee, 1999) and are higher in protein and other nutrients, except oil, than tannia (*Xanthosoma sagittifolium*). They also contains higher levels of protein and are also excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibre (Onwueme, 1978; Lambert, 1982; Hanson and Imamuddin, 1983; Bradbury and Holloway, 1988; Opara, 2001). The fresh taro leaf lamina and petiole contain 80% and 94% moisture, respectively. Taro corms and cormels are rich in starch, but low in fat and protein (Goplan *et al.*, 1977). Taro is recommended for gastric patients and taro flour is a good baby food (Parthasarathy,

1986). Taro corms are rich in calcium, phosphate, and vitamins A, B and C (Warid, 1970). The protein content in taro leaves is much higher in corms (Bradbury, Holloway, 1988). According to USDA National nutrient data base (USDA SR-21), nutrition value per 100g taro consists of carbohydrate, protein, energy, total fat and dietary fibre in the amount of 26.46g, 1.50g, 112Kcal, 4.1g respectively and the vitamins such as folates, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, Vitamin A, Vitamin C, Vitamin E, Vitamin K in the amounts 22μg, .600mg, 0.303 mg, 0.283 mg, 0.025 mg, 0.095 mg, 76 IU, 4.5mg, 2.38mg and 1 μg respectively.

2.5. SSR MARKERS

Molecular markers are broadly classified into three classes based on the method of their detection: (i) hybridization based markers such as restriction fragment length polymorphisms (RFLP), (ii) PCR based markers such as random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and microsatellite or simple sequence repeat (SSR), and (iii) sequence based markers such as single nucleotide polymorphisms (SNP) (Gupta and Rustgi, 2004). The sequences are abundant, dispersed throughout the genome and highly polymorphic in comparison with other molecular markers (Akkaya *et al.* 1992; Morgante and Olivieri 1993; Wang *et al.* 1994). The most advance form of genetic markers are molecular markers which reveal DNA sequence variations called 'polymorphisms' (Collard *et al.*, 2005). The size of repeat units, the number of repeats, the presence of variant repeats and the frequency of transcription in the area of DNA repeats are factors responsible for generating polymorphism (Somasundaram *et al.*, 2011). Polymorphic markers can be dominant or co-dominant markers based on whether markers can discriminate between homozygotes and heterozygotes loci (Collard *et al.*, 2005). SSRs have been used successfully in the genome mapping of a variety of crop species including maize, rice, barley and wheat (Senior and Heun 1993; Wu and Tanksley 1993; Saghai-Maroof *et al.* 1994; Roder *et al.* 1995). However, due to the technical difficulties in developing SSRs, these markers have not been commonly used for gene tagging in plants (Ratnaparkhe, 1998).

Microsatellites or SSRs are short tandem repeats of 1-6 nucleotides that occur with high frequency throughout the genomes of many organisms (Weber, 1990). In plants, the presence of SSRs was first demonstrated by the hybridization of oligonucleotide probes of poly (G-T) and poly (A-G) on the phage libraries of tropical tree genomes (Condit, 1991). SSRs have become the genetic markers of choice in mammalian and many plant species due to their abundance, high degree of polymorphism, and suitability for automation (Weber and May, 1989). SSR markers have several advantages over other molecular markers, which ensure a more reliable method for DNA fingerprinting (Yamamoto, 2001). A search of published DNA sequences reveals that SSRs are also show highly abundant in diverse plant genomes (Morgante, 1993; Wang, 1996). SSRs have therefore become the preferred molecular marker system for analysis in plant genetics and ecology. There polymorphisms consists of variations in the number of repeats, which was suggested to be due to slippage of polymerase (Kruglyak *et al.*, 1998). Simple sequence repeat (SSR) markers have become a preferred choice in recent years for several uses (Mahfooz *et al.*, 2012). SSRs have been reported to be superior to other molecular markers because multiple SSR alleles may be detected at a single locus using a simple PCR based screen, SSRs are evenly distributed all over the genome, they are co-dominant, only a very small quantities of DNA are required for screening and analysis may be semi-automated (Varshney *et al.*, 2005). Due to these features, SSRs have become valuable genetic markers for linkage mapping, QTL mapping, association mapping and diversity analysis (Jones *et al.*, 1997; Powell *et al.*, 1996; Varshney *et al.*, 2005). SSR primers are more useful for characterising taro landraces (Mabhaudhi, 2013).

2.6. MOLECULAR CHARACTERIZATION

Genetic markers are specific loci in chromosomes of particular organisms associated with a trait and can be used as tool for marker assisted selection (MAS) in plant breeding (Gupta, 2013). Genetic marker assisted breeding is more efficient, effective, reliable and cost effective as compared to conventional plant breeding (Collard *et al.*, 2005). Studies have used different molecular techniques, such as

isozyme, DNA hybridisation, RAPDs (Randomly Amplified Polymorphic DNAs) analyses, and microsatellites, to determine genetic diversity of Hawaiian, Pacific and Asian taro varieties (Irwin *et al.*, 1998; Lebot and Aradhya 1991; Matthews and Terauchi 1994; Mace and Godwin 2002). Simple sequence repeats (SSRs) markers were also used for germplasm management and marker-assisted selection in taro (Hu *et al.*, 2009; Lu *et al.*, 2011; Mace and Godwin, 2002; van Rensburg *et al.*, 2013). Characterization of genetic resources refers to the process by which accessions are identified or differentiated or genetic characterization refers to the description of attributes that follow a Mendelian inheritance or that involve specific DNA sequences (Vicente, 2005). In the agreed terminology of gene banks and germplasm management, the term 'characterization' stands for the description of characters that are usually highly heritable, easily seen by the eye and equally expressed in all environments (IPGRI/CIP, 2003). Molecular characterization, by itself or in conjunction with other data (phenotypic traits or geo-referenced data), provides reliable information for assessing, among other factors, the amount of genetic diversity (Perera, 2000), the structure of diversity in samples and populations (Shim, 2000; Figliuolo, 2004) rates of genetic divergence among populations (Maestri, 2002) and the distribution of diversity in populations found in different locations (Ferguson, 2004; Perera, 2000). Molecular characterization also helps to determine the breeding behaviour of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species, and its consequences (Papa, 2003). The increasing number of sequencing projects has resulted in an increasing possibility to develop markers linked to specific genes, which, in turn, may help identify novel functional variation (Han, 2004). There is a need for molecular characterisation, in addition to morphological characterisation, in order to determine the full nature and extent of differences (Mabhaudhi, 2013).

Molecular characterization of taro (*Colocasia esculenta*) was done by Irwin, 1998, using RAPD markers. Forty-four taro (*Colocasia esculenta*), two tania (*Xanthosoma* species) and one *Colocasia gigantea* accessions were evaluated for

genetic diversity using random amplified polymorphic DNA (RAPD) primers. One hundred and twelve RAPD primers were used in DNA amplifications. Seventy-three primers gave scorable, 24 gave unscorable, and 15 amplified few or no PCR bands. Band sizes ranged from 0.4 to 3.8 kb and the number of scorable bands per primer ranged from 1 to 13 with an average of 5 bands per primer. A total of 277 bands were scored. Thirty-two of 73 primers utilized were 'highly informative' because they either amplified more than 5 polymorphic bands. Eleven primers generated polymorphic patterns among Indonesian accessions. Almost every marker scored showed some polymorphism between *C. esculenta* accessions and the *Xanthosoma* sp. and *C. gigantea* accessions and a phylogenetic tree was generated using UPGMA cluster analysis.

Molecular characterisation studies are being done to analyse genetic diversity and phylogenetic studies in taro using different molecular markers. Taro germplasm accessions collected from different parts of India were subjected to RAPD (Random Amplified Polymorphic DNA) analysis to assess the genetic diversity prevalent and also to test the genetic basis of morphotypic classification (Lakhanpaul, 2003). Lakhanpaul analysed 13 random decamer primers which were used to analyse 32 taro accessions belonging to 28 morphotypes that were procured from Thrissur Regional Station of National Bureau of Plant Genetic Resources, India of which three out of these thirteen primers analysed showed 100 per cent polymorphism and he concluded that presence of a very close genepool of the wild, weedy and cultivated forms with extreme levels of phenotypic and genotypic variation is suggested as the reason for the high genetic diversity. According to him only one primer; OPW-12, gave monomorphic pattern, nine primers gave suboptimal levels, and OPW-8, OPW-10 and OPW-15 gave hundred percent polymorphism.

Morphological and molecular characterization of taro was done by Lebot *et al.*, (2004) using isozymes and AFLP for the genetic diversity analysis. Malate dehydrogenase, phosphogluco isomerases, isocitrate dehydrogenase, phosphogluconate dehydrogenase, mallic enzyme, shikimic dehydrogenase were

the isozymes used. For AFLP analysis, three primer combinations were used, and a total of 465 scorable amplification products were obtained but the genetic diversity obtained were low.

A germplasm stratification was done on taro (*Colocasia esculenta*) by Quero-Garcia, (2004), based on agro-morphological descriptors and AFLP markers. More than 450 accessions of taro collected throughout Vanuatu and established in a field collection were described using 19 descriptors. Three sampling strategies were tested and the variation captured within each sample was compared for the frequencies of characters. Sample (S1) was randomly selected; the second sample (S2) was conducted within the subgroups produced by the stratification method, and the third sample (S3) was based on UPGMA clustering within each subgroup, sample (S4) that included the parents of the Vanuatu breeding programme. AFLP markers were used to compare the diversity between S3 and a sample (S4). These studies have confirmed the narrow genetic base of the Vanuatu taro germplasm. The number of polymorphic bands per primer pair ranged from 7 to 30. S3 presented the most extreme values ranging from 28 to 74%. S4 had values between 38 and 60%.

Genetic diversity of taro, in Southeast Asia and the Pacific was done by Kreike *et al.*, (2004). The genetic diversity of 255 taro accessions, both cultivated and wild from Vietnam, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea and Vanuatu was studied using AFLPs. They used three AFLP primer combinations and generated a total of 465 scorable amplification products. In each country, the gene diversity within the groups of wild genotypes was the highest compared to the cultivars groups, of which the highest diversity in wild was observed in Thailand and the lowest diversity in cultivars were also observed in Thailand.

Genetic diversity of taro prevailing in Pacific Ocean islands, Vanua Lava, Vanuatu was studied by Caillon, (2006). He analysed the taro diversity using AFLP marker system. Taro cultivated in Ve^tuboso, a village of northern Vanuatu,

Melanesia, was surveyed to assess the extent of morphological and molecular variation being maintained by growers at the village level and to compare this diversity with the diversity found in the crops in Vanuatu. A total of 126 polymorphic bands were used for this study. The four accessions named 'Rov' were tightly clustered with only 3 polymorphic bands out of 126 and with a mean dissimilarity of 0.003. They got only a very less diversity which substantiate the work of Lebot and Kreike as it has already been demonstrated, using isozymes (Lebot and Aradhya, 1991) and then AFLPs (Kreike *et al.*, 2004), that Pacific taros share a very narrow genetic base.

Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta*) in Papua New Guinea was studied by Okpul *et al.*, 2005, using ISSR fingerprinting and agro-morphological characterization. Twenty-three discriminatory descriptors and ISSR primers (GA)9AT, (GA)9AC and (ACC)6Y, were used to characterize 13 taro accessions. ISSR primers generated complex banding patterns, with primer (GA) 9AC obtaining a fewer bands (10) but more polymorphism (90%) compared to 69% for both (GA)9AT and (ACC)6Y. Fingerprinting with three ISSR primers enabled the identification of closely related genotypes and the separation of those that were distantly related.

In the assessment and rationalization of genetic diversity of Papua New Guinea taro by Singh *et al.*, (2008), they used both morphological descriptors and SSR primers for the diversity analysis. Taro accessions for the study were collected from PNG. A total of 859 accessions were clustered for a characterisation and core collection. They used 30 agro morphological descriptors and a high variability was observed against 20 descriptor traits. 7 SSR primers resulted in the amplification of 35 total alleles of which 86% of them were polymorphic. The percentage of unique DNA fingerprints varied from 18.9% to 56.7%.

Assessment of genetic diversity in three taro landraces was done using agro-morphological and SSR primers by Mabhaudhi, (2013). Three taro landraces, namely, Dumbe Lomfula (wild), Kwangwanase and Umbumbulu, were collected

from two locations in Kwazulu-Natal (KZN), South Africa, and planted at two locations, Pietermaritzburg (KZN) and Roodeplaat, Pretoria. Agro-morphological characterisation of vegetative and corm characteristics were done four months after planting and at harvest, respectively. Agro-morphological characterisation was useful in showing differences between the wild landrace and the two cultivated landraces, as well as identification of dasheen and eddoe types. SSR primer characterisation showed that despite the significant morphological difference, the wild Dumbe Lomfula and Umbumbulu landraces were closely related but different from the KwaNgwanase landrace. DNA fingerprinting using five SSR primers showed that Dumbe-Lomfula and Umbumbulu landraces were more similar (0.82) to each other, while the KwaNgwanase landrace was distinctly different. SSR primer characterisation showed that despite significant morphological difference, the wild Dumbe Lomfula and Umbumbulu landraces were closely related but different from the Kwangwanase landrace.

Polymorphic microsatellites used for genetic diversity analysis in cultivars of taro from Brazil were developed by Nunes *et al*, (2012). The aim of this study was to perform a molecular characterization of the seven regional core collections. Genetic diversity of the cultivars was investigated by using SSR polymorphisms, in seven loci valuation by the software GelCompar II showed that the loci Xuqtem73, Xuqtem88 and Xuqtem110 were the most informative, featuring 7, 10 and 8 alleles, respectively, The percentage of cultivars with polymorphic alleles was greatest for the Xuqtem73 (85%) and Xuqtem 110 loci, the later had 100% polymorphic alleles. The polymorphism information content (PIC) ranged from 0.75 to 0.91, and the value of 0.91 was identical for the Xuqtem73, Xuqtem88 and Xuqtem110 markers.

Genetic structure and diversity of East African taro was studied by Macharea, (2014). This study utilizes six SSR primers developed by Mace and Godwin, (2002), to analyse and 5 population of taro from 3 different regions of East Africa namely Kenya, Uganda, Tanzania. 98 taro cultivars obtained from East Africa and SSR data indicated variation but did not show any distinct structure.

Population diversity estimate was relatively low with highest being 0.27. AMOVA revealed most variation among individuals within populations at 79%. In total 31 alleles were amplified from six SSR primers across 98 accessions and 85% were polymorphic. The highest proportion of percentage polymorphic loci was found in taro from Lake Victoria basin while the lowest was found in Central Kenyan taro.

Genetic diversity of Taro (*Colocasia esculenta* (L.) Schott) assessed with SSR markers by Noyer *et al*; (2003). Plant DNA was obtained from N. Kreike (Kreike *et al.*, 2003). Fifteen primer pairs were designed. Seven primer pairs were used to study the genetic diversity of 105 accessions and 100 alleles were revealed. One hundred alleles were identified ranging from 12 to 17 per locus with an average of 14.3. All of them exhibited polymorphism on a subset of 5 taro accessions with a number of alleles ranging from 2 to 8. The heterozygosity level ranged from 41.2 to 86.7 with an average of 68.7. Dendrograms were constructed using the NJTree method based on a similarity matrix computed with a Dice index. The NJTree based on microsatellite loci and the UPGMA dendrogram based on AFLP data (Kreike *et al.*, 2003) gave consistent results. A differentiation between Southeast Asian and Melanesian taros was observed, confirming AFLP and isozyme results as substantiated by Kreike, (2003).

Genetic diversity of taro (*Colocasia esculenta* (L.) Schott) in Vanuatu (Oceania) was studied with SSR markers by Sardos, (2012). A sample of 344 landraces referred as the National Sample (NS) was collected. Its genetic diversity was assessed using nine microsatellites markers. The nine SSR loci used to study the 344 landraces and revealed a total of 89 alleles, ranging from 6 to 17 alleles per locus with an average of 9.89. Among these alleles, 57.30% were rare, i.e. with a frequency q\0.05, with at the extremes, loci Ce1B03 and Ce1F04 exhibiting 33.33 and 88.23% of rare alleles, respectively. The molecular dataset revealed in 324 distinct multilocus genotypes, six genetic clusters mainly differentiated by rare alleles, a geographical structure of the genetic resources of taro based, within each village, on the dominance of one or two of these clusters rather that their exclusivity,

and an analogy between the patterns of dominant clusters between villages and the past and present social networks.

Genetic diversity of taro in Yunnan province was studied by Shen *et al.*, (1999). He observed abundant genetic diversity in protein expression and DNA molecular levels.

2.7. TARO LEAF BLIGHT

Taro is being replaced in many countries by other tuber crops such as sweet potatoes and cassava largely due to constrain of pests and disease, which are becoming a limiting factor for taro production (Ivancic, 1992). There are currently five viruses reported to infect taro with varying distribution in taro growing region and throughout the Pacific Islands i.e. *Dasheen mosaic virus* (*DsMV*), *Taro bacilliform virus* (*TaBV*) is a badnavirus, *Colocasia bobone disease virus* (*CBDV*), *Taro vein chlorosis virus* (*TaVCV*) and *Taro reovirus* (*TaRV*) (Revill *et al.*, 2005). The Oomycete water mould, *Phytophthora colocasiae* is another economically important pathogen which causes taro leaf blight (Sharma *et al.*, 2012). Taro leaf blight has been recorded in a number of countries in the Pacific region, most recently in Nigeria and West Africa (Bandyopadhyay *et al.*, 2011). Raciborski (1900) was the first person to study the leaf blight disease of taro in Java and was also responsible for naming the causal pathogen. There is limited information on the origin of *P. colocasiae* and the magnitude of the area of origin remains to be delineated (Zhang *et al.*, 1994). Ko (1979) has indicated that Asia may be the centre of origin of *P. colocasiae* given that it is the world's centre of origin for many wild and cultivated varieties of taro. One of the indications of the centre of origin of a fungus such as *Phytophthora* is the existence of an A1/A2 mating type ratio of about 1:1 (Zentmyer, 1988). Evidence for an Asian origin of *P. colocasiae* has come from China (Zhang *et al.*, 1994), where previous reports had indicated that only the A2 mating type occurred (Ho *et al.*, 1983). In India, only A1 mating type has been found indicating that it is not the centre of origin (Narula and Mehrotra, 1980). Taro leaf blight was first detected in the Western District highlands of Tutuila Island,

American Samoa on 15 June 1993 and the disease has severely constrained taro production in the country (Gurr, 1993). Leaf blight has become a limiting factor for taro production in the Solomon Islands, Ponape, Hawaii, Fiji, and Western Samoa and in India causing a 25-30% yield loss (Graham, 1965; Trujillo and Aragaki, 1964; Gollifer and Brown, 1974; Jackson *et al.*, 1980; Misra, 1997). The disease significantly reduces the number of functional leaves and can lead to yield reductions of the magnitude of 50% (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1977). The disease can also be spread on taro planting material and the fungus has been reported as remaining alive on planting tops for about 3 weeks after harvest (Jackson, 1977).

Worldwide, it is believed that crop diseases reduce agricultural productivity by more than 10%, equivalent to half a billion tonnes of food every year (Hunter, 1993). The epidemics associated with these diseases reduce food availability, increase food prices and pose a danger to rural livelihoods and regional food security. More than 600 M people could be fed each year by halting the spread of fungal diseases in the world's five most important crops alone (Fisher, 2012).

Taro is affected by at least 10 major diseases and pests in different parts of the world (Kohler, 1997). Of the various taro diseases, taro leaf blight (TLB) caused by the fungus-like Oomycete *Phytophthora colocasiae* Raciborski (*P. colocasiae*) is of prime importance because it can reduce corm yield by up to 50% (Jackson *et al.*, 1996, Singh *et al.*, 2006, Trujillo *et al.*, 1964 and Trujillo *et al.*, 1967) and leaf yield by 95% in susceptible varieties (Nelson, 2011). TLB can also deteriorate corm quality (Paiki, *et al.*, 1996 and Sar *et al.*, 1998). In addition to corm yield losses that occur as a consequence of the reduced leaf area (Jackson *et al.*, 1997) in diseased plants, a corm rot caused by *P. colocasiae* may also occur (Brunt *et al.*, 2001). Under some circumstances the disease invades harvested corms and causes heavy losses during storage (Jackson, 1975). The introduction of TLB to the Caribbean in 2004 led to the annihilation of the taro crop in the Dominican Republic, Cuba and Puerto Rico (Rao *et al.*, 2010).

The first sign of the disease are water-soaked lesions, which expand to form large brown spots; infection is generally first observed on those leaves where water collects. The lesions expand at night forming a 3-5 mm water soaked margin, which dries out during the day; the following night a newer water-soaked margin forms. At night large volumes of sporangia form around the expanding margin of the lesions such that they take on a white powdery appearance. TLB lesions exude droplets of a yellow to brown liquid which dries out during the day producing dark brown, hard deposits, which is a characteristic of the disease (Singh *et al.*, 2012). TLB is a very destructive disease, reducing the leaf area available for photosynthesis, and the number of functioning leaves. As seen in Samoa, susceptible varieties can be totally destroyed. Corm rots can usually be observed soon after harvest and entire corms can decay in 7 to 10 days. Areas damaged at harvest are sites for the rots to occur, and infection is encouraged by wet, warm conditions (Singh *et al.*, 2012).

Many countries had to undergo heavy economical loss. In Samoa taro was a major export and in 1993, taro exports to New Zealand from Samoa were 6300 tonnes, with an FOB value WST 9.5 million, representing 60 per cent of exports in that year (McGregor *et al.*, 2011). Due to TLB, Samoa suffered an annual loss in foregone domestic taro consumption valued at WST 11 million and a taro export market valued at WST 9 million (McGregor *et al.*, 2011).

Both cultural and chemical methods were instigated to reduce TLB infestation in plants and because of the high epidemic nature of the disease, defoliation is necessary.

Other cultural methods that have been recommended include delaying planting on the same land for a minimum of three weeks, avoiding plantings close to older infected ones and preventing the carryover of corms or suckers which can harbour the pathogen from one crop to the next (Jackson, 1999). Other methods are available, such as intercropping and planting during the dry season (Singh *et al.*, 2012). A range of fungicides, either protective or systemic have been found to

provide effective control, but there is evidence that results with chemicals can be variable (Jackson *et al.*, 1980).

Resistant cultivars offer the best sustainable management strategy against taro leaf blight. There was relative optimism with this approach as molecular studies clearly showed the existence of two distinct genepools in Asia and the Pacific, with the diversity in Southeast Asia being far greater than that found in the Pacific (Lebot and Aradhya, 1991; Mace *et al.*, 2006). In India, Misra (1996), conducted elaborate field trials to assess yield losses caused by taro blight. During 1990, yield loss of 28.75% was recorded in the farmer's field whereas on the CTCRI farm, a 46.75% yield loss was recorded in the susceptible variety and 22.75% in the tolerant variety (Misra, 1990, 1996). A variety 'Mukthakeshi' was released (in 2001) from Regional Centre of CTCRI is tolerant to taro leaf blight (Edison *et al.*, 2003).

2.8. MARKERS FOR ANALYSING TRAITS CORRESPONDING TO SPECIFIC GENES

The development of molecular techniques, in particular the use of molecular markers have been used to identify markers linked to useful traits and made great advances in recent years (Kamal *et al.*, 2008). Kamal *et al.* (2008), employed AFLP technique for marker analysis in taro leaf blight. Other studies related to marker analysis in TLB is not yet reported worldwide.

Amplified fragment length polymorphism (AFLP) analysis was used to analyse the geographical differentiation, phylogenetic relationships and to identify molecular markers linked to taro leaf blight resistance genes of Indian taro. Here, application of AFLP technique for evaluating and examining the genetic differentiation of 14 cultivars of taro including 4 highly susceptible, 6 susceptible, 2 moderately resistant and 2 highly resistant variety including Muktakeshi collected from different region of India by cluster analysis based on allele frequencies of gene loci and analysed the markers linked to leaf blight resistance gene by Sharma *et al.*, (2008). They used 5 primer combinations for the study. The five primer combinations produced bands ranging in size from 25 to 500 bp. Total 151

amplification bands were scored as polymorphic bands with an average of 41±3 bands per primer combination as indicated by their absence in atleast 1 of 14 accessions tested. The fragments observed in all the 14 accessions showed high polymorphism. Based on this dendrogram was constructed by UPGMA with a dissimilarity matrix, an *r* value of 0.90. The results of the molecular analysis with respect to the resistance of different genotypes to leaf blight indicated that the ALFP generated bands at approximately base pair of 75 are only detected in the highly resistant genotypes AFLP markers linked to the leaf blight resistance gene was identified.

Molecular markers linked to the apple scab resistance gene Vbj derived from *Malus baccata jackii* was done by Gygax, (2009) with RAPD, SCAR, SSR markers and he identified three RAPD markers, 3 SSR markers and 1 SCAR marker linked to Vbj were identified. The RFLP markers closely linked to the three dwarfing genes in distinct regions of the oat genome contributed to plant height and were used for characterizing new genetic sources for dwarfness in oats (Milach*et al.*, 1997). PCR-based markers were developed to detect the point mutations responsible for the two major semi dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) in wheat (Ellis *et al.*, 2002). Leaf tip necrosis using molecular markers viz., AFLP and SSR, was studied with the slow rusting resistance genes Lr46/Yr29 by Rosewarne *et al*, 2006. Fine mapping of a semi-dwarf gene in a centromeric region in rice (*Oryza sativa* L.) had done and identified a recessive gene, named *sd-c*, that controls plant height and tiller number helped in breeding semi dwarf *indica* rice cultivars (Chen *et al.*, 2013).

SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases was done by Mace, (2006) by employing SSR markers. *TaSTE* (wheat), the ortholog of *AtSTE1* (*Arabidopsis*), was cloned and mapped to wheat chromosome homologous group 3 in the interval between simple sequence repeats (SSRs) markers CWM48.1 and WMC532, with genetic distances of 17.7 and 7.6 cM, respectively. Both linkage and association analyses revealed that *TaSTE-A1* was significantly associated with plant height in bread

wheat (Zhang *et al.*, 2013). Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection was studied by Negi, (2000). Rajesh *et al.* (2013) developed RAPD-derived SCAR marker associated with tall-type palm trait in coconut. The marker OPA09260 was present exclusively in tall accessions and absent in the dwarf cultivar, so the marker was excised, purified, cloned, sequenced and converted to SCAR. Evaluation of cassava varieties for cassava mosaic disease resistance jointly by agro inoculation screening and molecular markers by using SSR and SCAR was studied by Huiping, (2010). Genetic effects of dwarfing gene *Rht-5* on agronomic traits in common wheat (*Triticum aestivum L.*) and QTL analysis on its linked traits was studied by Daoura, (2014), by employing SSR primers. Divashuk *et al.* (2013) studied reduced height genes and their importance in winter wheat cultivars grown in southern Russia by using SSR makers. Paran, (1993); worked on markers linked to downy mildew resistance genes in lettuce by employing SCARS and RAPD. Identification of markers linked to disease-resistance genes by bulked sergeant analysis was studied by Michelmore using RAPD and RFLP markers. Identification if molecular markers related to stem rust resistance gene *rpg4* in barley was studied by Borovkova *et al*, (1994), by using RAPD and RFLP.

Identification of Molecular Markers Linked to Leaf Rust Resistance Genes in Wheat was studied by Dhillon, (2011), by using RAPD primers. Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet was studied by Morgan *et al*, (1998); by using RAPD and RFLP. Identification of SRAP and RGA markers linked to powdery mildew (*Blumeria graminis*) resistance gene PmZB90 in common wheat was studied by Yanjie *et al*, (2013), and found out SRAP markers linked to the gene. Identification and characterization of RAPD and SCAR markers linked to anthracnose resistance gene in sorghum was studied by Singh, (2006), by employing RAPD and SCAR markers and found out a SCAR marker linked to anthracnose resistance gene and confirmed the marker by doing BLAST and found out that the marker showed 100 per cent alignment with the contig{_}3966 located on the longer arm of chromosome 8 of sorghum genome.

Penner, (1993) studied on RAPD marker linked to the oat stem rust gene *Pg3* and found out an RAPD marker closely linked to the gene. Molecular markers linked to brown stem rot resistance genes, *Rbs1* and *Rbs2*, in Soybean was studied by Bachman, (1999) using SSR markers and found out two SSR markers closely linked to the gene. Identification of molecular markers linked to head smut resistance gene *Shs* was studied by Frederiksen *et al.*, (1994) by using both RAPD and RFLP marker and found out 2 RFLPs and 1 RAPD marker closely linked to the gene. DNA markers for disease resistance breeding in peas was studied by Vaughan *et al.*, (1997).

2.9. ISSR MARKERS

Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. (Reddy, 2002). ISSR-PCR is a technique that overcomes most of these limitations (Zietkiewicz *et al.*, 1994; Gupta *et al.*, 1994; Wu *et al.*, 1994; Meyer *et al.*, 1993). It is rapidly being used by the research community in various fields of plant improvement (Godwin *et al.*, 1997). The technique uses microsatellites, usually 16-25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes (Reddy, 2002). The primers used can be either unanchored (Gupta *et al.*, 1994; Meyer *et al.*, 1993; Wu *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994).

ISSRs have high reproducibility possibly due to the use of longer primers (16-25 mers) as compared to RAPD primers (10 mers) which permits the subsequent use of high annealing temperature (45-60^{0}C) leading to higher stringency (Reddy, 2002). The sequences are abundant, dispersed throughout the genome and highly polymorphic in comparison with other molecular markers (Akkaya *et al.* 1992; Morgante and Olivieri 1993; Wang *et al.* 1994). Ratnaparkhe *et al.* (1998) were the first to demonstrate that ISSR markers are useful in gene

tagging and can be used for finding markers linked to the gene of interest. ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species which include rice (Joshi *et al.*, 2000), wheat (Nagaoka and Ogihara, 1997), finger millet (Salimath *et al.*, 1995), *Vigna* (Ajibade *et al.*, 2000), sweet potato (Huang and Sun, 2000) and *Plantago* (Wolff and Morgan-Richards, 1998).

Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a *fusarium* wilt resistance gene in chickpea was studied by Ratnaparkhe, (1998). They studied the inheritance of an inter-simple-sequence-repeat (ISSR) polymorphism in a cross of cultivated chickpea (*Cicer arietinum* L.) and a closely related wild species (*C. reticulatum* Lad.) using primers that anneal to a simple repeat of various lengths, sequences and non-repetitive motifs. Twenty two primers were used for analysis and yielded a total of 31 segregating loci. Primers based on $(GA)_n$ repeats were the most abundant while primers with a $(TG)_n$ repeat gave the largest number of polymorphic loci. They found an ISSR marker linked to the gene for resistance to *fusarium* wilt race 4. The marker concerned, UBC-855_{500}, was found to be linked in repulsion with the *fusarium* wilt resistance gene at a distance of 5.2 cM.

Marker assisted characterization of chickpea genotypes for wilt resistance was studied by Padaliya *et al.*, (2013); using RAPD, SCAR and ISSR. The inter simple sequence repeat (ISSR) marker UBC-825 produced amplification of 1200 bp in susceptible genotypes and intermediate genotype. The alleles of 265 bp amplified by primer TA- 96 (STMS primers) was present only in resistance genotypes and absent in other genotypes.

Molecular Markers for Resistance of Chocolate Spot Disease in Faba Bean (*Vicia faba* L.) using ISSR-PCR was studied by Khalaf *et al.*, (2015) and found out two markers linked to the disease resistance gene.

Identification and Validation of a New Male Sex-Specific ISSR Marker in Pointed Gourd was studied by Adhikari *et al.*, (2014) to allow gender determination

at any stage in the life cycle and found out a primer ISSR-6 closely linked to male bulk.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The taro accessions which were used in the work named "Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)" was carried out in ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram and the plant materials were taken from the germplasm collection of ICAR-CTCRI.

3.1 MANUAL METHOD FOR THE ISOLATION OF DNA

The CTAB extraction method developed by Doyle and Doyle (1987), modified by Sharma *et al.* (2008), with further slight modification made by Vinutha, K. B. (2014), was used for the isolation of DNA. Uncurled leaf of taro was selected for best results as it contains less polyphenols which results in high purity DNA. 2% PVP and 2% β-mercaptoethanol were added to the CTAB buffer (Appendix I) after preheating at a temperature of 65^{0}C. 0.3g of sample was cut by using a sterile knife. Liquid nitrogen was added to the sample and was ground to a fine powder. Before the powdered samples get thawed, 2ml buffer was added and the mixture was homogenized well. The homogenate was then transferred to a 2ml microfuge tube which was properly labelled with the appropriate accession numbers. 5µl of RNase (10mg/ml) was added to the mixture and was mixed thoroughly by slight inversion. The samples were then incubated in a water bath at 37^{0}C for 1 hour with intermittent shaking. 5 µl proteinase K (10mg/ml) was then added to the homogenate and incubated in a water bath for 37^{0}C for 30 minutes with intermittent shaking. Then the mixture was incubated in a waterbath (Memmert) at 65^{0}C for 30 mins with frequent shaking. The homogenate was then centrifuged at 10,000rpm for 10 mins in a centrifuge (Sigma laborzentrifge) and the supernatant was treated with equal volumes of 24:1 chloroform/isoamyl alcohol. The mixture was again centrifuged at 10,000rpm and the upper aqueous layer was collected which was then treated with 2/3rd volume of isopropanol for precipitation of DNA. The precipitated DNA was pelletized by centrifuging at 10,000rpm for 15 mins. The pellet was then washed with 70% ethanol and spinned down at 10,000rpm for 5 mins in a centrifuge

(Sigma laborzentrifge). The DNA pellet was air dried to remove ethanol traces completely followed by suspension in 100µl of 1X TE buffer.

3.2. KIT METHOD OF DNA ISOLATION - DNeasy Plant Mini Kit (Qiagen) Method

Young uncurled taro leaves were collected and about 100-200mg leaf was weighed to isolate DNA. The leaf samples were then grinded in pestle and mortar using liquid nitrogen. 400µl AP1 buffer and 4µl RNaseA were added to the sample. The homogenate was then vortexed and incubated for 10 mins at 65°C. The tubes were inverted twice or thrice during the incubation. 130µl of Buffer P3 was added to the homogenate after incubation. The samples were then mixed and incubated for 5 mins on ice. The lysate was centrifuged for 5 mins at 20,000xg (14,000rpm) followed by pipetting the lysate into a QIA shredder spin column placed in a 2ml collection tube. The samples were again centrifuged for 2 minutes at 20,000xg. The flow-through was then transferred into a new tube without disturbing the pellet if present. 1.5 volumes of Buffer AW1 was added and mixed by careful pipetting. 650µl of the mixture was transferred into a DNeasy Mini spin column placed in a 2ml collection tube and centrifuged for 1 minute at ≥6000xg (≥8000 rpm). The flow-through was then discarded. The previous step was repeated with the remaining sample. The spin column was placed into a new 2ml collection tube. 500µl Buffer AW2 was added, and centrifuged for 1 min at ≥6000xg. The flow-through was discarded. 500µl buffer AW2 was again added for further washing of the DNA pellet and again centrifuged for 2 mins at 20,000xg. The spin column was then removed from the collection tube carefully so that the column cannot come into contact with the flow-through. The spin column was transferred into a new 1.5ml or 2ml micro-centrifuge tube. 100µl Buffer AE was then added to the shredder for elution and was incubated for 5 mins at room temperature before centrifugation for 1 minute at ≥6000xg. The last step was repeated and the sample was kept in -20°C refrigerator. All the samples were checked for DNA in 0.8 % agarose gel and confirmed.

3.4. QUANTIFICATION OF DNA

20µl of DNA sample in 1X TE buffer was diluted to 2ml (2000 µl) with 1X TE buffer. The Optical Density (O.D) values were taken at 260nm and 280nm by spectrophotometer (Systronics, India). TE buffer was kept as blank. Purity was checked by calculating 260/280 of the respective sample. Best quality DNA would have the ratio 1.8 to 2.0. If the ratio is greater than 2.0, the value indicates that the sample is contaminated by RNA and if the ratio is lesser than 1.8, the value shows that the sample is contaminated by protein. An absorbance (A_{260}) of 1.0 corresponds to 50µg DNA/ml of sample. Concentration of the DNA is calculated by the formula,

Concentration of the DNA/ml of sample = A_{260} x Dilution factor x 50,

Where 50 corresponds to the concentration of DNA in µg/ml of unit absorbance.

Dilution factor = total volume/ volume of sample taken

3.5 CHECKING THE QUALITY OF DNA

To check the quality of DNA, agarose gel electrophoresis was performed. The agarose gel was prepared by weighing 0.8% agarose in 1X TBE buffer (Appendix III). The mixture was heated in an oven (IFB appliances) till the agarose is completely dissolved. Once the solution became warm, ethidium bromide was added to intercalate with the DNA sample and was mixed thoroughly with gentle swirling without the formation of bubbles. After the gel was set, 3µl DNA along with 3µl loading dye (bromophenol blue) was mixed and added to each well. Then 1X TBE buffer was added as electrophoresis buffer to the buffer tank (Scie-plas). The electrodes (anode and cathode) were connected to the respective slots in the power pack and was made to run at 80V for 1hr. The gel image was captured in a gel documentation system (Alpha Innotech, USA).

3.6 GENETIC DIVERSITY ANALYSIS

The thirty taro accessions used for genetic diversity were collected from Kerala which are conserved in the ICAR-CTCRI field gene bank. The details of the collection location in presented in table 1 and are as follows:

Table 1: Geographical location of Taro accessions

Sl. No.	SAMPLE	PLACE OF COLLECTION
1	C-218	Thrissur
2	C-203	Palghat
3	C-262	NBPGR, Thrissur
4	C-273	Kerala
5	C-370	Thrissur
6	C-371	Kollam
7	C-388	Alleppey
8	C-389	Unknown
9	C-390	Unknown
10	C-392	Idukki
11	C-406	NBPGR, Thrissur
12	C-681	Trivandrum
13	C-685	Kollam
14	C-688	Kottayam
15	C-691	Alappuzha
16	TCR 147	Kottayam
17	TCR 151	Kerala

18	TCR 155	Pathanamthitta
19	TCR 254	Kerala
20	TCR 296	Kannur
21	TCR 366	Kerala
22	TCR 369	Kozhikode
23	TCR 384	Kozhikode
24	TCR 424	Malappuram
25	TCR 429	Malappuram
26	TCR 443	Ernakulam
27	TCR 450	Ernakulam
28	TCR 545	Wayanadu
29	TCR 903	Malappuram
30	ADR 2014-20	Palghat

3.6.1. PRIMERS USED

Previously identified ten polymorphic SSR primers were used for genetic diversity analysis of 30 taro accessions from Kerala. The details of which are given in table 2.

Table 2: List of primers selected for genetic diversity analysis in taro

Sl. No.	SSR ID	Primer sequence	Repeat type	Expected product size (bp)
1.	Ce1 A06	F- GCTTGTCGGATCTATTGT R- GGAATCAGTAGCCACATC	$(CT)_{31}$	251
2.	Ce1 B03	F- TTGCTTGGTGTGAATG R- CTAGCTGTGTATGCAGTGT	$(GA)_3(GATA)_3(TA)_{12}$	175
3.	Ce1 C03	F- TGTTGGGAAAGAGGG R- GGGGAATAACCAGAGAA	$(CT)_{14}$	117
4.	Ce1 C06	F- CCAGAAGAGACGTTACAGA R- ACGACTTTGGACGGA	$(CT)_{16}$	166
5.	Ce1 F04	F- AGGGAATACAATGGCTC R- ACGAGGGAAGAGTGTAAA	$(CT)_{29}$	-
6.	Ce1 H12	F- TAGTTAGCGTGCCTTTC R- CAACAACTTAATGCTTCAC	$(GA)_{18}$	-
7.	uq73-164	F- ATGCCAATGGAGGATGGCAG R- CGTCTAGCTTAGGACAACATG	$(CT)_{15}$	164
8.	uq84-207	F- AGGACAAAATAGCATCAGCAC	$(CT)_{18}$	207

		R-CCCATTGGAGAGATAGAGAGA		
9.	uq97-256	F-GTAATCTATTCAACCCCCCTT R-TCAACCTTCTCCATCAGTCC	$(CA)_8$	256
10.	uq201-302	F-CTAAGGAGAGGAGATCCGAAC R-CAAGACGATGCTGAACCAC	$(C)_{15}$	283

3.6.2. SSR REACTION MIX FOR PCR STANDARDIZATION

Template DNA of 30 (Table 1) taro accessions were used. The PCR mixture composition is enlisted in Table III.

Table 3: PCR mixture composition

Components	Stock concentration	Required concentration	Volume for one reaction (20µl)
Taq buffer with 15mM (Finnzyme)	10X	1X	2
$MgCl_2$ (Thermo Scientific)	25mM	1mM	0.8
dNTP (Genei)	2mM each	0.1mM each	1.00
Primer (F) (IDT)	10µM	0.2µM	0.4
Primer (R) (IDT)	10µM	0.5µM	0.4

Template DNA	10ng/µL	20ng	2
Taq DNA Polymerase (Finnzyme)	2U/µl	1U/reaction	0.5
dH_2O			12.9
Total			20

PCR was carried out in a Biorad thermalcycler. The program was as follows:

94°C - 5 mins (initial denaturation)

95°C - 1min (denaturation)

56°C - 2mins (annealing) } 35 cycles

72°C - 3mins (extension)

72°C - 10mins (final extension)

4°C – α

The cycle was repeated 35 cycles and the amplified PCR products were electrophoresed in 2% agarose gel along with 100bp and 1kb DNA ladder and the gel was then viewed in a gel documentation system (Alpha imager, Innotech, USA).

3.6.3. DENATURING PAGE FOR SSR ANALYSIS

7 M urea denaturing PAGE (6% polyacrylamide) was performed using Biorad Sequi- Gen® Cell (38 X 50 cm) according to the Biorad manual with heat denatured samples and detected by silver staining (Pillen *et al.*, 2000).

3.6.4. INTEGRAL PLATE CHAMBER (IPC) UNIT ASSEMBLY

The unit consists of a larger plate and a smaller plate. The smaller plate in which the gel has to be casted was first cleaned well with a detergent named

labolene and was then rinsed thoroughly using both tap water and distilled water. Once the plate is dried, both the plates has to be laid down on the working table. With lint free tissue, both larger plate and the small plate was thoroughly cleaned with double distilled water followed by wiping with absolute alcohol. Then the larger plate was wiped separately using a repellent (labolene) and the smaller plate was wiped clean with bind silane thoroughly for the fine sticking of gel onto its surface. Then 0.4mm spacers were cleaned and laid on the long edge of the larger plate followed by mounting of the smaller plate which has bind silane coated surface facing towards the labolene coated surface of the larger plate. Then clamps were attached to both sides of the unit in order to hold both the larger and the smaller unit tight by moving the levers towards the IPC panel. The IPC assembly was then positioned vertically in a precision caster base containing the precision caster gasket and fixed to the base by locking the cam pegs. The entire assembly was laid flat on the working bench with the drain port facing the person doing the experiment.

3.6.5. GEL PREPARATION AND CASTING

The acrylamide solution was prepared according to the method given under gel preparation and casting in this chapter. 15ml of the acrylamide solution was taken. 42g of urea was weighed and the urea was transferred to a solution of 10ml 10X buffer and 15ml double distilled water. The mixture was then heated for 45 seconds. The urea solution was then sieved and poured into a beaker containing acrylamide and the solution was made up to 100ml. Simultaneously 0.1g of APS was weighed and dissolved in 1ml double distilled water by vortexing. Then 600µl of the APS solution and 60µl of TEMED was added to this mixture at the same time without any time lag. Then the solution was injected into the injection part of the caster base using a syringe. As soon as the gel front reached the other end of the unit, the vinyl comb was inserted between the plates. The gel was allowed to polymerase for 15-20 mins.

3.6.6. GEL RUNNING

When the gel is set, the IPC assembly was dislodged from the precision caster base and fit vertically into the universal base using a stabilizer bar. The temperature probe was then adhered to the surface of the outer plate to monitor the temperature during the run. The buffer tank and the buffer chamber of the larger plate were filled with require volume of 1X TBE buffer followed by attachment of electrodes to the power pack. The gel was then pre run for 10mins at 100W and a temperature of 50°C. During the time of pre run the samples were prepared for loading with bromophenol blue (8µl bromophenol blue for 20µl PCR reaction) and was made to denature at 95°C for 5 mins. After the pre run, the power supply was stopped and the top safety cover was removed. The wells were thoroughly rinsed using a pipette to remove any traces of the deposited urea or tiny pieces of dislodged gel. The denatured sample (4-6µl) was loaded along with 100bp DNA ladder. Then the upper safety cover was replaced and the samples were electrophoresed at 100W for 30-45 mins according to the PCR product size. The power supply was turned off after the completion of run. The upper safety cover was then removed and the 1X TBE was partially drained from the glass chamber by attaching the connector to the drain port. The clamps were then removed and the rest of the buffer was drained into the buffer tank. The plate was laid on the working bench and the smaller plate was lifted off carefully from the larger plate for staining.

3.6.7. SILVER STAINING FOR PCR PRODUCT DETECTION

The glass plate was transferred into a large trough containing fixer (Appendix VI) with the gel surface facing upwards and was shaken slowly for 20 mins on a shaker. After 20 mins, the glass plate was transferred into another trough which contained water for 5 mins for washing the fixer away. Then the gel plate was moved into a trough containing silver stain (Appendix VII) and shaken for 20 mins. The plate was then plunged into double distilled water and taken out immediately to wash off the stain adhering to the plate in the places other than the DNA bands. Subsequently the gel plate was transferred into a trough containing

developer (Appendix VIII) which was prepared on the day of the experiment and which was refrigerated. Once the gel plate was transferred into the developer, the trough has to gently be rocked manually in a to and fro motion. A white laminated sheet was kept underneath the plate for visualization of bands. After the bands were visibly developed, the plate was transferred immediately into the fixer for 5 mins for the thorough visualization of the bands followed by washing in double distilled water for another 5 mins. The plate was then dried for 2 days before scanning the image.

3.7. IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH TARO LEAF BLIGHT RESISTANCE

Six taro leaf blight susceptible and six tolerant taro varieties along with a TLB tolerant variety, Muktakeshi were used for the preliminary study aimed at identifying any molecular markers associated with taro leaf blight resistance. The samples used for the study was as follows:

Table 4: List of taro varieties for TLB analysis

Sl. No.	Taro accessions used	
	Susceptible	Tolerant
1	Sree Rashmi	Muktakeshi
2	C - 681	C - 545
3	C - 211	TCR 443
4	C - 688	C - 370
5	C - 82	C - 203
6	C - 185	C - 717

DNA from the susceptible and resistant taro plants (Table 4) were isolated using the above methods and quality of DNA was checked using 0.8% agarose and the gel image was taken in an alpha imager as mentioned earlier. The quantity of DNA was checked in a UV/VIS spectrophotometer at 260 and 280nm.

3.7.1. OPTIMIZATION OF ISSR REACTION

Different concentrations of template DNA varying from 10ng-50ng was tried. The PCR reaction mix was as follows:

Table: 5 PCR REACTION MIX

Components	Stock concentration.	Required concentration	Volume for one reaction (20µl)
Buffer with 15mM (Finnzyme)	10X	1X	2µL
$MgCl_2$ (Thermo Scientific)	25Mm	1mM	0.8µL
dNTP (Genei)	2mM	2mM each	0.5µL
Primer	10µM	0.3µM	0.6µL
Template DNA	10ng/µL	40ng	4µL
Taq DNA Polymerase (Finnzyme)	2U/µL	1U	0.5µL
dH_2O			11.6µL
Total			20µL

3.7.2. PRIMERS USED AND PCR CONDITIONS

A set of 14 UBC ISSR primers whose details are given below in table 6 were used for the study. Standardized concentrations of the template DNA and other PCR components as per table 5 were taken in a PCR tube and 30 different samples as listed in the Table 1 were subjected to PCR with the below mentioned primers.

Table 6: List of primers used for TLB analysis

Sl. No.	Primers used	Primer sequence (5'- 3')
1	UBC 808	AGAGAGAGAGAGAGAGC
2	UBC 810	GAGAGAGAGAGAGAGAT
3	UBC 817	CACACACACACACACAA
4	UBC 824	TCTCTCTCTCTCTCTCG
5	UBC 825	ACACACACACACACACT
6	UBC 827	ACACACACACACACACG
7	UBC 841	GAGAGAGAGAGAGAGAYC
8	UBC 847	CACACACACACACACARC
9	UBC 848	CACACACACACACACARG
10	UBC 857	ACACACACACACACACYG
11	UBC 861	ACCACCACCACCACCACC
12	UBC 871	TATTATTATTATTATTAT
13	(AG)9AC	AGAGAGAGAGAGAGAGAGAC
14	(AG)9AT	AGAGAGAGAGAGAGAGAGAT

PCR was carried out in a Biorad thermalcycler. The program was as follows:

94°C - 5 mins (initial denaturation)

95°C – 30 seconds (denaturation)
56°C - 1 min (annealing) } 35 cycles
72°C – 1 min (extention)

72°C - 10 mins (final extention)

4°C – α

The PCR products were then analysed in a 2% agarose gel along with 100bp and 1kb ladder and the images were documented in an alpha imager for further analysis.

3.7.3. SSR REACTION

The samples were subjected to PCR as mentioned above in the genetic diversity analysis study.

The PCR products thus obtained were checked in a 2% agarose gel and the image was checked in an alpha imager. The products were then denatured for doing PAGE by incubating the samples at 95°C for 5mins and the bands were analysed by staining using silver staining method (As described in genetic diversity analysis). Here bands specifically linked with resistance were marked and the band sizes were determined (Padaliya *et al*, 2013; Mace, 2006 Zhang *et al*., 2013).

3.8. ANALYSIS OF MOLECULAR MARKER DATA

Scoring of bands and data analysis using NTSys including cluster analysis for analysis of genetic diversity in taro.

For genetic diversity analysis, clear and reproducible bands obtained using SSR markers were only selected for scoring. Binary scoring was carried out by assigning

"1" for presence of band and "0" for absence of band. The data matrix was created in excel format and was used as the input file for cluster analysis. This binary data was statistically analyzed used NTSYS pc Ver. 2.2. Pair-wise distance (similarity) matrices were computed using sequential, agglomerative, hierarchical, and nested (SAHN) clustering option of the NTSYS-Pc (Numbering Taxonomy and Multivariate Analysis System, Biostatistic, New York, U.S.A, Software Version 2.02 package).The program also generated dendrogram, which grouped the accessions on the basis of Nei genetic distance (Nei, 1979) using un weighted pair group method with arithmetic average (UPGMA) cluster analysis.

3.8.1. PIC

Polymorphic information content is a software which helps in measuring heterozygosity (H), and polymorphic information of the SSR primers employed for studying genetic diversity analysis of the taro.

3.8.2. CLUSTER ANALYSIS

The R statistical package was used for hierarchical cluster analysis based on Euclidean distance. A dendrogram grouping of the 30 accessions based on SSR marker data was constructed based on this method.

For identification of molecular markers using ISSR and SSR markers linked to taro leaf blight resistance, bands repeated only in the resistant lines and not present in the susceptible variety was noted for comparison and the molecular weight was calculated using Alpha imager software available with gel documentation system (Alpha Imager).

RESULTS

4. RESULTS

The results of study entitled "Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)" carried out at the Division of Crop improvement, CTCRI, Sreekariyam, Trivandrum are explained in this chapter.

4.1. GENETIC DIVERSITY ANALYSIS

4.1.1. DNA ISOLATION PROTOCOL

For DNA isolation, samples were collected from fields of CTCRI and is represented as plate 1. DNA isolation was done by employing two methods viz., DNA isolation mini kit (Amnion AMpreP plant gDNA Mini kit) and the method standardized by Doyle and Doyle (1987), modified by Sharma *et al*; (2008), with a further modification made by Vinutha, (2014), (data unpublished). Both the methods gave intact and unsheared DNA and showed a good absorbance value with purity ranging from 1.65-2.1.The spectrophotometer readings of the DNA samples extracted from 30 taro accessions are listed in the table 7.

Table 7 : DNA QUANTIFICATION AND YIELD OF DNA

Sl NO:	SAMPLE	ABSORBANCE A260	ABSORBANCE A280	A260/280	DNA YIELD (ng/µl)
1	C-218	0.02	0.11	1.68	853
2	C-203	0.02	0.01	1.66	112
3	C-262	0.63	0.04	1.76	331.5
4	C-273	0.10	0.06	1.78	505
5	C-370	0.062	0.04	1.76	310

6	C-371	0.03	0.02	2.0	149.5
7	C-388	0.07	0.04	1.7	356
8	C-389	0.02	0.01	1.61	101.5
9	C-390	0.03	0.02	2.01	170.5
10	C-392	0.06	0.03	2.00	310.5
11	C-406	0.05	0.02	2.12	272.5
12	C-681	0.06	0.03	2.09	290.5
13	C-685	0.03	0.02	1.62	146.5
14	C-688	0.06	0.03	1.94	290.5
15	C-691	0.11	0.05	1.97	545.5
16	TCR 147	0.04	0.03	1.70	228.5
17	TCR 151	0.08	0.042	1.86	390.5
18	TCR 155	0.03	0.02	1.81	161
19	TCR 254	0.03	0.18	1.93	171
20	TCR 296	0.035	0.02	2.16	176
21	TCR 366	0.02	0.01	1.8	100
22	TCR 369	0.18	0.09	1.97	885
23	TCR 384	0.17	0.08	1.97	820
24	TCR 424	0.26	0.14	1.82	1275
25	TCR 429	0.04	0.02	1.82	209.5

26	TCR 443	0.06	0.03	1.9	285
27	TCR 450	0.07	0.04	1.84	333
28	TCR 545	0.05	0.03	1.77	226
29	TCR 903	0.04	0.02	2.11	180
30	ADR 2014-1	0.01	0.06	2.02	545.5

The agarose gel profile of the DNA extracted from the accessions are represented as Plate 2 and 3.

4.1.2. SSR PCR AMPLIFICATION FOR GENETIC DIVERSITY ANALYSIS

PCR condition, already standardized by molecular biology lab, crop improvement division, CTCRI was done to obtain crisp and clear amplicons with an annealing temperature of 56^{0}C. 18 SSR primers were screened to analyse its amplification profile, out of which 10 primers were used to study genetic variation. After the preliminary analysis from the primer screening data, 14 primers were selected from the 18 screened. From this 10 were selected for genetic diversity analysis. The primers Cel B12, Cel D12 were eliminated from the study due to its poor amplification strata and uq 110-283, uq 132-147 were discarded because of its monomorphic banding pattern. The primers selected for genetic diversity analysis are Cel H12, Cel A06, Cel B03, Cel F04, Cel C03, Cel C06, uq 97- 256, uq 201- 302, uq 84-207, uq 73-164. The amplicons obtained after SSR primer screening and various gradient studies are resolved in 2% agarose gel profiles are shown in plates 4-8.

4.1.3. DENATURING PAGE FOR SSR ANALYSIS

Denaturing PAGE gives higher resolution and separation of bands comparing to agarose gel electrophoresis, so all the 10 SSR primers amplified DNAs were

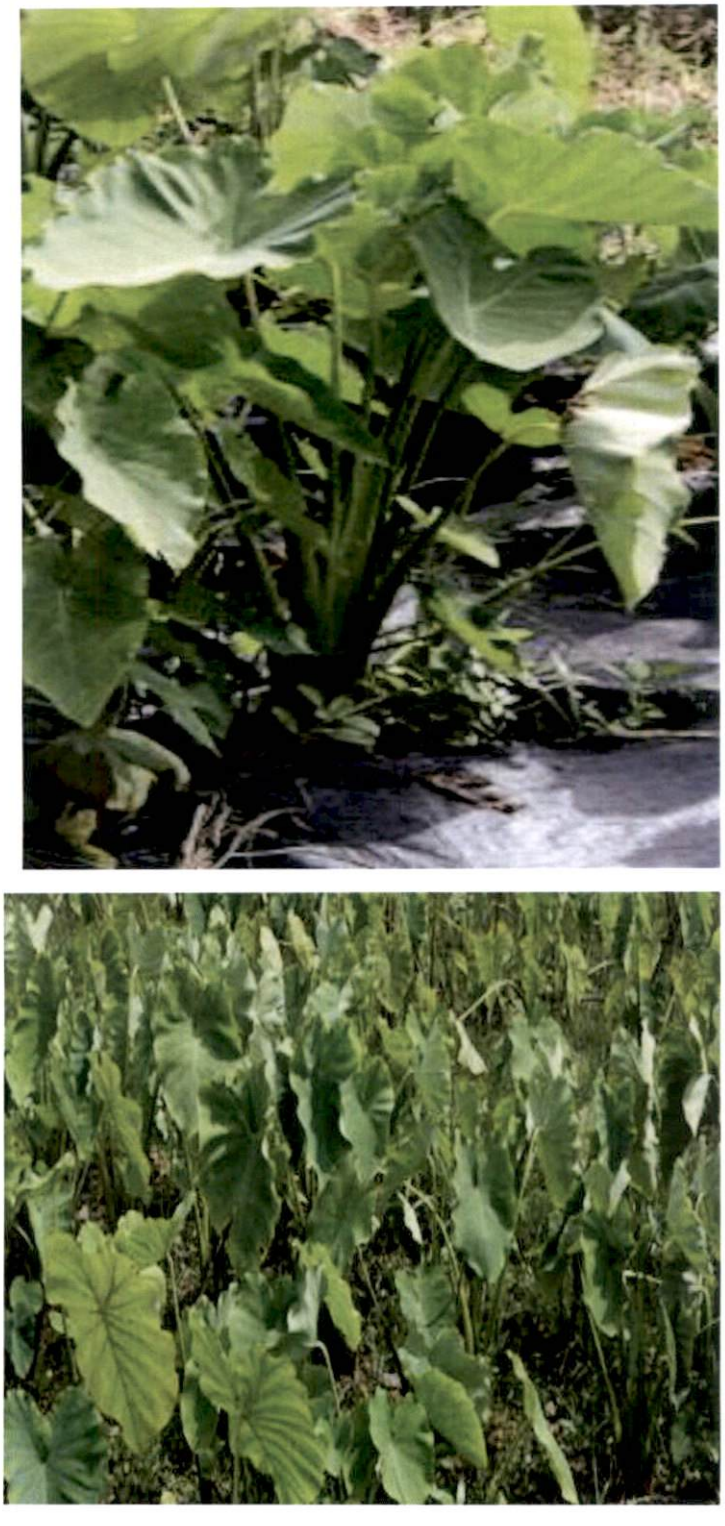
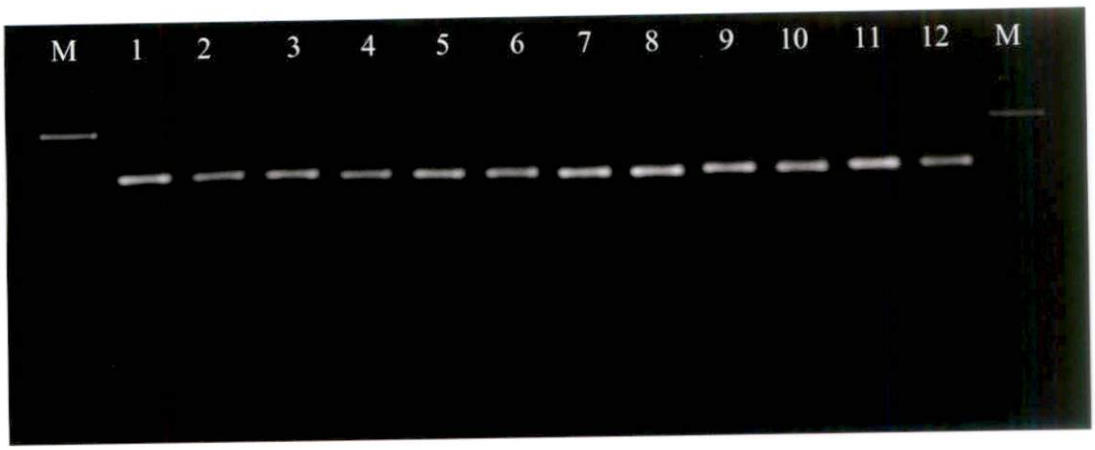


Plate 1: Taro field, ICAR- CTCRI



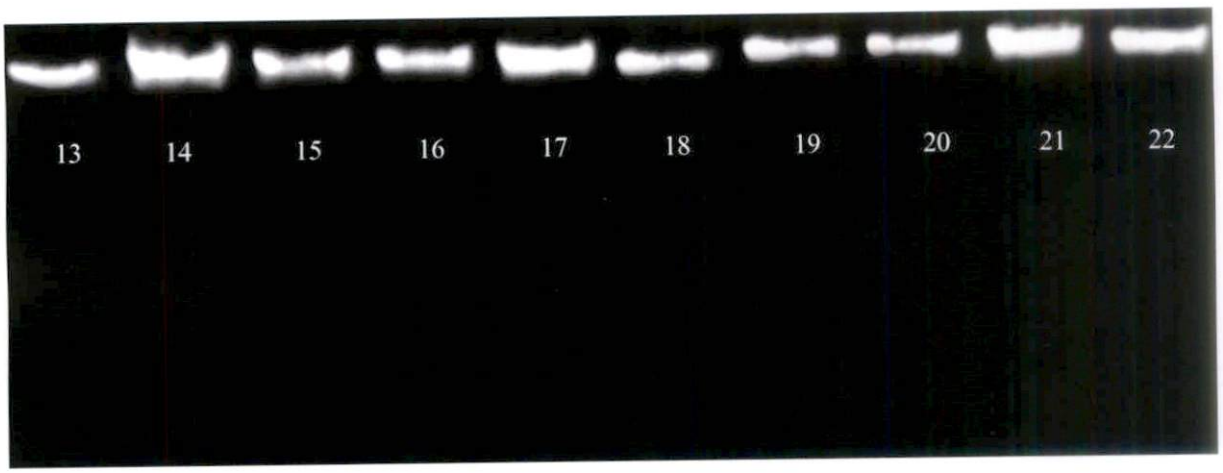
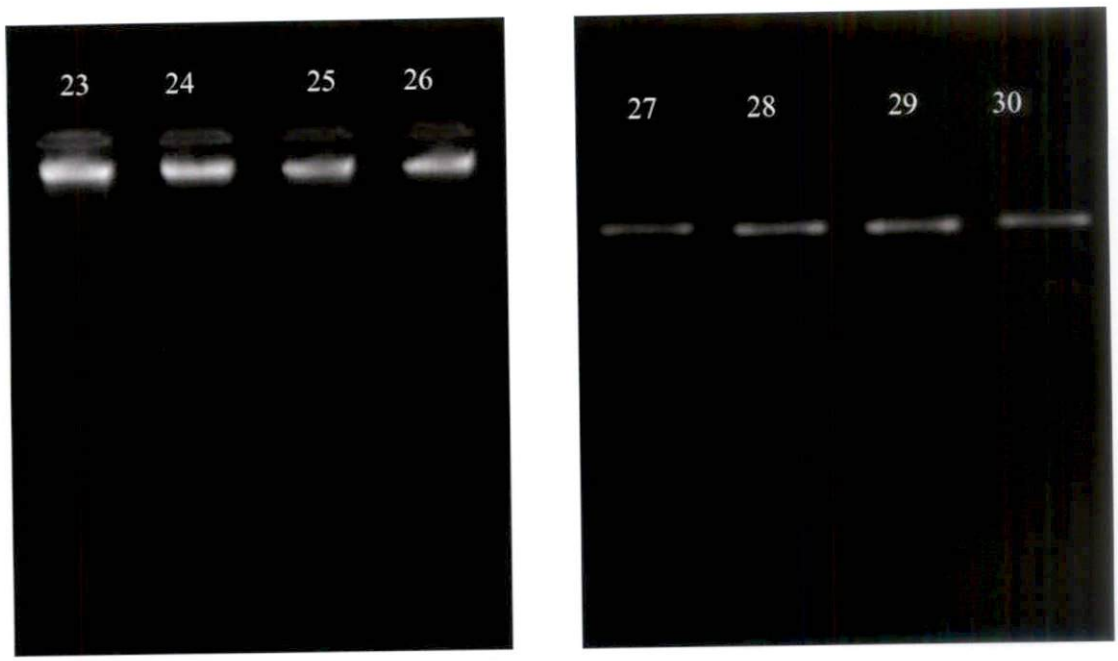


Plate 2: Agarose gel profile of DNA (0.8%) extracted from taro accessions

1. C390 2. TCR 147 3. TCR 443 4. C-203 5. C-262 6. C-681 7. C-273 8. C-371 9. TCR 545 10. TCR 903 11. TCR 429 12. TCR 450 13. C-388 14. TCR 384 15. TCR 151 16. TCR 369 17. TCR 366 18. C- 218 19. TCR- 296 20. C- 254 21. C-406 22. C-389

a



b

Plate 3: Agarose gel profile of DNA (0.8%) extracted from taro accessions

a. 23. C-392 24. C-370 25. ADR2014-1 26. C-691 27. TCR 155 28. C-685 29. C-688 30. TCR 424
b. 1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK

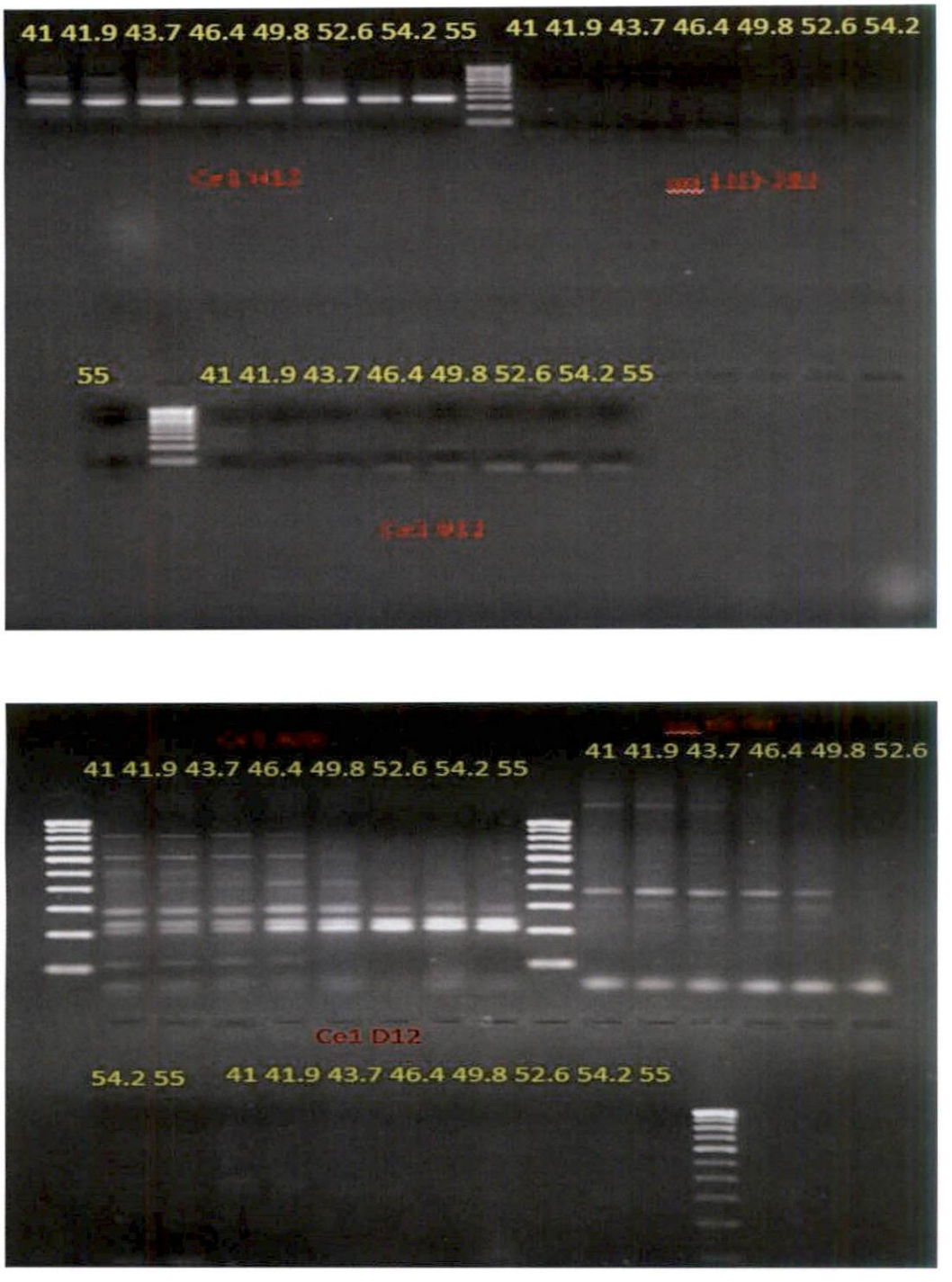


Plate 4: Gel profile obtained after various annealing temperatures

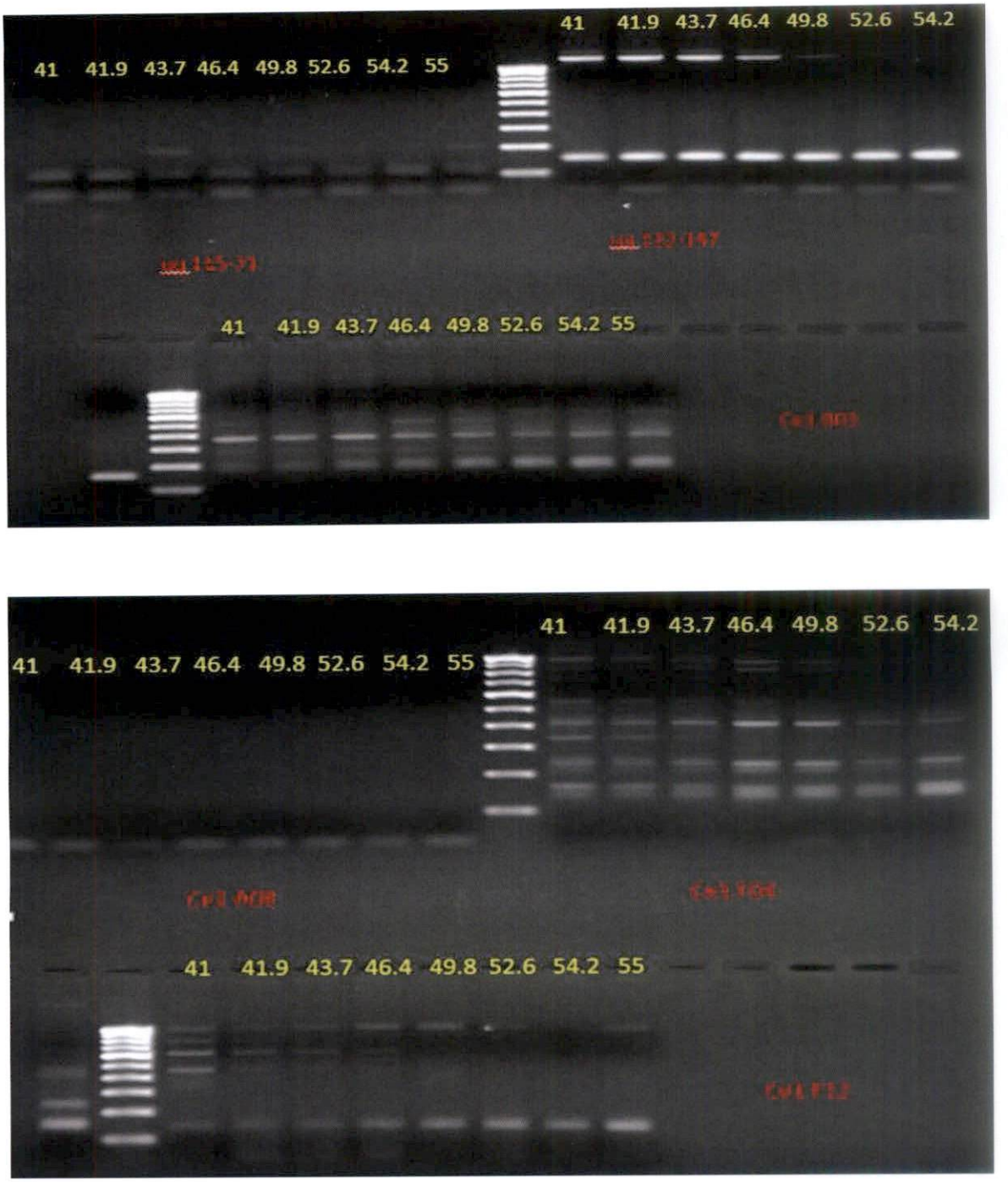
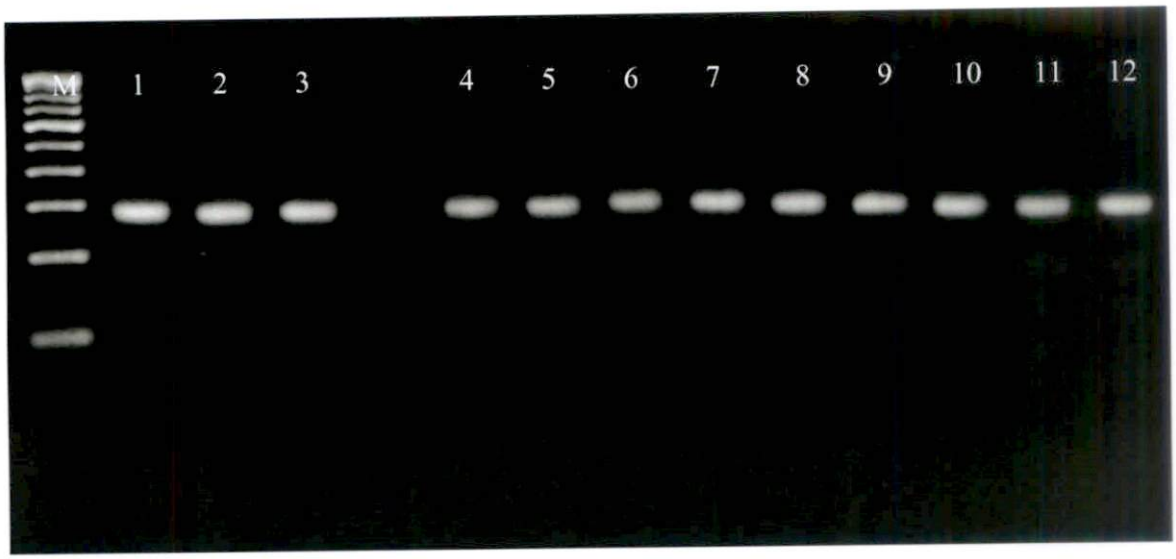


Plate 5: Gel profile obtained after various annealing temperatures



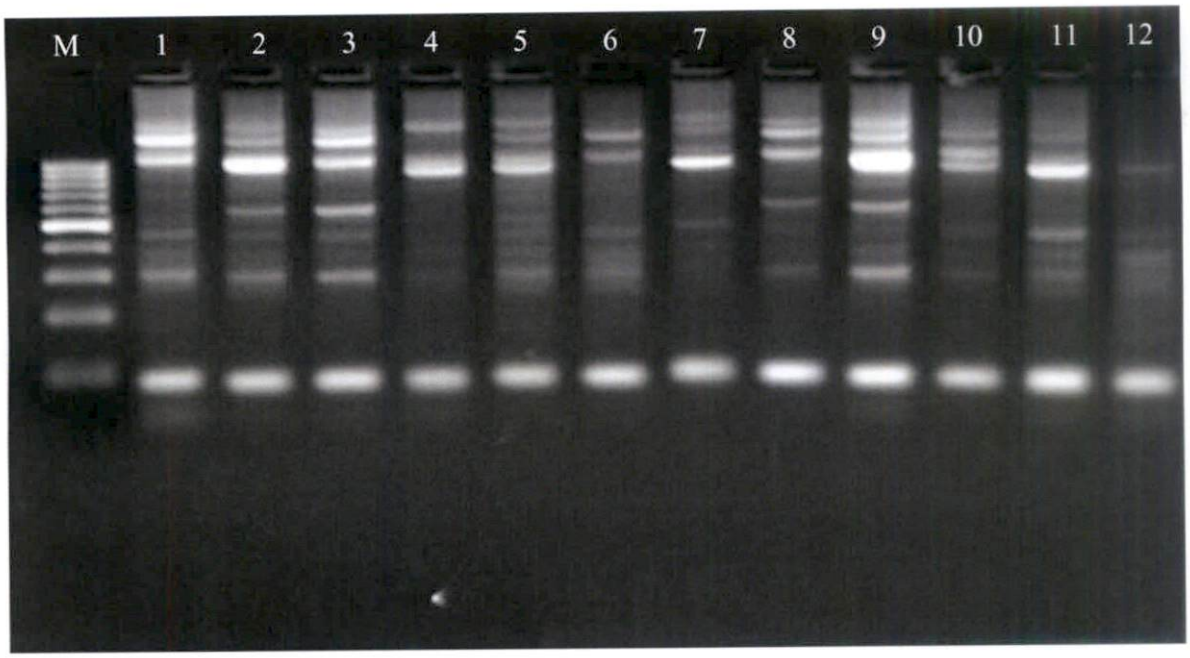
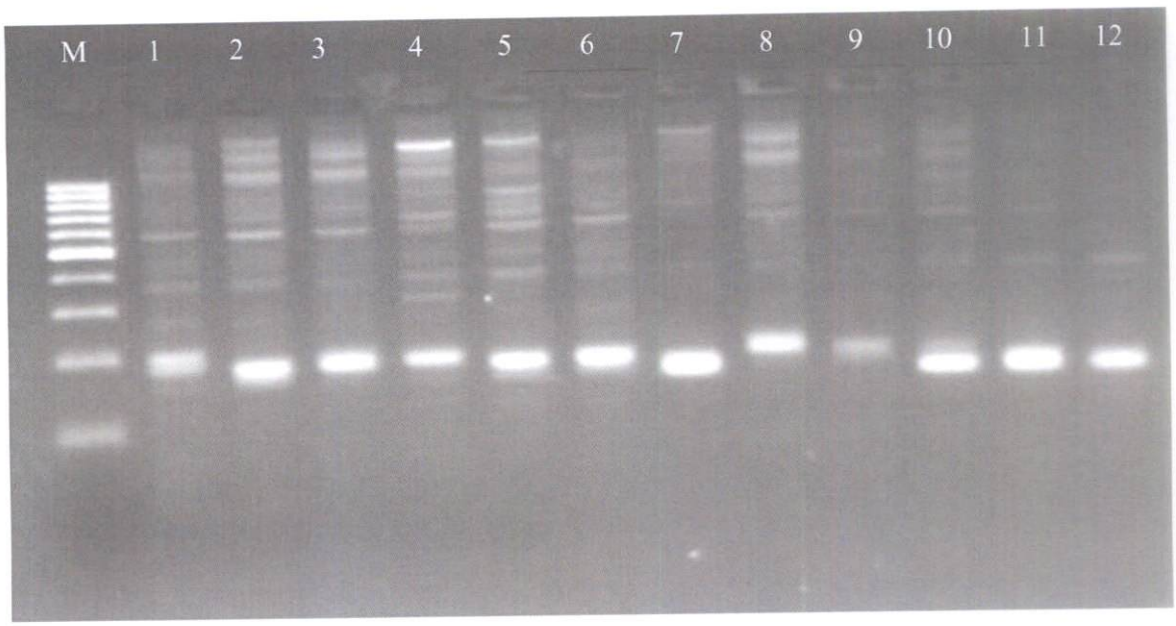


Plate 6: Agarose gel profile of DNA (2%) extracted from taro accessions with primers Ce1 A08 and uq 88-94 respectively and accessions in their order are represented below:

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK



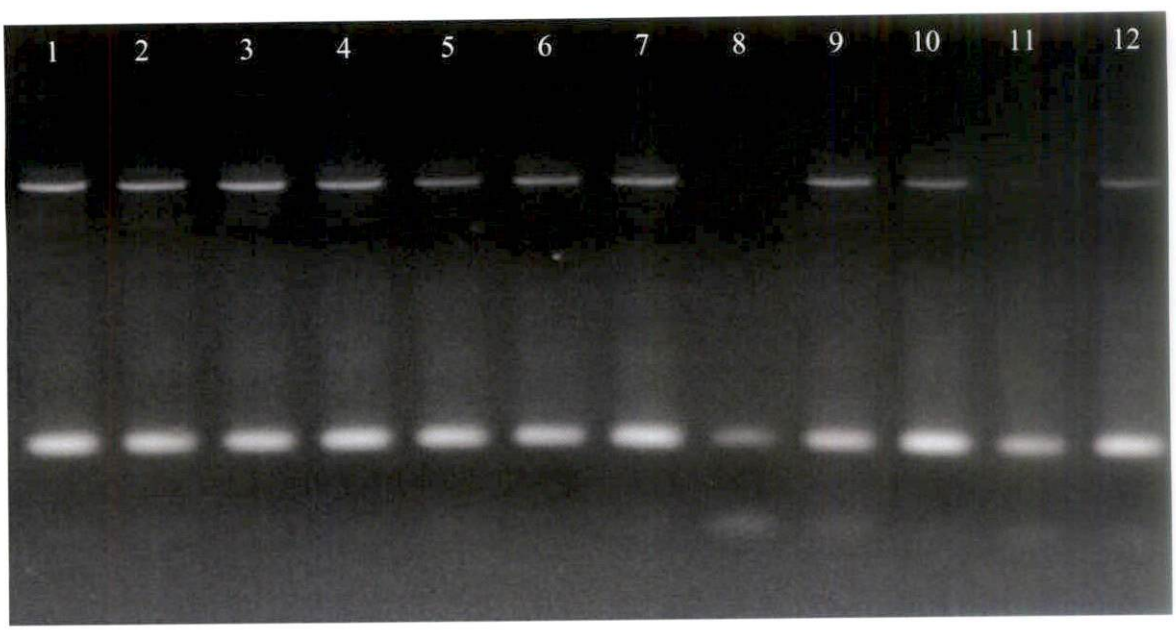


Plate 7: Agarose gel profile of DNA (2%) extracted from taro accessions with primers uq 97-256 and Cel C03 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK

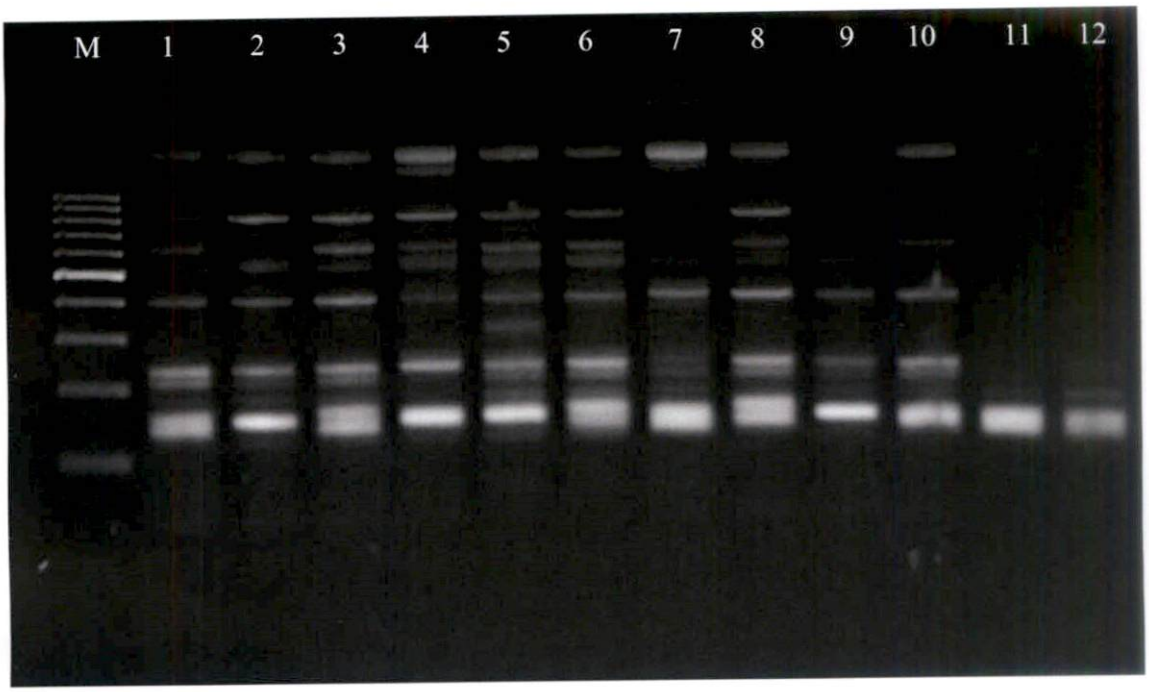
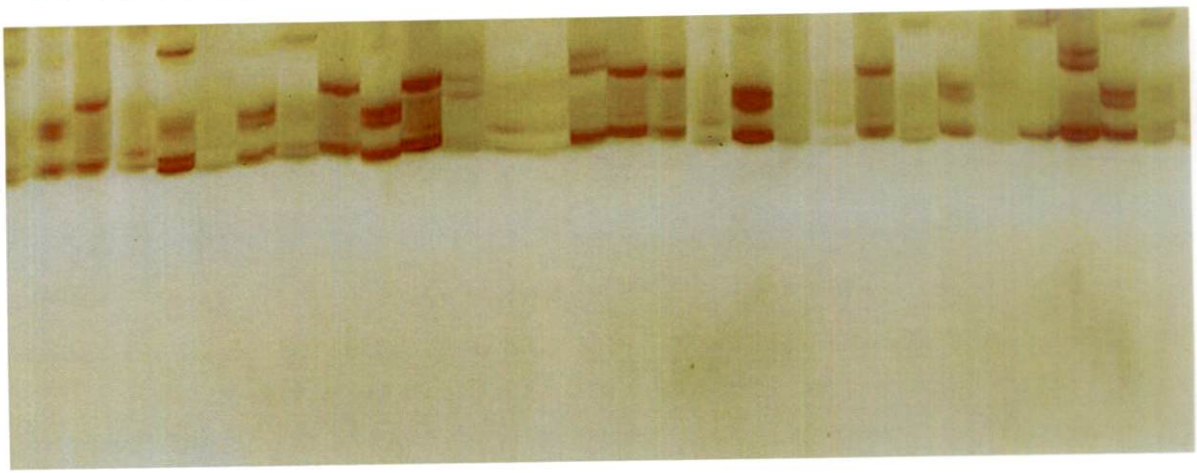


Plate 8: Agarose gel profile of DNA (2%) extracted from taro accessions with primers uq 73-164 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 M

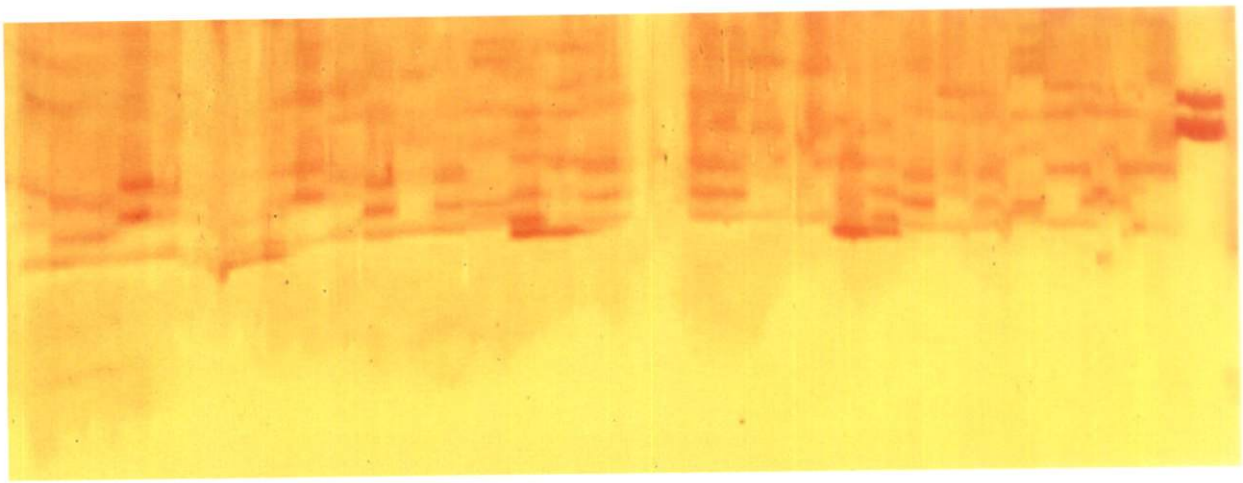
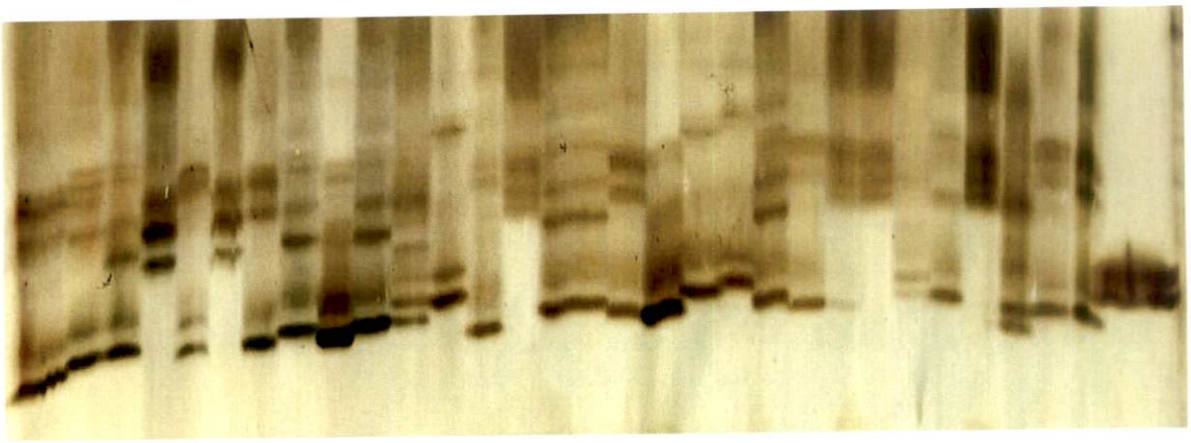


Plate 9: Denaturing Urea-Acrylamide (6%) gel profile of uq 73-164 and Ce1 B03 respectively

1. C390 2. TCR 147 3. TCR 443 4. C-203 5. C-262 6. C-681 7. C-273 8. C-371 9. TCR 545 10. TCR 903 11. TCR 429 12. TCR 450 13. C-388 14. TCR 384 15. TCR 151 16. TCR 369 17. TCR 366 18. C- 218 19. TCR- 296 20. C- 254 21. C-406 22. C-389 23. C-392 24. C-370 25. ADR2014-1 26. C-691 27. TCR 155 28. C-685 29. C-688 30. TCR 424

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 252627 282930 M



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

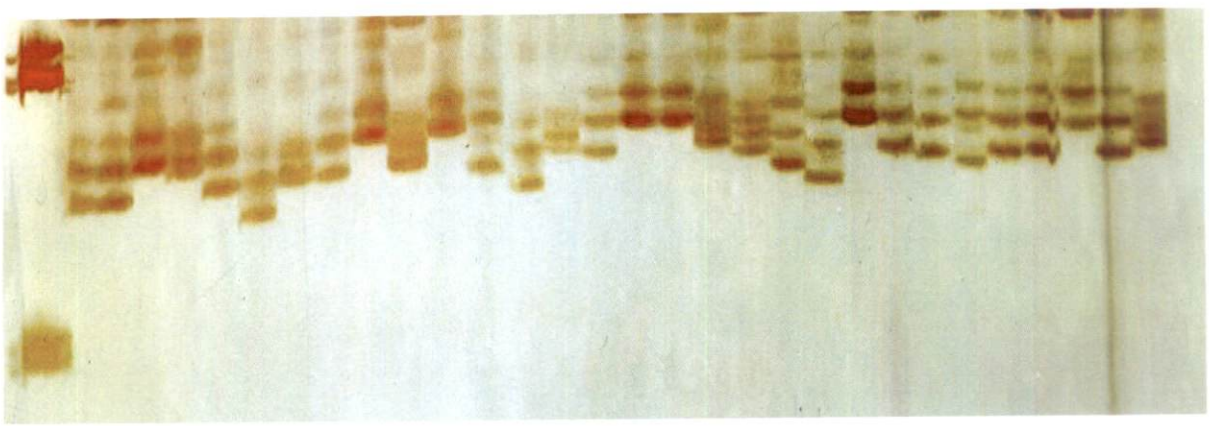
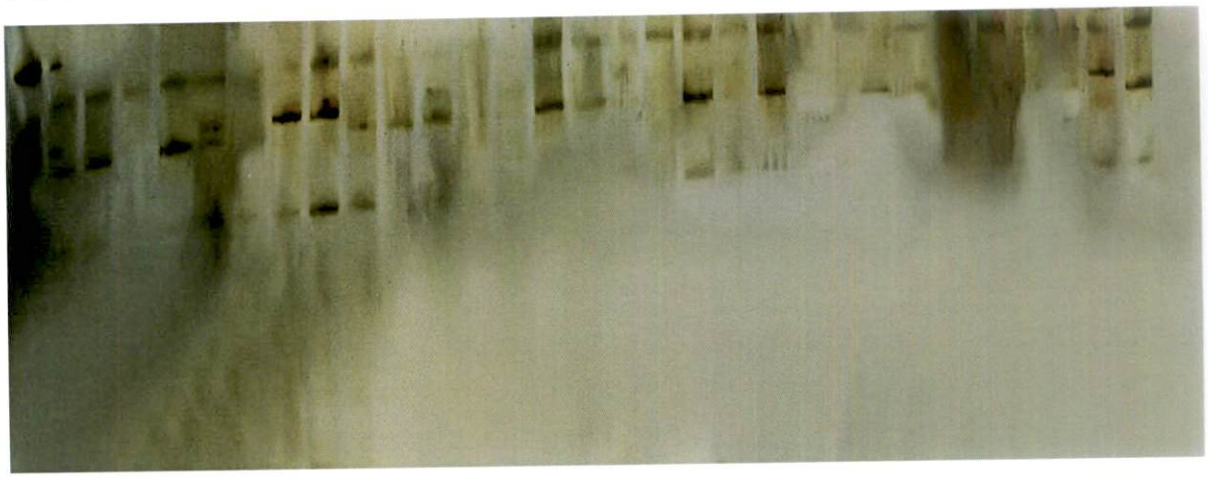


Plate 10: Denaturing Urea-Acrylamide (6%) gel profile of Ce1 C03 and Ce1 F04 respectively

1. C390 2. TCR 147 3. TCR 443 4. C-203 5. C-262 6. C-681 7. C-273 8. C-371 9. TCR 545 10. TCR 903 11. TCR 429 12. TCR 450 13. C-388 14. TCR 384 15. TCR 151 16. TCR 369 17. TCR 366 18. C- 218 19. TCR- 296 20. C- 254 21. C-406 22. C-389 23. C-392 24. C-370 25. ADR2014-1 26. C-691 27. TCR 155 28. C-685 29. C-688 30. TCR 424

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 2930 M

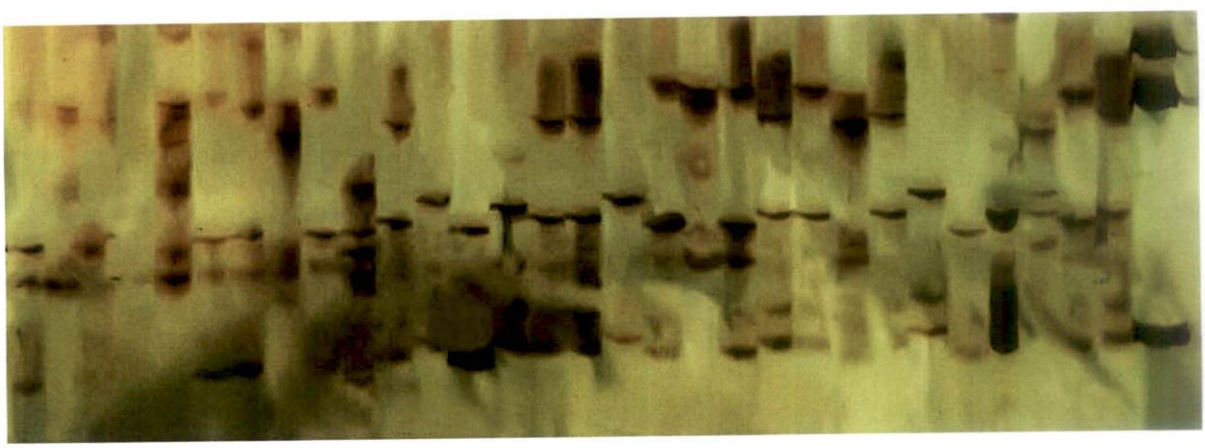
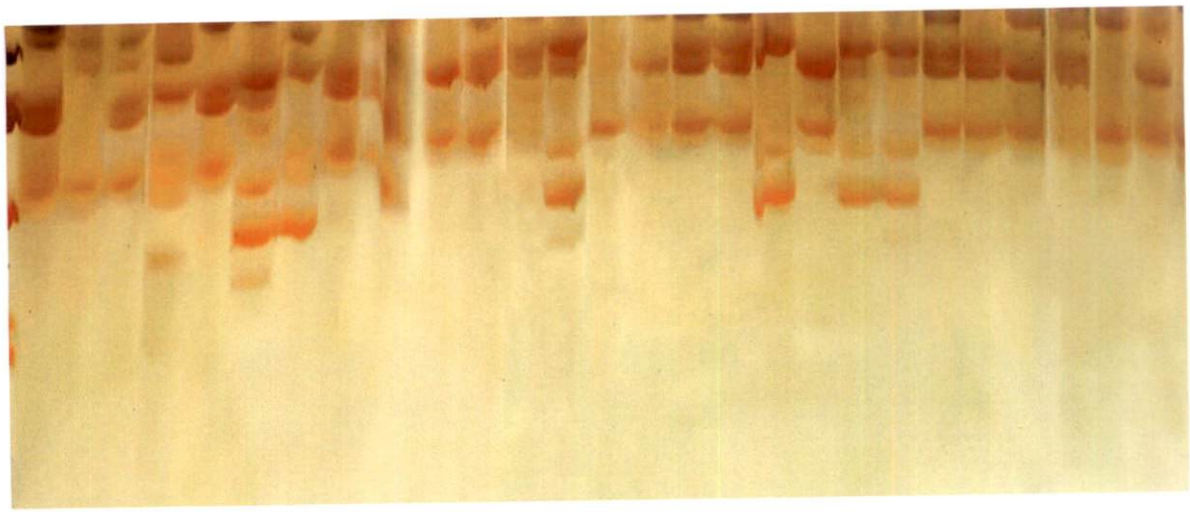


Plate 11: Denaturing Urea-Acrylamide (6%) gel profile of uq84-207 and uq 97-256 respectively.

1. C390 2. TCR 147 3. TCR 443 4. C-203 5. C-262 6. C-681 7. C-273 8. C-371 9. TCR 545 10. TCR 903 11. TCR 429 12. TCR 450 13. C-388 14. TCR 384 15. TCR 151 16. TCR 369 17. TCR 366 18. C- 218 19. TCR- 296 20. C- 254 21. C-406 22. C-389 23. C-392 24. C-370 25. ADR2014-1 26. C-691 27. TCR 155 28. C-685 29. C-688 30. TCR 424

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

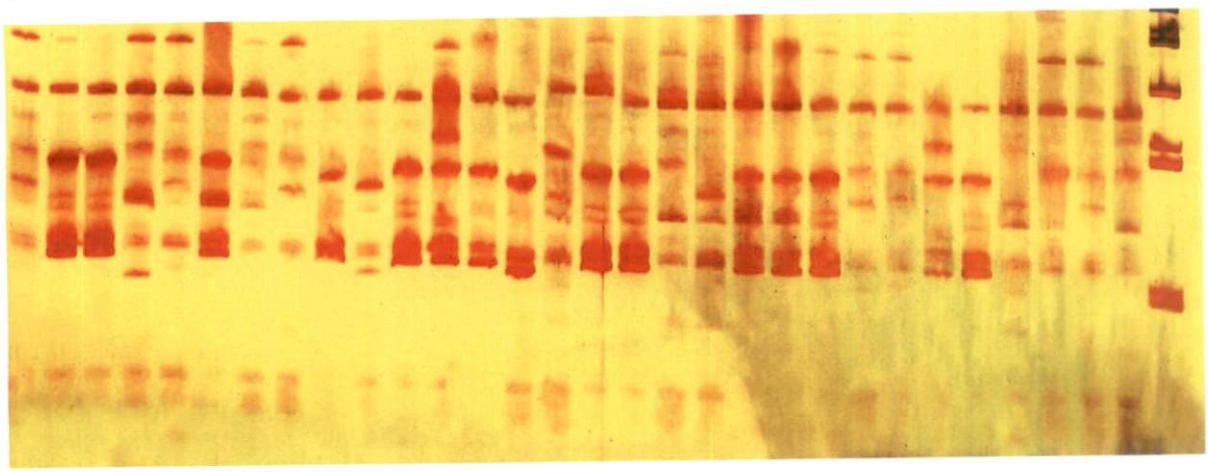


Plate 12: Denaturing Urea-Acrylamide (6%) gel profile of uq 201- 302 and Ce1 H12 respectively

1. C390 2. TCR 147 3. TCR 443 4. C-203 5. C-262 6. C-681 7. C-273 8. C-371 9. TCR 545 10. TCR 903 11. TCR 429 12. TCR 450 13. C-388 14. TCR 384 15. TCR 151 16. TCR 369 17. TCR 366 18. C- 218 19. TCR- 296 20. C- 254 21. C-406 22. C-389 23. C-392 24. C-370 25. ADR2014-1 26. C-691 27. TCR 155 28. C-685 29. C-688 30. TCR 424

separated by PAGE. All the primers gave high polymorphic bands and show high allelic frequency than their respective agarose gels. The primer uq 97-256 gave the highest number of alleles and the lowest was obtained from the primer uq 84-207. The denaturing urea- acrylamide gel profiles are represented as plate 9-12.

4.1.4. ANALYSIS OF MOLECULAR MARKER DATA

For analysis of genetic diversity, the bands obtained after PAGE were considered. The presence of band were marked as "1" and the absence was marked as "0". This data was used for construction of dendrogram and PIC analysis.

4.1.4.1. POLYMORPHIC INFORMATION CONTENT

The PIC value ranged from 0.639 to 0.857. The highest heterozygosity was obtained for the primer uq 97-256 with a value of 0.871 and the lowest was obtained for the primer uq 84-207 with a value of 0.694. Number of alleles per locus ranged from 1.9-4.56 with the maximum alleles shown by uq 97-256 and the minimum showed by uq 84-207 and uq 201-302. The heterozygosity, PIC and number of alleles of all primers are represented in the table 8.

Table 8: PIC ANALYSIS

SL NO:	GENOTYPES	HETEROZYGOSITY	PIC	ALLELES PER LOCUS
1	uq 73-164	0.833	0.8137	3.57
2	Ce1 C03	0.844	0.825	2.1
3	uq 97-2556	0.871	0.857	4.36
4	Ce1 B03	0.81	0.784	2.73
5	Ce1 F04	0.805	0.777	3.63
6	Ce1A06	0.803	0.774	3.73

7	Ce1 C06	0.768	0.734	1.866
8	Ce1 H12	0.805	0.777	3.6
9	uq 84-207	0.694	0.647	1.9
10	uq 201-302	0.871	0.639	1.9

4.1.4.2. DENDROGRAM ANALYSIS

Based on the scoring data, a dendrogram tree was constructed using R Package software. It grouped the varieties into 3 main clusters. According to the fig.1, the first cluster comprised of the accessions from C-406 to TCR 450. The second cluster consists of C-218 to C-384 and the third large cluster groups most of the genotypes ranging from TCR 424 to TCR-296 and ADS2014-20 has formed an outlier. The third cluster is further subdivided into four sub-clusters and the second sub-cluster consists of major number of genoytpes and has an outlier C-273. The dendrogram tree is represented as fig 1.

4.2. MOLECULAR MARKERS ASSOCIATED WITH TLB

Molecular marker linked with TLB was studied using two markers viz., ISSR and SSR.

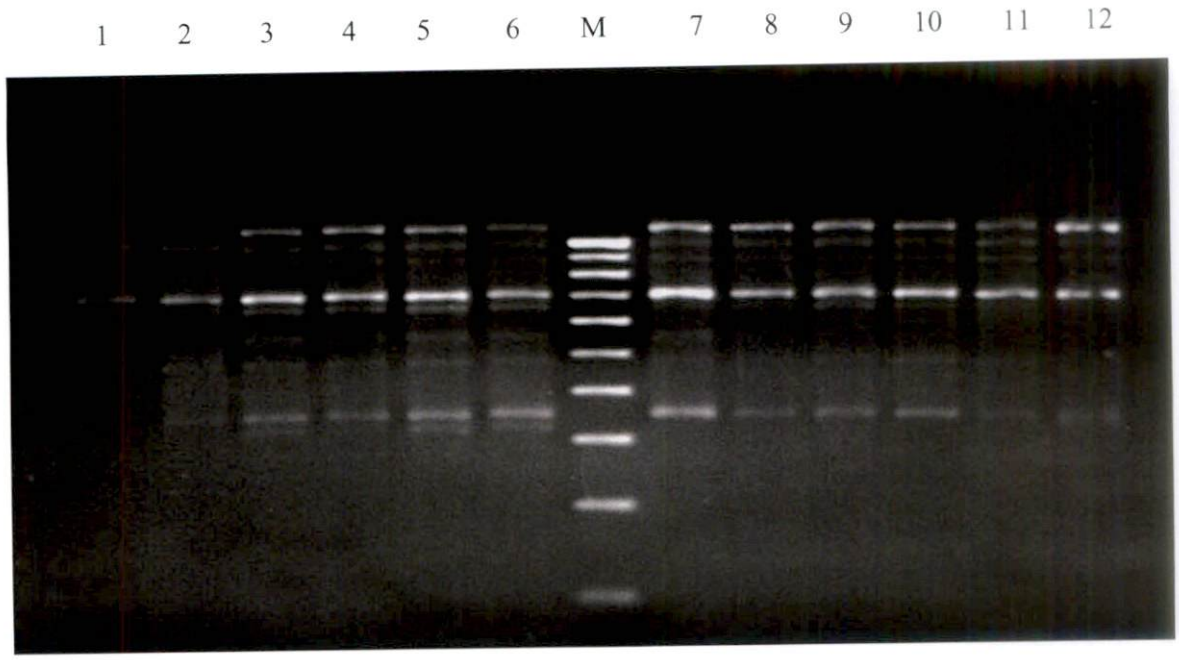
4.2.1. DNA ISOLATION

DNA was isolated using DNA isolation mini kit (Amnion AMpreP plant gDNA Mini kit). The method was found to be easy and efficient. The method gave intact and clear and crisp DNA. The spectrophotometer readings of the DNA samples extracted from 6 susceptible and 6 resistant taro accessions are listed in the table.

Table 9: DNA QUANTIFICATION AND YIELD OF DNA

Sl NO:	SAMPLE	ABSORBANCE A260	ABSORBANCE A280	A260/280	DNA YIELD (ng/µl)
1	Sree Reshmi	0.19	0.09	1.94	925
2	C- 681	0.13	0.06	2.08	635
3	C-211	0.04	0.02	1.95	215
4	C-688	0.06	0.03	1.94	290.5
5	C-82	0.09	0.04	2.00	430
6	C-185	0.10	0.05	1.90	495
7	Muktakeshi	0.08	0.04	1.86	420
8	C – 545	0.04	0.03	1.77	226
9	TCR 443	0.09	0.04	2.04	444.5
10	C - 370	0.07	0.04	1.76	310
11	C - 203	0.02	0.01	1.66	112
12	C - 717	0.07	0.03	1.94	340

The method has resulted in obtaining a good quality DNA with a purity in the range of 1.8 -2.0. The DNA profiles resolved in 0.8% agarose gel is represented in plate 2.



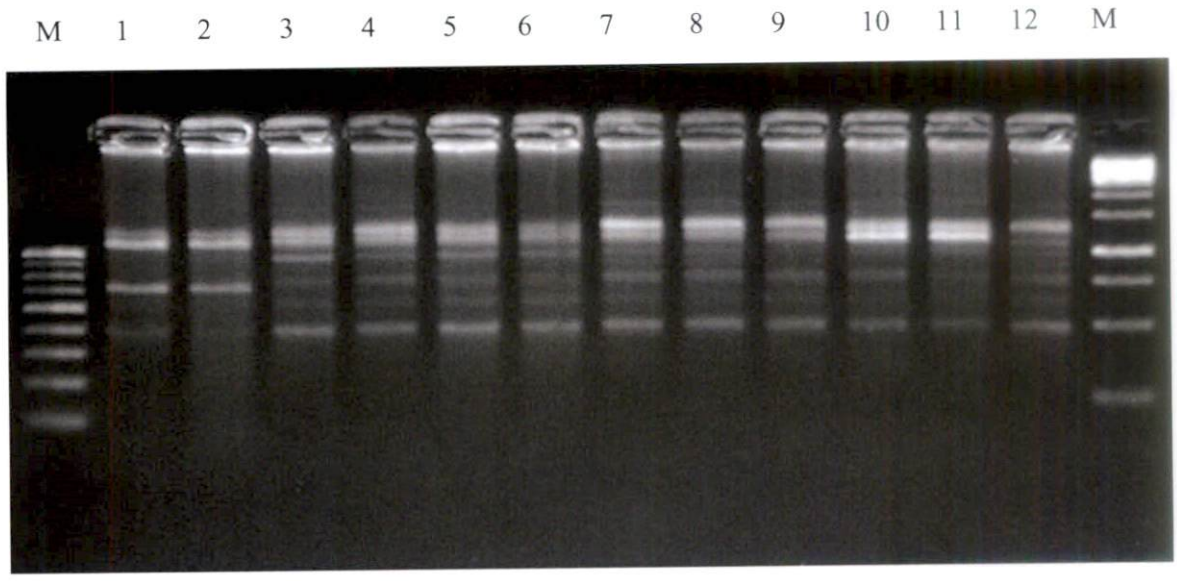


Plate 13: Agarose gel profile of DNA (2%)of UBC 841 and UBC 848 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK . In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.

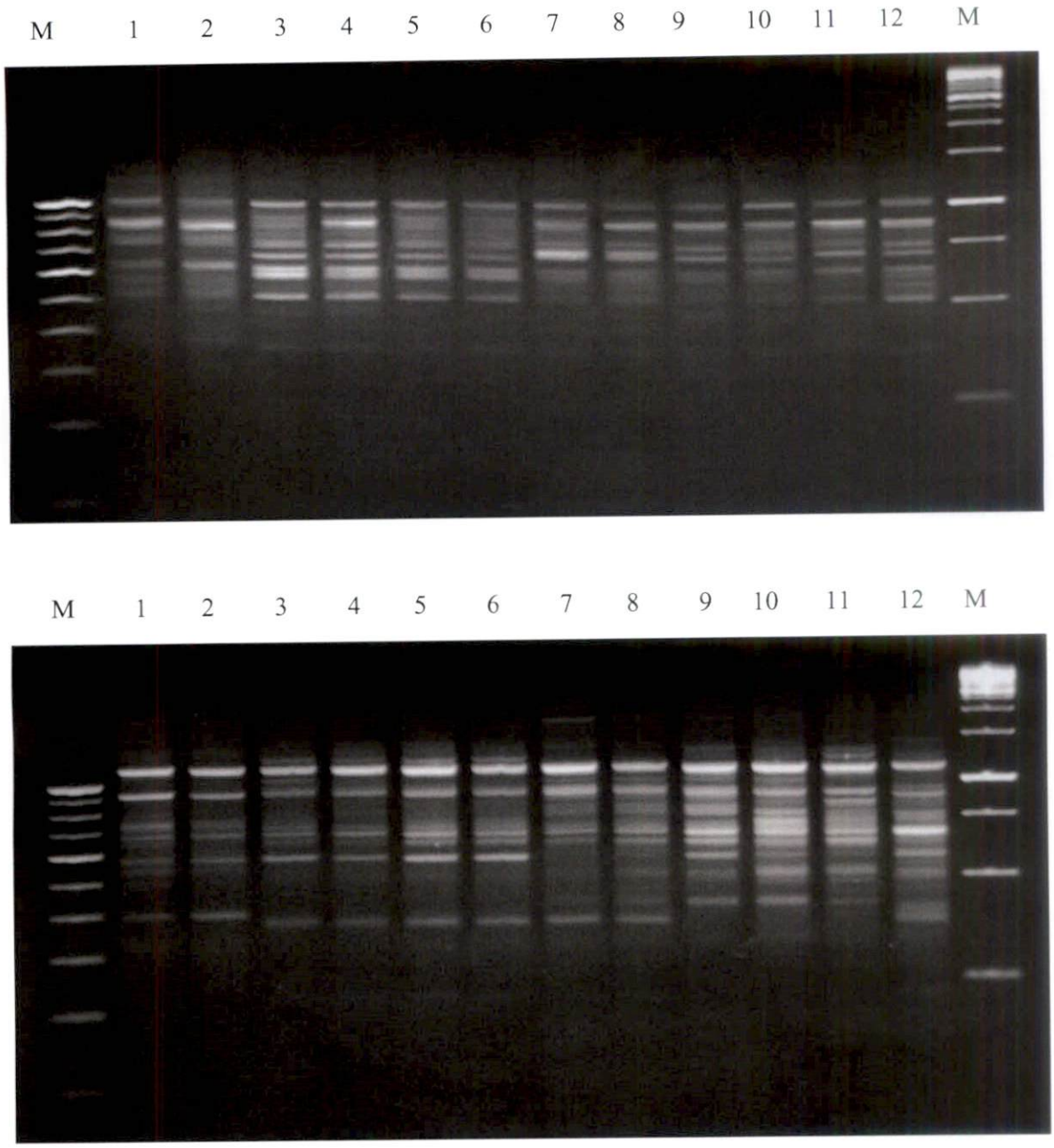


Plate 14: Agarose gel profile of DNA (2%) of UBC 810 and $(AG)_9AC$ respectively. In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK

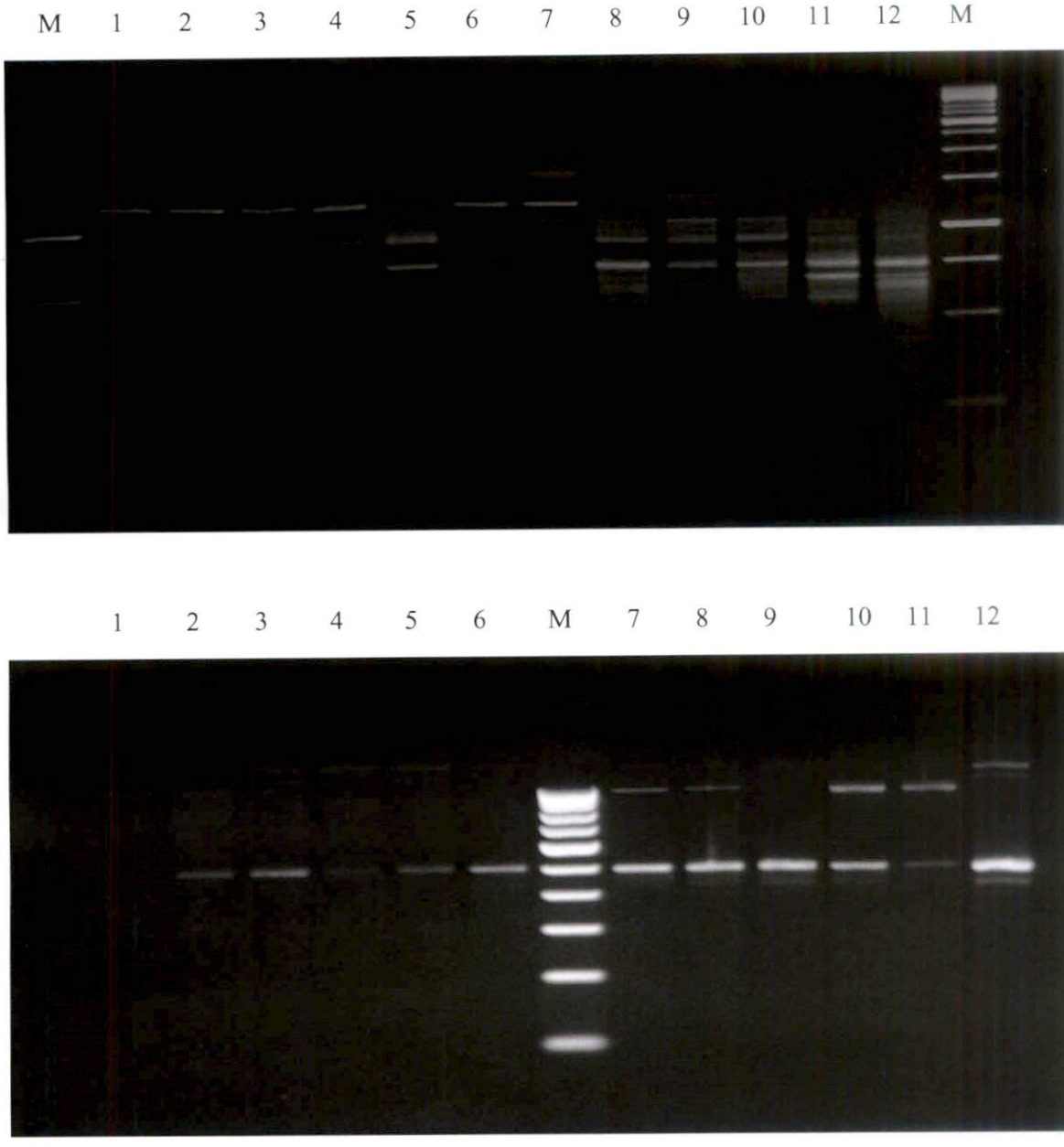
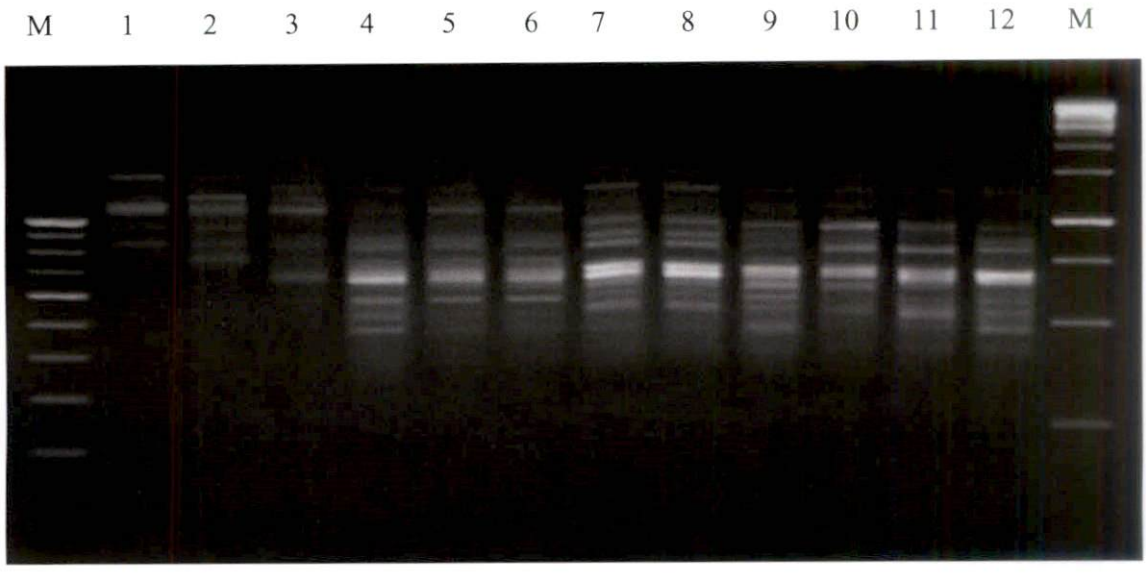


Plate 15: Agarose gel profile of DNA (2%) of UBC 808 and UBC 847 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK. In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.



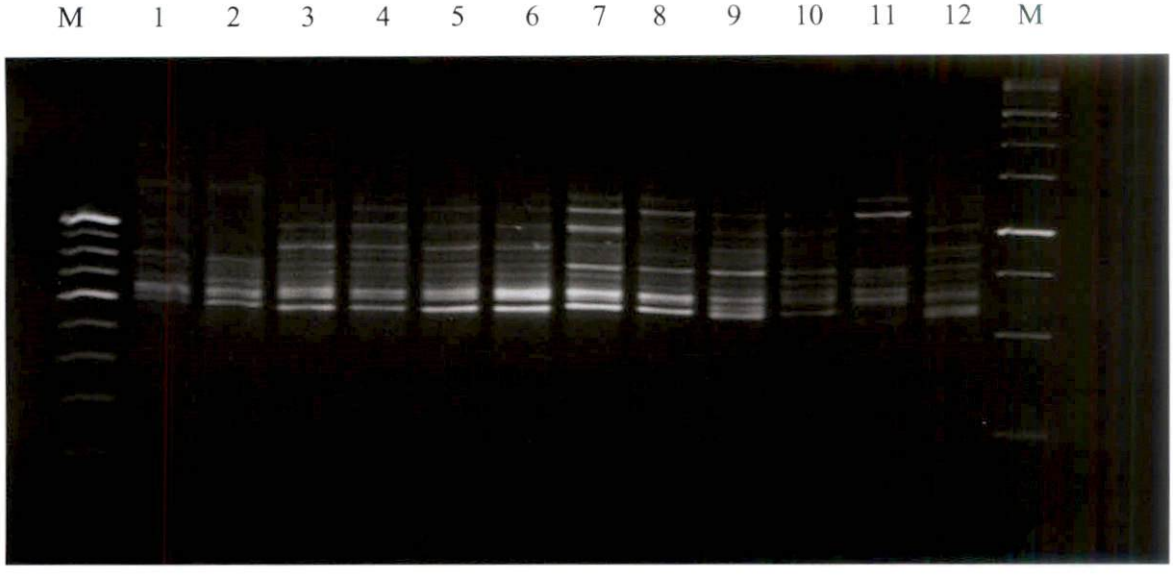
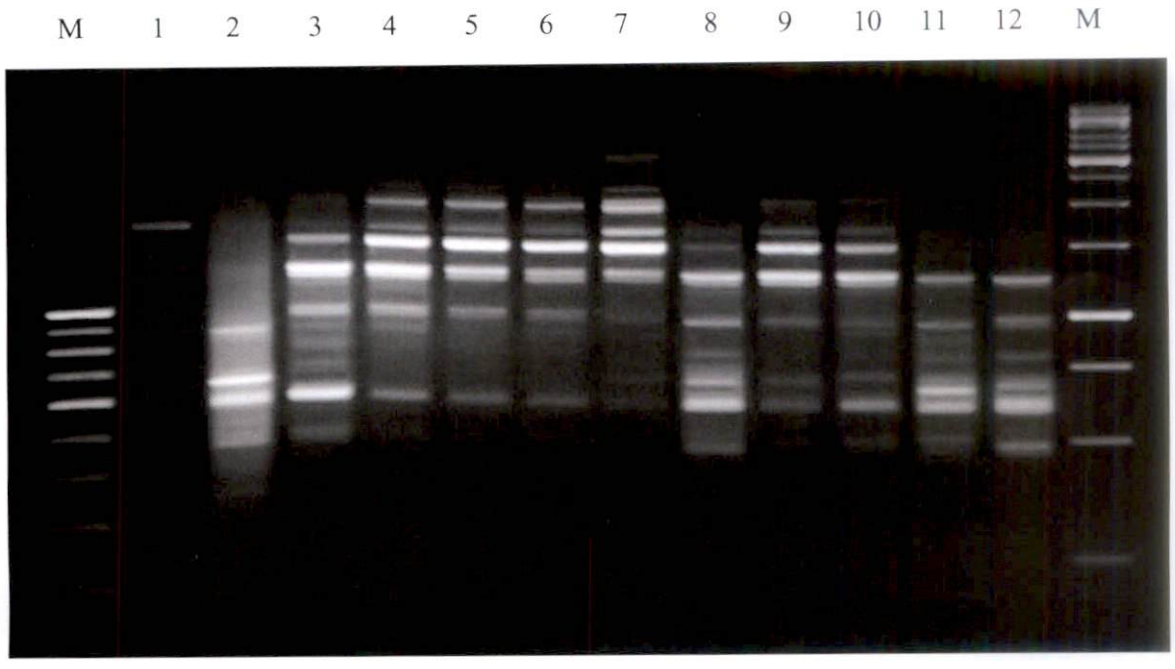


Plate 16: Agarose gel profile of DNA (2%) of UBC 837 and UBC 871 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK. In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.



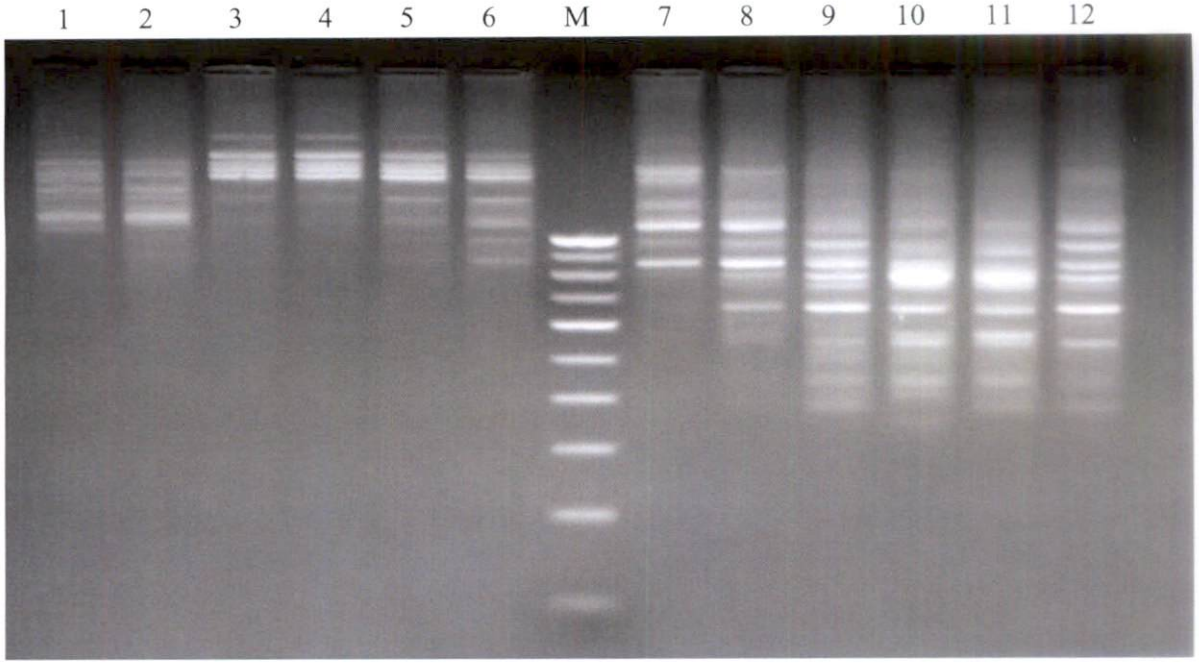


Plate 17: Agarose gel profile of DNA (2%) of UBC 857 and UBC 825 respectively. 1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK . . In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.

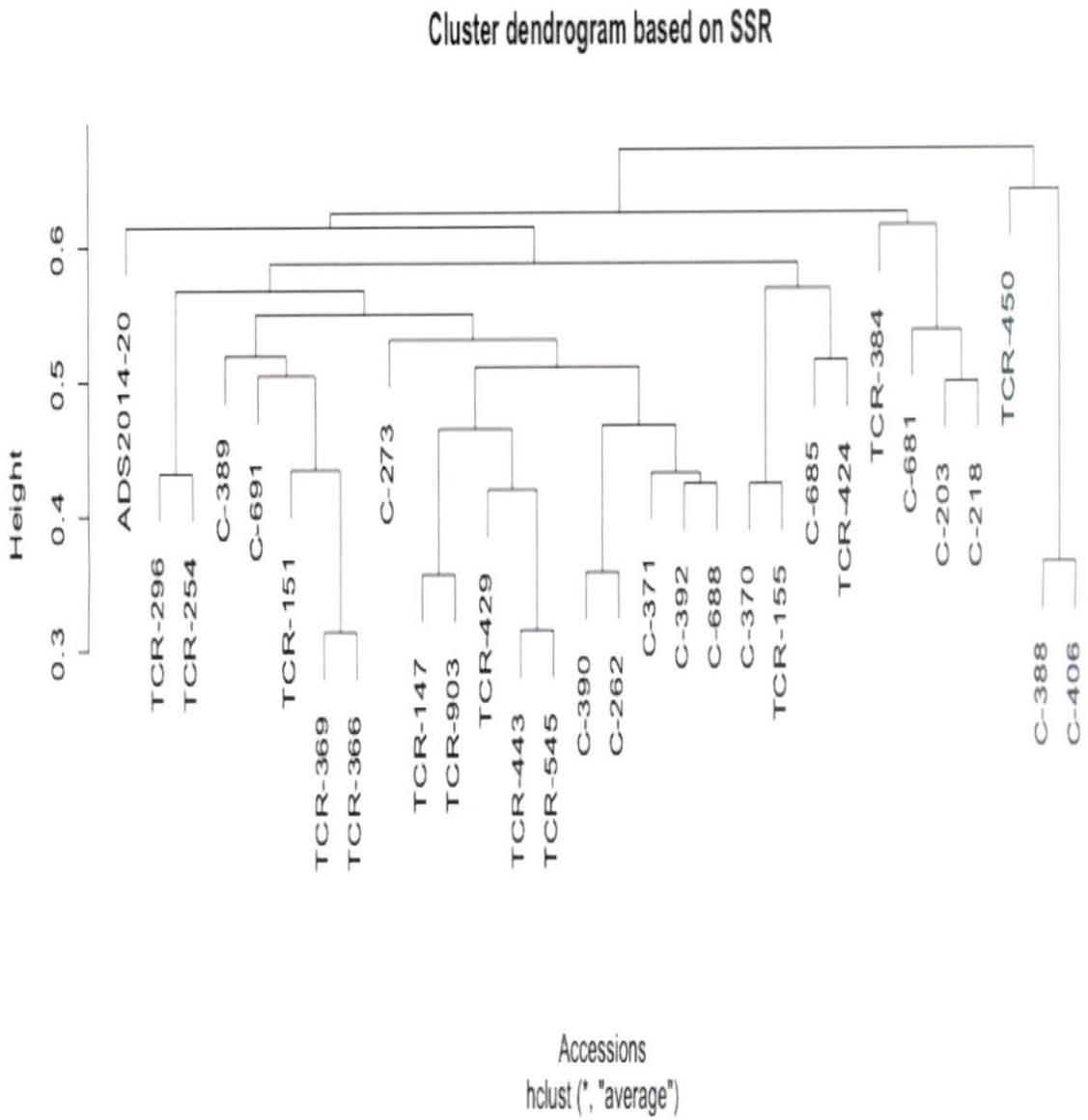


Figure 1: Dendrogram clustering of accessions based on genetic diversity analysis

4.2.2. ISSR PCR AMPLIFICATION FOR TLB MARKER ANALYSIS

PCR condition, already standardized by molecular biology lab, crop improvement division, CTCRI was done to obtain crisp and clear amplicons with an annealing temperature of 56^0C. 17 ISSR primers were screened to analyse its amplification profile, out of which 13 primers were used for the study. After the preliminary analysis from the primer screening data, 13 primers were selected from the 17 screened. Rest of the primers viz., UBC 11, UBC 811, UBC 818, $(ACC)_6Y$ were eliminated because of its poor amplification profile. The primers selected for the TLB screening were UBC 841, UBC 848, UBC 810, UBC 808, UBC 817, UBC 847, UBC 871, UBC 827, UBC 857, UBC 825, UBC 809, $(GA)_9AC$, $(AG)_9AT$.

4.2.3. ISSR GEL ELECTROPHORESIS FOR PCR PRODUCT ANALYSIS

The PCR products were made to resolve in 2% agarose gel for the identification of molecular markers associated with leaf blight resistance in taro. Out of the 13 primers, 5 of them gave an extra band for resistant varieties. UBC 825 and $(AG)_9$ AC gave an extra band in all the resistant varieties in 684.99 bp and 808.30 bp region respectively. The primers UBC 827, UBC 817and UBC 808 gave bands in 5 resistant varieties in the region, 597.92, 674.95 and 667 respectively. The agarose gel profiles are represented as plate 13-17.

4.2.4. SIMILARITY INDEX

The similarity index values obtained for each pair wise comparison among the 12 taro accession based on thirteen ISSR marker data is given in the table 10. The similarity coefficient based on ISSR markers ranged from 0.42 to 0.93. Most of the similarity coefficients ranges between 0.53 to 0.76. Among the 12 taro accessions, the lowest similarity index (0.42) was observed between Muktakeshi and Sree Rashmi, whereas, the highest similarity index (0.93) was observed between C-211 and C-688 along with C-688 and C-82.

Table 10: Similarity coefficient of 12 taro accessions using thirteen ISSR markers

	Sree Rashmi	C 681	C 211	C 688	C 82	C 185	TCR 545	TCR 443	C 370	C 203	C 717	Muktakeshi
Sree Rashmi	1											
C 681	0.78	1										
C 211	0.68	0.76	1									
C 688	0.65	0.74	0.93	1								
C 82	0.63	0.74	0.89	0.93	1							
C 185	0.68	0.68	0.84	0.85	0.86	1						
TCR 545	0.61	0.63	0.74	0.74	0.74	0.81	1					
TCR 443	0.53	0.61	0.66	0.66	0.68	0.72	0.87	1				
C 370	0.49	0.56	0.59	0.61	0.64	0.69	0.78	0.87	1			
C 203	0.49	0.55	0.56	0.57	0.59	0.66	0.74	0.80	0.90	1		
C 717	0.47	0.55	0.53	0.56	0.57	0.62	0.68	0.77	0.84	0.87	1	
Muktakeshi	0.42	0.5	0.49	0.52	0.54	0.59	0.64	0.73	0.82	0.84	0.89	1

4.2.5. DENDROGRAM ANALYSIS

A dendrogram generated using UPGMA cluster analysis separated the 12 taro accessions into two major clusters with the susceptible varieties clustering together and so also the resistant (Fig. 2). The clusters, cluster I and cluster II have around 60% similarity. Cluster I comprised of six accessions, Sree Rashmi, C-681, C-211, C-688 C-82 C-185, whereas, Cluster II comprised the resistant six accessions, TCR

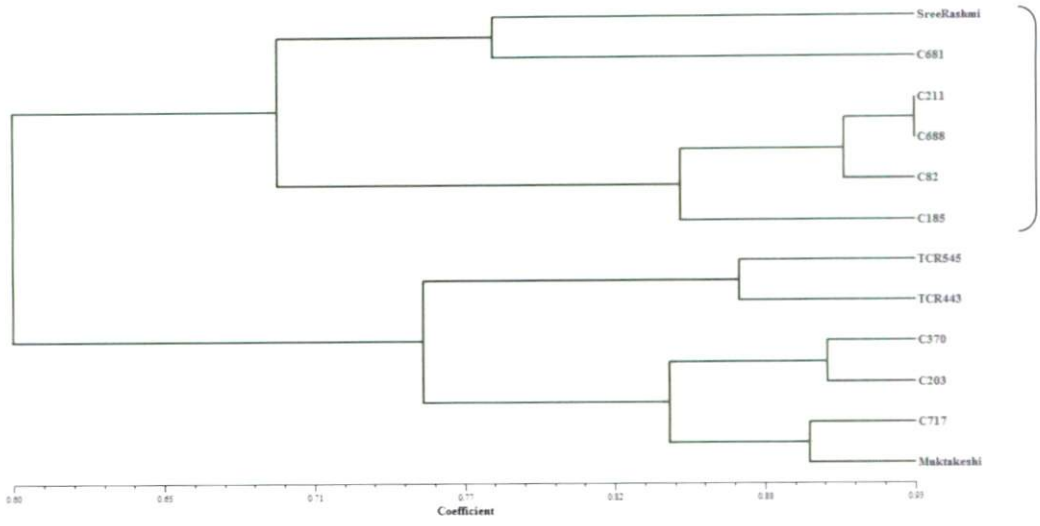


Fig. 2 Dendrogram showing the clustering pattern in 12 taro accessions using thirteen ISSR markers

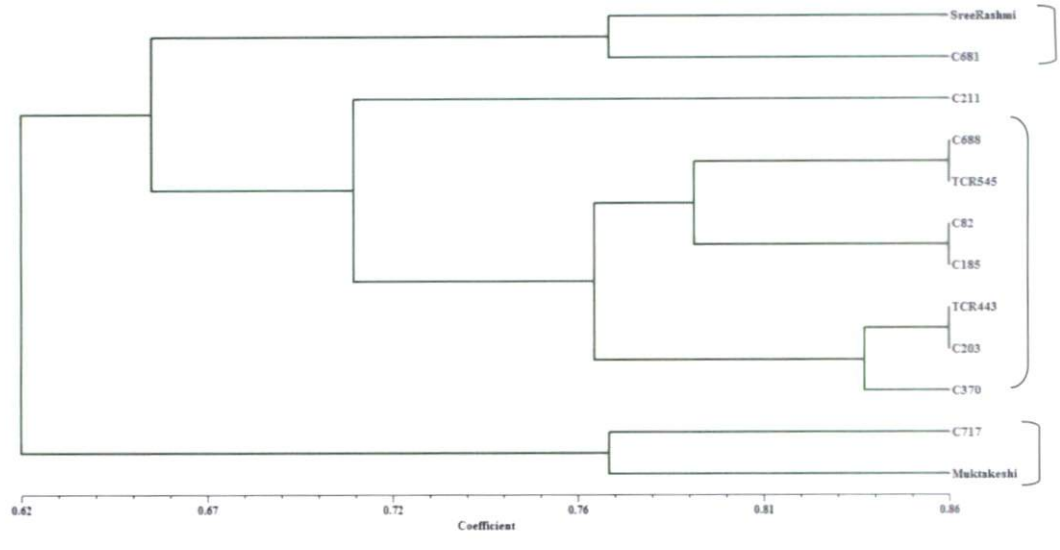


Fig. 3 : Dendrogram showing the clustering pattern in 12 taro accessions using 7 SSR markers

173755

545, TCR 443, C-370, C-203, C-717, Muktakeshi. The dendrogram tree is represented as fig 2.

Table 11: Details of 13 TARO accessions grouping under the two major clusters

Cluster	**Accessions**
Cluster I	Sree Rashmi, C-681, C-211, C-688 C-82 C-185
Cluster II	TCR 545, TCR 443, C-370, C-203, C-717, Muktakeshi

4.2.6. SSR PCR AMPLIFICATION FOR TLB MARKER ANALYSIS

PCR condition, already standardized by molecular biology lab, crop improvement division, CTCRI was done to obtain crisp and clear amplicons with an annealing temperature of 56^{0}C. As mentioned in 4.1.2, 18 SSR primers were screened to analyse its amplification profile, out of which 10 primers were used to study TLB marker analysis. The primers selected were Ce1 A08, Ce1 F04, Ce1 CO3, Ce1 F12, Ce1 H12, uq 97- 256, uq 201- 302, uq 73-164, uq 115-71 , uq 88-94.

4.2.7. DENATURING PAGE FOR SSR ANALYSIS

As mentioned in the part, 4.1.3, denaturing PAGE was done to analyse the bands as it gives higher resolution than agarose gel electrophoresis. But it was found out that no SSR primers gave any specific band for resistant varieties. The SSR gel profiles are represented as plate 18-19.

4.2.7.2 SSR MARKERS ASSOCIATED WITH TLB

No specific bands were obtained while performing SSR marker study. The primers Ce1 A08, Ce1 F04 and Ce1 C03 failed to give any specific band in the respective product size, so they were not taken for the study and was not involved in the scoring.

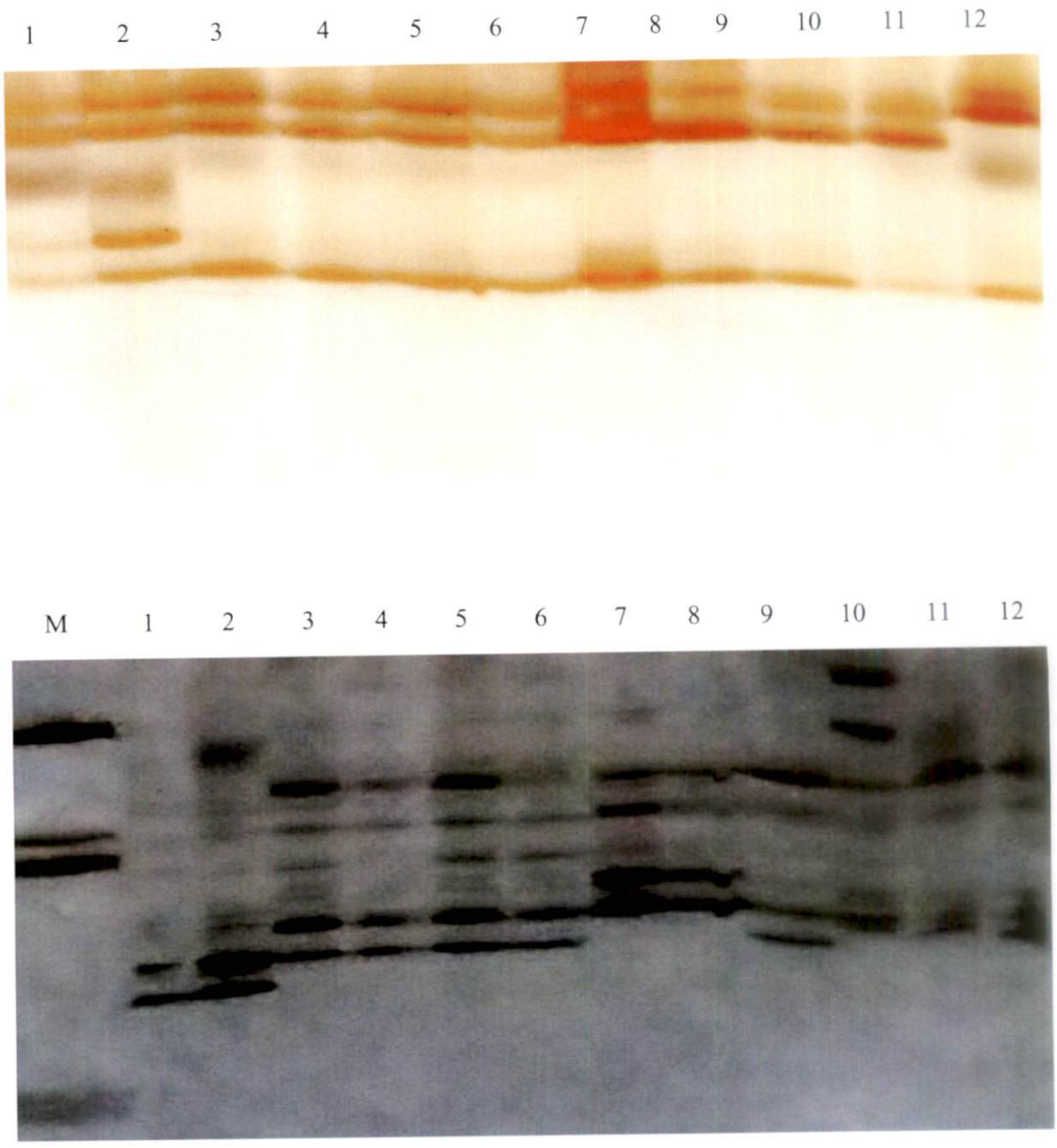


Plate 18: Denaturing Urea-Acrylamide (6%) gel profile of uq 88 and uq 97 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK. . In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.

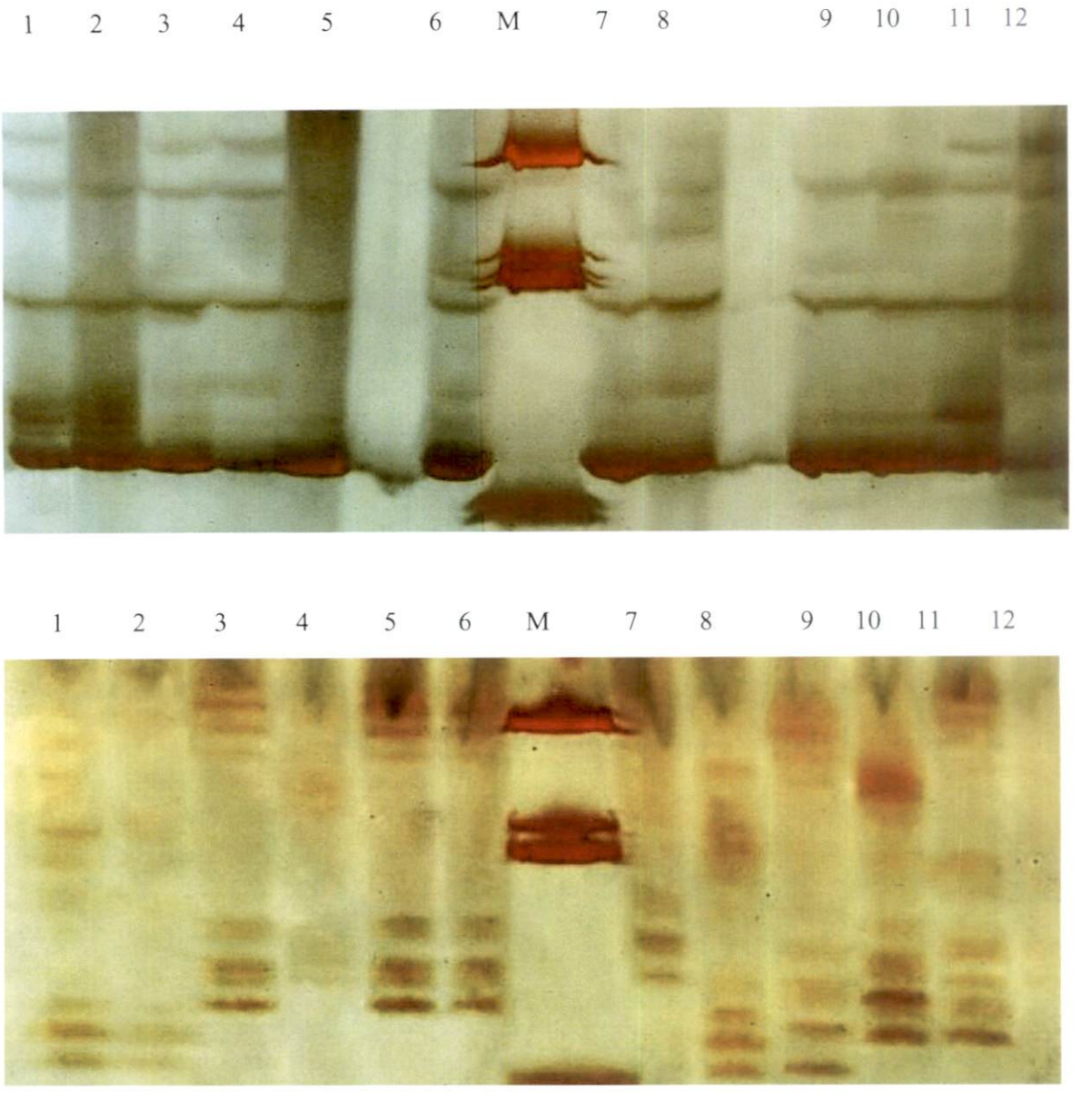


Plate 19: Denaturing Urea-Acrylamide (6%) gel profile of Ce1 H12 and F12 respectively.

1. SR 2. C-681 3. C-211 4. C-688 5. C-82 6. C-185 7. C-545 8. TCR 203 9. C-370 10. C-203 11. C-717 12. MUK. . In this, 1-6 comprises of susceptible varieties and 7-12 comprises of resistant varieties.

4.2. SIMILARITY INDEX

The similarity index values obtained for each pair wise comparison among the 12 taro accession based on 7 SSR marker data is given in the table 12. The similarity coefficient based on SSR markers ranged from 0.48 to 0.86. Most of the similarity coefficients ranges between 0.61 to 0.84. Among the 12 taro accessions, the lowest similarity index (0.48) was observed between Sree Rashmi and Muktakeshi, whereas, the highest similarity index (0.86) was observed between TCR 443 and C - 203 and along with C - 82 and C - 185 and C - 688 and TCR 545.

Table 12: Similarity coefficient of 12 taro accessions using 7 SSR markers

	Sree Rashmi	C 681	C 211	C 688	C 82	C 185	TCR 545	TCR 443	C 370	C 203	C 717	Muktakeshi
Sree Rashmi	1.00											
C 681	0.77	1.00										
C 211	0.64	0.68	1.00									
C 688	0.52	0.70	0.80	1.00								
C 82	0.59	0.68	0.82	0.84	1.00							
C 185	0.64	0.68	0.73	0.80	0.86	1.00						
TCR 545	0.57	0.61	0.66	0.86	0.75	0.80	1.00					
TCR 443	0.68	0.68	0.59	0.80	0.64	0.73	0.84	1.00				
C 370	0.70	0.66	0.66	0.73	0.75	0.80	0.82	0.84	1.00			
C 203	0.68	0.68	0.68	0.80	0.73	0.82	0.80	0.86	0.84	1.00		
C 717	0.52	0.61	0.57	0.64	0.61	0.66	0.55	0.66	0.59	0.75	1.00	
Muktakeshi	0.48	0.61	0.52	0.73	0.57	0.66	0.64	0.70	0.55	0.70	0.77	1.00

A dendrogram generated using UPGMA cluster analysis separated the 12 taro accessions into three major clusters (Fig. 3). The clusters, cluster I and cluster II and cluster III have around 67% similarity. Cluster I comprised of two accessions, Sree Rashmi and C- 681, whereas, Cluster II comprised six accessions, viz., C-211, C-688 C-82 C-185, TCR 545, TCR 443, C-370, C-203. Here, it is observed that the highly susceptible Sree Rashmi is clustering with another susceptible accession, C-681 and the tolerant Muktakeshi clusters with another tolerant line, C-717. However, the rest of the susceptible and tolerant accessions are grouped together in the cluster II. Accession C-211, a susceptible line forms an outlier with the cluster II. The dendrogram is analysis is obtained as fig 3.

Table 13: Details of 12 taro accessions grouping under the three clusters

Cluster	**Accessions**
Cluster I	Sree Rashmi, C 681
Cluster II	C-211, C-688 C-82 C-185, TCR 545, TCR 443, C-370, C-203
Cluster III	Muktakeshi, C-717

DISCUSSION

5. DISCUSSION

5.1 GENETIC DIVERSITY ANALYSIS

Taro (*Colocasia esculenta* L. Schott) is one of the major root crop belonging to the family *Araceae* and sub-family *Aroidea*. It's one of the earliest crop and is used as a major food in south Pacific and African countries. The molecular characterization of germplasm is a major factor in rationalizing the national collections and in establishing a core collection for the region, which will consist of a limited number of genotypes that are more accessible to regional breeding programs selected to represent the genetic diversity of the region (Mace *et al.*, 2002). Taro is believed to have had its origin in northeast India and so the probability of finding a high genetic diversity is more in India. So, in this study we have incorporated 30 taro accessions from Kerala, a Southern state of India, to analyse its diversity. If there is a high degree of diversity existing within a population, it can be utilized for further breeding experiments in order to widen its genetic base and to achieve more elite hybrid varieties. In addition, taro germplasm characterization using molecular markers will contribute to the knowledge of genetic relationships between accessions of wild and cultivated gene pool, and hence facilitate the breeding of taro cultivars to satisfy the market needs and respond to diverse biotic (e.g., taro leaf blight) and abiotic (e.g., drought and salinity) challenges (Sharma *et al.*, 2008).

Microsatellites have great potential in this respect, owing to their multi-allelic and co-dominant nature, which allows individuals to be uniquely genotyped (Mace *et al.*, 2002). High information content, definitive allele assignment, and rapid analysis by PCR make SSR markers well suited for both linkage mapping and genetic diversity studies in plants (Akkaya *et al.*, 1995; Plaschke *et al.*, 1995; Bell and Ecker, 1994; Saghai-Maroof *et al.*, 1994; Morgante *et al.*, 1994; Wu and Tanksley, 1993).

5.1.2. DNA ISOLATION PROTOCOL

To isolate pure and intact DNA from plant tissues, numerous protocols have been established (Maroof *et al.*, 1984; Doyle and Doyle, 1990; Scott and Playford, 1996; Sharma *et al.*, 2000: Haymes, 1996). But it can vary with plant species, thus, these DNA extraction protocols cannot be reproduced for all plant species (Porebski *et al.*, 1997). Mucilage, a highly viscous secondary metabolite composed of a polar polymer of glycoprotein is present in tubers, seeds, and stems of taro, which co-precipitates with DNA and inhibits the action of Taq polymerase and reduce its purity (Jose and Usha, 2000; Ghosh *et al.*, 2009). The cetyl trimethylammonium bromide (CTAB) method and its modifications have been used to obtain good quality total DNA for polymerase chain reaction (PCR). CTAB is considered to be the best method to isolate the DNA. Modifications in CTAB may enable it to fulfil the quality and quantity requirements of PCR reactions (Tiwari *et al.*, 2012). High concentrations of NaCl, Ethylene Diamine Tetra Acetic acid (EDTA) and mercaptoethanol enhanced the CTAB extraction and purification activity. As additional quantities of NaCl and mercaptoethanol enhanced the DNA extraction and proteins degradation respectively, while increased concentration of EDTA protected DNA. Considering this, a method standardized by Sharma *et al.*, (2008) with slight modifications made by Vinutha (2014) was chosen for the study. A purity range of 1.65-2.00 was obtained, however, for the majority of the DNA samples, the purity ranged from 1.8-2.00 which was considered as a good quality DNA. Typically A260/280 absorbance ratios of the DNA ranging from 1.8-2.0 indicated that little or no protein contamination has occurred (Schultz *et al.*, 1994). The DNeasy kit (Qiagen) also gave a good quality DNA in the range of 1.8-2.0. The kit also uses the above reagents for isolation of DNA. Here, the main advantage was the time saved as the kit method took only 2 hrs to complete. Considering this added advantage, kits are now being commonly used in many experiments.

5.1.3. PCR STANDARDIZATION AND PRIMER SCREENING

We chose SSR markers for studying genetic diversity in taro accessions from Kerala because SSR markers have been applied to analyse genetic diversity (Cho *et al.*, 2000; Harrington, 2000). SSRs can be used as highly informative markers for genome mapping and gene tagging (Ratnaparkhe, 1998). SSR is a single locus amplifying marker and more alleles can be detected at a single locus with microsatellites ie., more than with other molecular markers presently used, and this results in an average index of similarity between individuals which is generally much lower (Noyer *et al., 2003*). The development of molecular markers as a tool for taro germplasm characterization and early progeny selection is highly desirable for developing an efficient breeding program to speed the integration of new genetic material into elite germplasm (Mace *et al.*, 2003).

The PCR condition already standardized at the molecular biology lab of ICAR-CTCRI was utilized for PCR amplification. It is critical to optimize the reaction conditions prior to large-scale application of each locus, such as phylogenetic or genetic diversity studies (Narina, *et al.*, 2011). 20ng/µl DNA for SSR reaction mixture was also standardized for one reaction. Higher concentrations of DNA can hinder the amplification process because high amount of DNA can contain more amount of polyphenols which can interact with Taq polymerase. According to Ahmed, *et al.*, (2009), too much template DNA may decrease PCR efficiency due to contaminants in DNA preparations. 0.5mM $MgCl_2$ was used in the study which was optimum and gave good amplification profile. Increased Mg^{+2} concentration enhances Taq. activity up to a certain limit, above which it may act as a depressant of it (Kramer *et al.*, 2004). The SSR primers selected for genetic diversity analysis are Cel H12, Cel A06, Cel B03, Cel F04, Cel C03, Cel C06, uq 97- 256, uq 201- 302, uq 84-207, uq 73-164 because they detected distinct and polymorphic bands, whereas, the eliminated primers showed monomorphic amplification. The development of an ideal molecular DNA marker system which is genetically co-dominant and highly polymorphic allows precise discrimination

even of closely related individuals and is important due to the high levels of heterozygosity in individual genotypes (Elizabeth, *et al.*, 2003).

5.1.4. DENATURING PAGE FOR SSR PRODUCT SEPERATION

For resolving the PCR products denaturing gel electrophoresis was done because the resolution of acrylamide gels compared to agarose gels, could result in the detection of a larger number of bands per locus. This is of particular concern for SSR loci containing dinucleotide repeats whose amplification products which differ by 2 bp could not be resolved using agarose gels (Senior *et al.*, 1998). In our study, stutter bands were also visible with the actual bands after staining with silver nitrate. However, only bands of the expected product size was considered for scoring in some previous studies (Nunes *et al*, 2012, Rallo *et al.* 2000). A marker was deemed suitable when a single locus amplified efficiently at the expected size and with a minimum of stutter bands when assayed on silver-stained denaturing polyacrylamide gels (Echt *et al.*, 1996). Length of flanking sequences correlate positively with the shadow band contribution and the possibility of stutter bands generation is increased with the use of low DNA template concentration (Olejniczak *et al.*, 2006). DNA replication slippage can also occur during *in vitro* amplification of the SSRs to produce 'stutter bands', which often obscure non-parental SSR bands in genetically segregating populations (Ellegren, 2004). In this study, though stutter bands were obtained, they were eliminated and the bands in the range of its product size was only considered.

5.1.5. POLYMORPHIC INFORMATION CONTENT

The highest PIC value was obtained for the primer uq 97-256 with a value of 0.857 and high heterozygosity value of 0.871. Nunes *et al.*, 2012 investigated the genetic diversity of seven regional core collections of Brazilian taro using seven microsatellite loci. The polymorphism information content (PIC) ranged from 0.75 to 0.91, and the polymorphism observed in this study was able to differentiate between the taro cultivars and showed a high polymorphism. This study is thereby in accordance with the values attained by Nunes. The polymorphic alleles per locus

obtained in the study ranged from 1.9 to 4.6 which is also comparable with the data observed by Nunes. Taro from lake Victoria basin Macharia *et al.*, 2014, showed a hetrozygosity value of 0.248 which signifies a low genetic base in the area. The variability has been indicated in diversity studies based on quantitative traits (Ivancic *et al.*, 1995), isozyme variations (Lebot and Aradhya, 1991), variations in ribosomal and mitochondrial DNA restriction sites (Matthews, 1990), Randomly Amplified Polymorphic DNA (Irwin *et al.*, 1998), Amplified Fragment Length Polymorphisms (AFLP) (van Eck *et al.*, 1998) and microsatellites (Mace and Godwin, 2000). Thus it proves that SSR markers can be used for genetic diversity analysis of taro and show enough polymorphism to differentiate the accessions. This is possible because the complementary flanking regions to the primers are frequently conserved within the same species or among species and correlated genera, although the microsatellite regions are subject to high mutation rates (Hopkins *et al.*, 1999).

5.1.6 DENDROGRAM ANALYSIS

Based on the scoring data, a dendrogram tree was constructed using R software. It grouped the genotypes into 3 main clusters and one outlier. The third cluster is further subdivided into four sub clusters and the second sub cluster had an outlier C-273. But no congruence was seen between accession grouping and geographical origin. The results agrees with the result published by Lakahanpaul *et al.*, (2003), where, clustering pattern did not show any strict relationship between geographical distribution and genotypic diversity as the accessions from different geographic regions were placed in nearly all the clusters. Macharia *et al.*, (2014), also got similar results, where they found no distinguishable pattern of the clustering groups either as dasheen or eddoe or in accordance with its geographical origin. Isozymes and RAPD studies performed by Sharma *et al.*, (2008), also showed no similarity between the clusters and the geographical origin. The previous study by Vinutha (2014), where taro accessions from the Centre of Origin was studied, no accessions were grouped based on their geographical similarities in both molecular and morphological clustering. It should be noted that taro inhabits

diverse environments. Geographically close habitats can be ecologically quite different and conversely, habitats that are geographically distant from one another can be very similar in their environmental conditions.

5.2. MOLECULAR MARKERS ASSOCIATED WITH LEAF BLIGHT RESISTANCE IN TARO

Taro is being replaced in many countries by other tuber crops such as sweet potatoes and cassava largely due to constrain of pests and disease, which are becoming a major limiting factor for taro production (Ivancic, 1992). TLB constitutes a significant threat to food security and economy in those Pacific Island countries which do not have resistant varieties and where taro is a major staple and an export commodity (Sharma *et al.*, 2008). Leaf blight has become a limiting factor for taro production in all taro growing-countries including India causing yield loss of 25-30 % (Thankappan, 1985). There are studies going around the world to develop resistant varieties to taro leaf blight. But no studies have yet been reported to identify markers associated to TLB disease with SSR and ISSR primers. The one study done till date was by Sharma *et al.*, 2008 by employing AFLP markers. It was identified that major constraints for taro breeding programs including TLB resistance breeding are the lack of knowledge of the genetic diversity in the cultivars, the limitations in access to and knowledge of additional sources of disease resistance as well as the absence of information on the potential agronomic and processing value of genotypes (Ivancic, *et al.*, 2000). The development of molecular techniques, in particular the use of molecular markers have been used to identify markers linked to useful traits and made great advances in recent years (Sharma *et al.*, 2008).

The varieties Sree Reshmi and Muktakeshi which are highly susceptible and tolerant respectively, were taken for the study. For breeding resistant varieties, the germplasm was screened for TLB resistance. Six of the tolerant lines identified were used along with six susceptible ones for this study. The long-term strategy for the management of taro leaf blight is the breeding of more resistant varieties which,

together with the introduction of resistant exotic varieties, is the most sustainable approach in managing the disease (Hunter *et al.*, 1993). For the present study, both SSR and ISSR markers were employed.

In a previous study done at ICAR-CTCRI, Sharma *et al.*, (2008), examined the genetic differentiation of 14 cultivars of taro including 4 highly susceptible, 6 susceptible, 2 moderately resistant and 2 highly resistant varieties including Muktakeshi and performed molecular analysis with respect to the resistance of different genotypes to leaf blight. It indicated that the ALFP generated bands at approximately 75bp were only detected in the highly resistant genotype but the band was absent in moderately resistant varieties and he concluded that there is more than one gene involved in taro leaf blight resistance. In our study we used 6 susceptible and 6 resistant varieties by employing 13 ISSR markers and 10 SSR markers.

5.2.1. ISSR MARKER STUDY

ISSR primers were used to find out molecular markers associated with leaf blight resistance in taro. The primers selected for the molecular screening for developing markers associated with TLB were UBC 841, UBC 848, UBC 810, UBC 808, UBC 817, UBC 847, UBC 871, UBC 827, UBC 857, UBC 825, UBC 809, $(GA)_9AC$ and $(AG)_9AT$. Ratnaparkhe *et al.*, (1998) were the first to demonstrate that ISSR markers are useful in gene tagging and can be used for finding markers linked to the gene of interest. They had found out that the simple sequence repeat $(AC)_8YT$ when used directly in a PCR reaction, amplifies a marker, UBC-855, which is linked to the gene for resistance to *fusarium* wilt race 4. The repeat $(AC)_8T$ amplified a marker UBC-825 which was located 5.0 cM from the gene for resistance to *fusarium* wilt race 4, which was closer than the UBC-855 and CS-27 markers (Mayer *et al.*, 1997; Ratnaparkhe *et al.*, 1998). In soybeans, Lohnes *et al.* (1993) reported that two resistance genes (*Rmd* and *Rps2*) were closely linked to each other, and to a non-nodulation gene (*Rj2*) was characterized using ISSR. In lettuce, ISSR markers were used to identify the 13 resistance genes for

downy mildew and have been mapped in four clusters (Hulbert *et al.*, 1985). In the present study, the primers UBC 827, UBC 817and UBC 808 gave bands in 5 resistant varieties in the region, 598, 675 and 667 bp, respectively. UBC 825 and $(AG)_9AC$ gave an extra band in all the resistant varieties in 685 bp and 808 bp regions, respectively. This analysis is important because this marker if validated, can be utilized for further breeding programs to screen for resistant progeny at a very early stage itself and thus speedup the process of developing varieties which are resistant to TLB. The clustering of resistance genes at a specific chromosomal region is advantageous in a breeding program, as this block of genes can be transferred to an adapted background via backcrossing, and the desired trait can be selected using the ISSR markers (Ratnaparkhe, 1998).

5.2.2. CLUSTER ANALYSIS

The cluster analysis in the present study utilizing ISSR markers proved to be a successful one with the formation of 2 clusters. The first cluster consisted of susceptible varieties viz, Sree Rashmi, C-681, C-211, C-688 C-82 C-185 and the second cluster comprised of the resistant varieties TCR 545, TCR 443, C-370, C-203, C-717, Muktakeshi. Though the studies done in this aspect was a preliminary one, we got specific clustering and specific bands for the resistant ones. This varieties can be further screened and the level of degree of resistance needs to be ascertained before it can be utilized for breeding purpose. Nevertheless, hybridizations between accessions of these two different groups might give a heterotic response. The remarkably high level of polymorphism around the disease resistance gene cluster indicates an association between the molecular mechanism of disease resistance and rapid sequence divergence in plants (Sudupak *et al.*, 1993; Yu *et al.*, 1996). Similar to the application of the marker assisted selection (MAS) using DNA markers tightly linked to wilt resistance genes which was used to screen a large number of germplasm lines for the presence of these genes without actually subjecting them to the pathogen and to pyramid them into agronomically superior varieties (Padaliya *et al.*, 2013), the present result could also pave way for use of these ISSR markers after proper validation in MAS applications in future.

5.2.3. SSR MARKER STUDY

Studies on disease resistance genes have indicated a high level of polymorphism and the presence of SSRs at certain loci for crops like maize, where resistance genes to rust were found in five clusters (Yu *et al.*, 1996). Disease resistance genes have been found to form clusters in crops like flax, lettuce (Sheperd and Mayo, 1972; Islam *et al.*, 1993; Kesseli *et al.*, 1993). However, finding SSR markers linked to disease resistance genes is very expensive and involves screening the library, sequencing the clone and synthesizing the primers. This has prevented the broad use of microsatellites in plants (Ratnaparkhe, 1998). In the present study, previously identified SSR makers were used among which primers Ce1 A08, Ce1 F04 and Ce1 C03 didn't give any specific band in the respective product size, so they were not taken for the study and was not involved in the scoring. The primers chosen for the study were Ce1 H12, Ce1 F12, uq 201- 302, uq 97- 224, uq 73-164, uq 115- 71 and uq 88-94 since they gave polymorphic bands. However, no specific abnd could be obtained associated with TLB resistance. RAPD markers were used by Gygax, *et al.*, (2004) on apple scab resistance gene *Vbj* derived from *Malus baccata jackii*. He quoted that breeding for scab-resistant apple cultivars by pyramiding several resistance genes in the same genetic background is a promising way to control apple scab caused by the fungus *Venturia inaequalis*. After further studies, a similar type of marker system could be developed and employed in taro to breed new varieties where resistance to TLB could be confered by pyramiding several resistance genes. Gygax found out three RAPD markers linked to *Vbj*. SSR analysis of cultivated groundnut germplasm resistant to rust and late leaf spot diseases was done by Mace, (2006) by employing SSR markers. Negi, (2000) identified AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection. Such studies are being done worldwide to find out markers linked to disease resistance gene and to incorporate this genes into new varieties by breeding. Though in this study, no specific bands were obtained in the respective product size, further studies will be done to identify the marker by incorporating more number of markers and genotypes.

5.2.4. CLUSTER ANALYSIS

Three clusters were obtained while mapping dendrogram using NTsys software. The first cluster consisted of the highly susceptible variety Sree Reshmi along with C-681 which points out that C-681 also show high susceptibility towards TLB which was similar to that of ISSR study. The second cluster showed a mixed pattern which clustered both susceptible and resistant groups together. The third cluster grouped the resistant variety Muktakeshi and C-717 together. Further studies has to be done to analyse their relation, as these accessions were also grouped together in ISSR studies. Similar results were also obtained while studying chickpea genotypes for wilt resistance by Padaliya *et al.*, (2013). He found out that the moderately resistant genotype Chaffa was grouped in a separate cluster while resistant genotype WR-315 was grouped in a sub-cluster of cluster-I. Moreover, another resistant and tolerant genotype such as, ICCV-2 and GG-1 was grouped in the same sub-cluster of cluster-II, the susceptible genotypes GG-4 and JG-62 were present in one cluster.

It is difficult to pyramid two or more disease resistance genes through conventional means, particularly where the resistance genes in question are effective against all the prevalent pathotypes. However, recent advances in molecular biology has made it possible to pyramid several genes in single line using marker assisted selection (MAS) and tagging of genes with molecular markers is pre-requisite for MAS (Dhillon *et al.*, 2011).

The future strategy should be to develop new elite taro varieties in along with enrichment of the germplasm from different regions to further diversify the existing germplasm diversity and conserve taro collection. Use of this germplasm for crop improvement needs will strengthen the capacities of the national taro breeding programmes and to develop high disease resistant varieties.

SUMMARY

6. SUMMARY

The project titled "Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)" was carried out at the Division of Crop improvement, ICAR-CTCRI, Sreekariyam, during 2014-15. The objective of the study was to assess the genetic diversity among taro accessions from Kerala, using SSR markers and to identify molecular markers associated with leaf blight resistance in taro by SSR and ISSR markers. 30 accessions maintained at ICAR-CTCRI which was collected from different districts of Kerala were used for genetic diversity analysis. 12 genotypes, 6 each of both susceptible and resistant varieties screened in the fields of ICAR-CTCRI for TLB was employed for the study to identify molecular markers associated with leaf blight resistance in taro.

DNA of all the 30 accessions were isolated by using two methods- the method standardized by Doyle and Doyle, (1987) and later by Sharma *et al.*, (2008), which was modified slightly by Vinutha (2014). The other method used for the study was the kit method - DNeasy Plant Mini Kit (Qiagen). Purity of most of the DNA varied from 1.7-2.0.

PCR condition already standardized at the molecular biology laboratory, Division of Crop Improvement, ICAR-CTCRI was used for SSR-PCR amplification. The annealing temperature of the PCR condition was optimized at 56°C. Ten primers which showed polymorphic bands were selected for the study based on its agarose gel profile. The primers selected for genetic diversity analysis were Cel H12, Cel A06, Cel B03, Cel F04, Cel C03, Cel C06, uq 97- 256, uq 201- 302, uq 84-207 and uq 73-164.

Denaturing PAGE of 6% acrylamide concentration was prepared to separate the PCR products. The apparatus was electrophoresed for 45 minutes at 100W. The amplified products were observed by staining with silver nitrate. Binary scoring of 1, 0 was tabulated in an excel sheet and was subjected to R-package and PIC program to quantify the diversity of the genotypes.

All the primers gave polymorphic bands and showed high allelic frequency than their respective agarose gels. By calculating polymorphic information content, the highest heterozigosity was obtained for the primer uq 97-256 with a value of 0.871 and the lowest was obtained for the primer uq 84-207 with a value of 0.694. Number of alleles per locus ranged from 1.9-4.56 with the maximum alleles shown by uq 97-256 and the minimum showed by uq 84-207 and uq 201-302.

For the analysis of genetic variation among 30 Kerala accessions, a dendrogram was constructed for phylogenetic analysis and the first cluster of the tree comprised of the accessions from C-406 to TCR 450. The second cluster consists of C-218 to C-384 and the third large cluster groups most of the genotypes ranging from TCR 424 to TCR-296 and ADS2014-20 has formed an outlier. The third cluster is further subdivided into four sub-clusters and the second sub-cluster consists of major number of genoytpes and has an outlier C-273.The analysis found no correlation between geographical origin of the accessions and the cluster grouping. The accessions in the different clusters can be included as parents in breeding programs depending upon traits of interest possessed by them in the future as they have a wide genetic base.

The other study conducted was to identify molecular markers associated with leaf blight resistance in taro by employing two marker systems - SSR and ISSR markers. The DNA was isolated by the same methods as done for the genetic diversity study. 13 ISSR markers were selected for the study which gave good amplification profile in agarose gels. PCR conditions already standardized in the ICAR-CTCRI molecular biology lab with an annealing temperature of 56°C was employed for the study. The primers selected for the TLB screening were UBC 841, UBC 848, UBC 810, UBC 808, UBC 817, UBC 847, UBC 871, UBC 827, UBC 857, UBC 825, UBC 809, $(GA)_9AC$ and $(AG)_9AT$. Out of the 13 primers, 5 of them gave an extra band for resistant varieties. UBC 825 and $(AG)_9AC$ gave an extra band in all the resistant varieties in 685 bp and 808 bp regions, respectively. The primers UBC 827, UBC 817 and UBC 808 gave bands in 5 resistant varieties in the regions, 598, 675 and 667 bp, respectively.

Analysis of the binary data using NTSYS pc, a dendrogram was constructed where all the susceptible varieties clustered together into cluster I and all the resistant varieties into cluster II. A similarity matrix generated using NTSys showed a similarity index ranging from 0.42 to 0.93. The lowest similarity index (0.42) was observed between Muktakeshi and Sree Rashmi, whereas, the highest similarity index (0.93) was observed between C-211 and C-688 along with C-688 and C-82.

SSR markers were also employed to study the marker associated with TLB resistance. The PCR conditions employed in the genetic diversity analysis was used to amplify the PCR products. The primers selected were Ce1 A08, Ce1 F04, Ce1 CO3, Ce1 F12, Ce1 H12, uq 97- 256, uq 201- 302, uq 73-164, uq 115-71 and uq 88-94. The products were then subjected to 6% acrylamide (denaturing urea gel) for its separation. But no specific bands were obtained in the range of its expected product size. The primers Ce1 A08, Ce1 F04 and Ce1 C03 didn't give any specific band in the respective product size, so they were eliminated from the study.

A dendrogram generated using UPGMA cluster analysis separated the 12 taro accessions into three major clusters. Here, it was observed that the highly susceptible Sree Rashmi was clustered with another susceptible accession, C 681 and the tolerant Muktakeshi clustered with a tolerant line, C 717. However, the rest of the susceptible and tolerant accessions were grouped together in the cluster II. Accession C 211, a susceptible line formed an outlier with the cluster II. The similarity coefficients based on SSR markers ranged from 0.48 to 0.86. The lowest similarity index (0.48) was observed between Sree Rashmi and Muktakeshi, whereas, the highest similarity index (0.86) was observed between TCR 443 and C 203 along with C 82 and C 185 as well as C 688 and TCR 545.

Further studies have to done by employing more accessions and primers to screen for markers associated with the TLB resistant gene to arrive at a definite consensus.

REFERENCES

REFERENCE

Abraham, A. and Ramachandran, K. 1960. Growing *Colocasia* embryos in culture. *Curr. Sci.* 29: 342-343.

Acevedo-Rodríguez. P., Strong, M. T., 2005. Monocots and Gymnosperms of Puerto Rico and the Virgin Islands. Contributions from the United States National Herbarium 52:415.

Adhikari, S., Saha, S., Bandyopadhyay, T. K., and Ghosh, P. Identification and Validation of a New Male Sex-Specific ISSR Marker in Pointed Gourd (*Trichosanthes dioica Roxb.*). *The Sci. World J.* 10: 1155-1161.

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

Ahmed, I., Matthews, P. J., Biggs, P. J. M., Naeem, M., McLenachan, P. A. and Lockhart, P. J. 2013. Identification of chloroplast genome loci suitable for high-resolution phylogeographic studies of *Colocasia esculenta* (L.) Schott (Araceae) and closely related taxa. *Mol. Ecol. Resour.* 13:929-937.

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.

Akkaya, M. S., Bhagwat, A. A., Cregan, P. B. 1992. Length polymorphisms of simple-sequence repeat DNA in soybean. *Genetics* 132: 1131-1139.

Akkaya, M. S., Shoemaker, R. C., Specht, J. E., Bhagwat, A. A., and Cregan, P. B. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Sci.* 35: 1439-1445.

Ashkanil, S. Rafii, M. Y., Sariah, M., Siti, A., Akmar, N., Rusli, I., Rahim, H. and Latil. M. A. 2011. Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genet. Mol. Res.* 10 (3): 1345-1355.

Bachman, M. S., Tamulonis, J. P., Nickell, C. D. and Bent, A. F. 2001. Molecular Markers Linked to Brown Stem Rot Resistance Genes, Rbs1 and Rbs2, in Soybean. *Crop Sci.* 41:527-535.

Bandyopadhyay, R., Sharma, K., Onyeka, T. J., Aregbesola, A., Kumar, P. L. 2011. First report of taro (*Colocasia esculenta*) leaf blight caused by *Phytophthora colocasiae* in Nigeria. *Plant Dis.* 95-618.

Barrau, J. 1965. L'humideet le sec. *J. Polyn. Soc.* 74: 329-346.

Barth, F. 1975. Rituals and knowledge among the Baktaman of New Guinea. Yale University Press, New Haven, pp. 292.

Bayliss-Smith, T. P. 1982. Taro (Colocasia esculenta) in the New Guinea highlands. In: Bourke, R. M. and Kesavan, V. (eds). *Proceedings of the Second Papua New Guinea Food Crops Conference*, Department of Primary Industry, Port Moresby. pp. 134-147.

Bayliss-Smith, T. P. 1985. Pre-ipomoean agriculture in the New Guinea highland above 2000 metres: some experimental data on taro cultivation. In: Farrington, E. S. (ed.). *Prehistoric Subsistence Agriculture in the Tropics*, BAR International Series 232, Oxford. pp. 285-320.

Bell, C. J. and Ecker, J. R. 1994. Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics* 19: 137-144.

Bourke, R. M. and Vlassak, R. 2004. Estimates of Food Crop Production in Papua New Guinea. Australian National University, Canberra, Australia.

Bradbury, H. J. and Holloway, W. D. 1988. Chemistry of tropical root crops. ACIAR. Canberra. 201 pp.

Brunt, J., Hunter, D. and Delp, C. 2001. A bibliography of taro leaf blight. Noumea, New Caledonia: Secretariat of the Pacific Community. 93 p.

Caillon , S., Quero-Garcia, J., Lescure, J.P. and Lebot, V. 2006. Nature of taro (*Colocasia esculenta* (L.) Schott) genetic diversity prevalent in a Pacific Ocean island, Vanua Lava, Vanuatu. *Genet. Resour. Crop Evol.* 53(6): 1273-1289.

Chen, M., Zhao, Z. Chen, L., Zhou, F., Zhong, Z., Jiang, L. and Wan, J. 2013. Genetic analysis and fine mapping of a semi-dwarf gene in a centromeric region in rice (*Oryza sativa* L.). *Breed Sci.* 63(2): 164-168.

Cho, M., Ishii, Y. G., Temnykh, S., Chen, X. 2000. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 100: 713-722.

Clarke, W. C. 1977. A change of subsistence staple in pre-historic Papua New Guinea. In: Leaky, C. L. A. (ed.), *Proceedings of the Third International Symposium on Tropical Root Crops*, International Society for Tropical Root Crops and International Institute for Tropical Agriculture, Ibadan. pp. 159-163.

Coates, D. J., Yen, D. E. and Gaffey, P. M. 1988. Chromosome variation in taro, *Colocasia esculenta*: Implications for the origin in the Pacific. *Cytologia* 53: 551-560.

Collard, B. C. Y, Jahufer, M. Z. Z., Brouwer, J. B., Pang, E. C. K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts. *Euphytica* 142:169-196.

Condit, R. Hubbell, S. P. 1991. Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome* 34(1): 66-71.

Daoura, B. G., Chen, L., Du, Y. and Hu, Y. G. 2014. Genetic effects of dwarfing gene Rht-5 on agronomic traits in common wheat (*Triticum aestivum L.*) and QTL analysis on its linked traits. *Field Crop. Res.* 156: 22-29.

Darkwa, S. and Darkwa, A. A. 2013. TARO "*Colocasia esculenta*": It's Utilization in Food Products in Ghana. *J. Food Process. Technol.* 4: 225-432.

Dhillon, N. K. and Dhaliwal, H. S. 2011. Identification of Molecular Markers Linked to Leaf Rust Resistance Genes in Wheat and Their Detection in the Local Near-Isogenic Line. *Am. J. Plant Sci.* 2: 433-437.

Diegues, A. C. and Arruda, R. S. V. 2010. In: Wagner W. L., Herbst, D.R. and Sohmer, S.H. (eds.) *Saberes tradicionais e biodiversidade no Brasil. Brasília: Manual of the flowering plants of Hawaii.* Honolulu, Hawai'i, Bishop Museum Press, USA. 1919 pp.

Divashuk, M. G., Vasilyev, A. V., Bespalova, L. A., Karlov, G. I. 2012. Identity of the Rht-11 and Rht-B1ereduced plant height genes. *Rus. J. Genet.* (7): 761-763.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Plant Mol. Biol. Rep.* 20:161-175.

Deo, C. P., Tyagi, T. P., Taylor, M., Becker, D. K. and Harding. 2009. Improving taro (*Colocasia esculenta* var. esculenta) production using biotechnological approaches. *S. Pacif. J. Nat. Sci.* 27: 6-13.

Dwivedi, N. K., Asha, K. I., Asokan, N. R., Indiradevi, A. and Suma, A. 2013. Collection and Conservation of Genetic Resources of Tropical Root and Tuber Crops for Sustainable Livelihood – A Status Report. *J. Root crops.* 39(2): 21-28.

Echt, C. S., Yay-Matquardt, P., Hseih, M. and Zahorchak, R. 1996. Characterization of microsatellite markers in eastern white pine. *Genome* 40: 9-17.

Edison, S., Sreekumari, M. T., Santha, V. P. And Sheela, M. N. Diversity and genetic resources of taro in India. In: Guarino, L., Taylor, M. and Osborn, T. (eds.), *Proceedings of Third Taro Symposium.* Secretariat of the Pacific Community, Nadi, Fiji Islands, pp. 85-88.

Elizabeth, S. J., Leonie, J. H., Michelle, C. D., Michael, T. A. and Terry, P.T. 2003. An SSR and AFLP molecular marker- based genetic map of white clover (*Trifolium repens* L.). *Plant Sci.*165: 531-539.

Ellegren, H. 2004. Microsatellites: Simple sequences with complex evolution. *Nat. Rev. Genet.* 5:435-445.

Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzeke, G. J., Richards, R. A., 2002. Perfect markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. *Theor. Appl.Genet.* 105: 1038-1042.

FAOSTAT [Food and Agriculture Organization Statistical Database]. 2010. [online] Available :http://www.ibb.unesp.br/servicos/publicacoes/rbpm/pdf_v7_n1_2005/artigo_11_v7_n1.pdf.

Figliuolo, G. and Perrino, P. 2004. Genetic diversity and intra-specific phylogeny of *Triticum turgidum* L. subsp. dicoccon (Schrank) Thell. revealed by RFLPs and SSRs. *Genet. Resour. Crop Evol.* 51: 519-527.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McGraw, S. L.,Gurr, S. J. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186-194.

Ghani, F. D. 1979. The status of Keladi china (*Colocasia esculenta*) cultivation in peninsular Malaysia. *IFS Provisional Rep.* 5: 35-54.

Ghani, F. D. 1979. The status of Keladi china (*Colocasia esculenta*) cultivation in peninsular Malaysia. *IFS Provisional Rep.* 5: 35-54.

Ghosh, R., Paul, S., Ghosh, S. K. and Roy, A. 2009. An improved method of DNA isolation suitable for PCR-based detection of *begomo* viruses from jute and other mucilaginous plants. *J. Virol. Met.* 159:34-39.

Godwin, I. D., Aitken, E. A. B., and Smith, L. W. 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis* 18: 1524-1528.

Gollifer, D. E and Brown, J. F. 1974. *Phytophthora* leaf blight of *Colocasia esculenta* in the British Solomon Islands. *Papua New Guinea Agric. J.* 25: 6-11.

Golson, J. 1977. The making of the New Guinea Highlands. In: Winslow, J. H. (ed.), The Melanesian Environment, The Australian National University Press, Canberra. pp: 45-56.

Graham, K. M. 1965. FAO Plant Protection Committee for SE Asia and Pacific Region. *Quarterly Newsl.* 8-6.

Griffin, G. J. L. 1982. Potential applications of taro starch. Paper presented at the Regional Meeting on Edible Aroids, Suva, Fiji.

Guman, Z. and Dongxiao, Z. 1990. The relationship between geographic distribution and ploidy level of taro, *Colocasia esculenta*. *Euphytica* 47: 25-27.

Gupta, P. K, Rustgi, S. 2004. Molecular markers from the transcribed /expressed region of the genome in higher plants. *Funct. Int. Gen.* 4:139-162.

Gupta, H. S., Raman, B., Agrawal, P. K., Mahajan, V., Hossain, F., Thirunavukkarasu, N. 2013. Accelerated development of quality protein maize hybrid through marker-assisted introgression ofopaque-2 allele. *Plant Breeding* 132: 77–82.

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.

Gurr, P. 1993. The taro leaf blight in American Samoa. Proceedings of Taro Leaf Blight Seminar, Alafua Campus, USP, Samoa, pp 35-38.

Gygax, M., Gianfranceschi, L., Liebhard, R., Kellerhals, M., Gessler, C. and Patocchi, A. 2004. Molecular markers linked to the apple scab resistance gene Vbj derived from *Malus baccata jackii*. *Theor. Appl. Genet.* 109: 1702-1709.

Han, Z. G., Guo, W. Z., Song, X. L. and Zhang, T. Z. 2004. Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboretum* in allotetraploid cotton. *Mol. Gen. Genomics* 272:308-327.

Hanson, J. and Imamuddin, H. 1983. Germination of *Colocasia gigantean*. Hook. In: Shideler, F. S. and Rincon, H. (eds). Paper presented at the Proceedings of the 6th Symposium of the International Society for Tropical Root Crops, Peru. pp. 187-188.

Harrington, S. 2000. A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers. MS thesis Cornell University, Ithaca.

Haymes, K. M. 1996. Mini-prep method suitable for a plant breeding program. *Plant Mol. Biol. Rep.* 14:280-284.

Ho, H. H., Hu, Y. N., Zhuang, W. Y. and Liang, Z. R. 1983. Mating types of heterothallic species of *Phytophthora* in China. I. *Acta Mycol. Sin.* 2(3): 187-191.

Hopkins, M., Mitchell, S., Casa, A., Wang, T., Dean, R., Kochert, G. and Kresovich, S. 1999. Discovery and Characterization Of Polymorphic Simple Sequence Repeats (SSR) in Peanut. *Crop Sci.* 39: 1243-1247.

Huang, J. and Sun, S. M. 2000. Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theor. Appl. Genet.* 100: 1050-1060.

Huiping, B., Aileni1, M. and Zhang, P. 2010. Evaluation of cassava varieties for cassava mosaic disease resistance jointly by agro-inoculation screening and molecular markers. *Afr. J. Plant Sci.* 4(9): 330-338.

Hulbert, S. H. and Michelmore, R .1985. Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae Regal*) in lettuce (*Actuca sativa L.*). *Theor. Appl. Genet.* 70: 520-528.

Hunter, D. G. and Pouono, K. 1998. Evaluation of exotic taro cultivars for resistance to taro leaf blight, yield and quality in Samoa. *J. of South Pacific Agr.* 5: 39-43.

IPGRI/CIP. 2003. Descriptores del Ulluco (Ullucus tuberosus). Instituto internacional de Recursos Fitogenéticos, Roma, Italia; Centro Internacional de la Papa, Lima, Peru.

Irwin, S. V., Kaufusi, P., Banks, K., de la Peña, R. and Cho, J. J. 1998. Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica* 99: 183-189.

Islam, M. R., Sheperd, K. W. and Mayo, G. M. E. 1993. Recombination among genes at the , group in flax conferring resistance to rust. *Theor. Appl. Genet.* 77: 540-546.

Ivancic, A. 1992. Breeding and genetics of taro (*Colocasia esculenta* (L.) Schott. Ministry of Agriculture and Lands, Solomon Islands UNDP, Food and Agriculture Organizations of the United Nations. pp. 97.

Ivancic, A. and Lebot, V. 2000. The Genetics and Breeding of Taro. CIRAD Publication Series, Montpellier, France, 194p.

Jackson, G. V. H., Gollifer, D. E. and Newhook, F. J. 1980. Studies on the taro leaf blight fungus *Phytophthora colocasiae* in the Solomon Islands: control by fungicides and spacing. *Ann. Appl. Biol.* 96: 1-10.

Jackson, G. V. H. 1999. Taro Leaf Blight. *Pest Advisory Leaflet* No. 3. Plant Protection Service, Secretariat of the Pacific Community, Suva, Fiji Islands. 2 pp.

Jackson, G. V. H., Ball, E. A. and Arditti, J. 1977. Seed germination and seedling proliferation of taro, *Colocasia esculenta* (L.) Schott in vitro. *J. Hort. Sci.* 52: 169-171.

Jackson, G.V. H. and Gollifer, D. E. 1975. Storage rots of taro, *Colocasia esculenta*, in the British Solomon Islands. *Ann. Appl. Biol.* 80: 217-230.

Jackson, G. V. H. Brief summary of situation in the region and comments on available assistance for long-term regional projects on taro leaf blight control. 1996. In Proceedings of Taro Leaf Blight Seminar, Alafua, Western Samoa, New Caledonia, pp. 71-74.

Johnson, R. C., Johnston, W. J., Nelson, M. C., Simon, C. J. and C. T. Golob. 1999. Core utilization and development - an example with Poapratensis. In: Johnson, R. C. and Hodgkin, T. (eds.). *Core collections for today and tomorrow*. IPGRI, Rome, Italy. Pp. 49-60.

Jones, C. J., Edwards, K. J., Castaglione, S., Winfield, M. O., Sala, F., van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M. and Daly, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed.* 3: 381-390.

Jose, J. and Usha, R. 2000. Extraction of geminiviral DNA from a highly mucilaginous plant (*Abelmoschus esculentus*). *Plant Mol. Biol. Rep.* 18:349-355.

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 intersimple sequence repeat (ISSR) polymorphism in the genus Oryza. *Theor. Appl. Genet.* 100: 1311-1320.

Kesseli, R., Witsenboer, H., Stanghellini, M., Vandermark, G. and Michlmore, R. 1993. Recessive resistance to *Plasmoparalactucae*-radicis maps by bulked segregant analysis to cluster of dominant disease resistance genes in lettuce. *Mol. Plant Microbe In.* 6: 722-728.

Khalaf, A. E. A., Afiah, S. A., Khalil, R. M. A. and Ahmed, A. I .S. 2015. Molecular Markers for Resistance of Chocolate Spot Disease in Faba Bean (*Vicia faba* L.) Using ISSR-PCR. *Middle-East J. Sci. Res.* 23(4): 558-567.

Ko, W. H. 1979. Mating-type distribution *of Phytophthora colocasiae* on the island of Hawaii. *Mycologia* 71(2): 434-437.

Kohler, F., Pellegrin, F., Jackson, G.V. H. Mac Kenzie, E. 1997. Taro. In: Diseases of Cultivated Crops in Pacific Island Countries; Secretariat of the Pacific Community: Noumea, New Caledonia.

Kramer, M. F. and Coen, D. M. 2004. Enzymatic Amplification of DNA 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.

Kreike, C. M., VanEck, H. and Lebot, V. 2003. Genetic diversity in taro (*Colocasia* esculenta (L.) Schott) from South East Asia and Oceania. *Theor. Appl. Genet.* 109: 761-768.

Kruglyak, S., Durrett, R., Schug, M. D., Aquadro, C. F. 1998. Equilibrium distribution of microsatellite repeats length resulting from a balance between slippage events and point mutations. *Proc. Natl. Acad.* Sci. 95:10074-10078.

Kuruvilla, K. M. and Singh, A. 1981. Karyotypic and electrophoretic studies on taro and its origin. *Euphytica* 30: 405–413.

Lakhanpaul, S., Velayudhan, K. C. and Bhat, K. V. 2003. Analysis of genetic diversity in Indian taro (*Colocasia esculenta* (L.) Schott.) using random amplified polymorphic DNA (RAPD) markers. *Genet. Resour. Crop Evol.* 50: 603- 609.

Lambert, M. 1982. Taro cultivation in the South Pacific. In Lambert, M. (eds.). SPC Handbook (SPC). Noumea, New Caledonia: South Pacific Commission. 144 p.

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

Lebot, V., Prana, M.S., Kreike, N., van Heck, H., Pardales, J., Okpul , T.,Gendua, T., Thongjiem, M., Hue , H., Viet, N. and Yap. T. C. 2004. Characterisation of taro (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. *Genet. Resour. Crop Evol.* 51: 381-392.

Lebot, V. and Aradhya, K. M. 1991. Isozyme variation in taro (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. *Euphytica* 56: 55-66.

Lebot, V., Hartati, S., Hue, N. T., Viet, N. V., Nghia, N. H., Okpul, T., Pardales, J., Prana, M. S., Prana, T. K., Thongjiem, M., Kreike, C. M., VanEck, H., Yap, T. C. and Ivancic. A. 2000. Genetic variation in taro (*Colocasia esculenta*) in South East Asia and Oceania. Twelfth Symposium of the ISTRC. Potential of root crops for food and industrial resources. Tsukuba, Japan, pp: 524-533.

Lee, W. 1999. Taro (*Colocasia esculenta*) [Electronic Version]. Ethnobotanical Leaflets. http://www.siu.edu/~ebl/leaflets/taro.htm

Lohnes, D. G., Wagner, R. E. and Bernard, R. L. 1993. Soybean genes Rj2, Rmd, and Rsp2 in linkage group. *J. Hered.* 84:109-111.

Loy, T. H., Spriggs, M. and Wickler, S. 1992. Direct evidence for human use of plants 28,000 years ago: starch residues on stone artefacts from the northern Solomon Islands. *Antiquity* 66: 898-912.

Mabhaudhi, T. and Modi, A. T. 2013. Preliminary Assessment of Genetic Diversity in Three Taro (*Colocasia esculenta* L. Schott) Landraces UsingAgro-morphological and SSR DNA Characterisation. *J. Agric. Sci. Technol.* B3: 265-271.

Mace, E. S and Godwin, L. D. 2002. Development and characterization of polymorphic microsatellite markers in taro (*Colocasia esculenta*). *Genome* 45: 823-832.

Mace, E.S., Mathur, P. N., Izquierdo, L., Hunter, D., Taylor, M. B., Singh, D., DeLacy, I. H., Jackson, G. V. H. and Godwin, I. D. 2006. Rationalization of taro germplasm collections in the Pacific Island region using simple sequence repeat (SSR) markers. *Plant Genet. Resour.* 4: 210-220.

Madeira, N. R. 2008. Contribuiçãoportuguesa à produção e aoconsumo de hortaliças noBrasil: umarevisãohistórica. *Hort. Bras.* 26: 428-432.

Maestri, E., Malcevschi, A., Massari, A. and Marmiroli, N. 2002. Genomic analysis of cultivated barley (*Hordeum vulgare*) using sequence-tagged molecular markers. Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes, and simple-sequence repeats (SSRs). *Mol. Gen. Genomics* 267(2): 186-201.

Mahfooz, S., Maurya, D. K, Srivastava, A. K., Kumar, S. and Arora, D. K. 2012. A comparative in silico analysis on frequency and distribution of microsatellites in coding regions of three formaespeciales of *Fusarium oxysporum* and development of EST-SSR markers for polymorphism studies. *Microbiol. Lett.* 328(1): 54-60.

Matthews, P. J. and Terauchi, R. 1994. The genetics of agriculture: DNA variation in taro and yams. In: J. G. Hather (ed.), *Tropical Archaeobotany: Applications and New Developments*, Routledge, London. pp. 251-262.

Matthews, P. J. 1990. The Origins, Dispersal and Domestication of Taro. Ph.D. thesis, The Australian National University, Canberra.

Matthews, P. J. 1991. A possible tropical wildtype taro: *Colocasia esculenta* var. aquatilis. Indo-Pacif. *Prehistory Ass. Bull.* 11:69-81.

Mayer, M. S., Tullu, A., Simon, C. J., Kumar, J. S., Kraft, J. M., Kaiser, W. J. and Muehlbauer, F. J. 1997. Development of DNA marker for *Fusarium* wilt resistance in chickpea. *Crop Sci.* 37: 1625-1629.

McGregor, A. 2011. Assessing the social and economic value of germplasm and crop improvement as a climate change adaptation strategy: Samoa and Vanuatu case studies. A background case study prepared for IUCN's report. In: Lal, P.N. (ed.). *Climate change adaptation in the Pacific: Making informed choices*. Australian Department of Climate Change and Energy Efficiency. Suva, Fiji: IUCN, pp. 35.

Meyer, W., Mitchell, T. G., Freedman, E. Z. and Vilgays, R. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. *J. Cli. Microbiol.* 31: 2274-2280.

Michelmore, R.W., Paran, I., Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked seggregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci.* 88: 9828-9832.

Milach, S. C. K., Rines, H. W., Phillips, R. L., Stuthman, D. D. and Morikawa, T. 1997. Inheritance of a new dwarfing gene in oat. *Crop Sci.* 783-790.

Misra RS. 1990. Studies on *Phytophthora* leaf blight disease of *Colocasia*. Central Tuber Crops Research Institute (India) Annual Report, pp 103-104.

Miyasaka, S. C. 1979. Calcium nutrition of taro (*Colocasia esculenta* (L.) Schott) and its possible relationship to guava seed disease. MSc Thesis, University of Hawaii.

Morgan, R. N, Wilson, J. P., Hanna, W. W. and Ozais-Akins, P. 1998. Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet. *Theor. Appl. Genet.* 96:413-420.

Morgante, M. and Olivieri, A. M. 1993. PCR- amplified microsatellite markers in plant genetics. *Plant J.* 3:393-427.

Morgante, M., Rafalski, A., Biddle, P., Tingey, S. and Olivieri, A. M. 1994. Genetic mapping and variability of seven soybean simple sequence repeat loci. *Genome* 37: 763-769.

Morren, G. E. B. and Hyndman, D. C. 1987. The taro monoculture of Central New Guinea. *Hum. Ecol.* 15(3): 301-315.

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. Appl. Genet.* 94: 597-602.

Narina, S. S., Orgeix, C. A. and Sayre, B. L. 2011. Optimization of PCR conditions to amplify microsatellite loci in the bunchgrass lizard (*Scelopo russlevini*) genomic DNA. *BMC. Res. Notes* 4: 26.

Narula, K. L. and Mehrotra, R. S. 1980. Occurrence of A1 mating type of *Phytophthora colocasiae. Indian Phytopathol.* 33(4): 603-604.

Negi, M. S., Devic, M., Delseny, M. and Lakshmikumaran, M. 2000. Identifcation of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection. *Theor. Appl. Genet.* 101: 146–152.

Noyer, J. L., Billot, C., Weber, A., Brottier, P., Quero-Garcia, J. and Lebot, V. 2003. Genetic diversity of taro (Colocasia esculenta (L.) Schott.) assessed by SSR markers. In: Proceedings of Third Taro Symposium. Guarino, L., Taylor, M. and Osborn, T. (Eds.). Secretariat of the Pacific Community, Nadi, Fiji Islands. pp. 174 – 180.

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.

O'Sullivan, J. N., Asher, C. J., and Blamey, F. P. C. 1995. Diagnostic criteria for nutritional disorders in taro. In: Asher, C. J. and Crasswell, E. T. (eds.). *Diagnosis and correction of mineral nutrient disorders of root crops in the Pacific.* Proceedings of Workshop, ACIAR Proceedings No.65. Australian Centre for International Agricultural Research, Nuku'alofa, Tonga.

Oke, O. L. 1990. Food security indeveloping countries. In: *Roots, tubers, plantains and bananas in human nutrition.* No: 24: FAO Corporate Documentary Repository, Food and Agriculture Organization of the United Nations, Rome.

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. *PGR Newsl.* 143: 8-16.

Olejniczak and W. J. Krzyzosiak. 2006. Genotyping of simple sequence repeats--factors implicated in shadow band generation revisited. *Electrophoresis* 27: 3724-3734.

Onwueme, I. C. 1978. The Tropical Tuber Crops Yam, Cassava, Sweet potatoes and Cocoyams. Chichester: John Wiley and Sons, pp. 215–219.

Onwueme, I. 1999. Taro cultivation in Asia and the Pacific. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific. Bangkok, Thailand. 15 pp.

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

Padaliya, R. V., Suthar, K. P., Diwakar S., Mahatma, M. K. and Patil, V. R. 2013. Marker assisted characterization of chickpea genotypes for wilt resistance. *Afr. J. Biotechnol.* 12(50): 6907-6912.

Paiki, F.A. 1996. Symptoms of Taro Leaf Blight disease (*Phytophtora colocasiae*) and the Relationship with Yield Components in Biak. *Sci. New Guinea* 21(3): 153-157.

Panoff, F. 1972. Maenge taro and cordyline: Elements of Melanesian key. *J. Polyn.* Soc. 81: 375-390.

Papa, R. and Gepts, P. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. *Theor. Appl. Genet.* 106:239-250.

Paran, I. and Michelmore, R. W. 1993. Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet.* 85: 985-993.

Penner, G. A., Chong, J., Levesque-Lemay, M., Molnar, S. J. and Fedak, G. 1993. Identification of a RAPD marker linked to the oat stem rust gene Pg3. *Theor. App.l Genet.* 85:702-705.

Perera, L., Russell, J. R., Provan, J. and Powell, W. 2000. Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). *Genome* 43:15–21.

Pillen, K., Binder, A., Kreuzkam, B., Ramsy, L., Waugh, R., Forster, J. and Leon, J. 2000. Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. *Theor. Appl. Genet.* 101: 652-660.

Plaschke, J., Ganal, M. W., and Roder, M. S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theor. Appl. Genet.* 91: 1001-1007.

Plucknett, D. L. 1970. Colocasia, Xanthosoma, Alocasia, Cyrtosperma and Amorphophallus. In: Proceedings of the Second International Symposium on Tropical Root and Tuber Crops. Honolulu, Hawaii, College of Tropical Agriculture, University of Hawaii. pp. 127 – 135.

Plucknett, D. L. 1976. Edible aroids: Alocasia, Colacasia, Cryptosperma, Xanthosoma. In: Simmonds, N. W. (eds.). Evolution of Crop Plants. Longman press, London, U. K., pp. 10-12.

Porebski, S., Bailey, L. G. and Baum, B. R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.* 15:8–15.

Powell, W., Machray, G. C. and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* 1:215-222.

Purseglove, J. W. 1972. Tropical Crops. In: Longman, I. (ed.). *Monocotyledons.* London, UK. 334pp.

Queensland Department of Primary Industries and Fisheries, 2011. Special edition of Environmental Weeds of Australia for Biosecurity Queensland., Australia: The University of Queensland and Department of Primary Industries and Fisheries.

Raciborski, M. 1900. Parasitic algae and fungi, Java. Batavia Bulletin of the New York State Museum, pp. 189.

Rajesh, M. A. Aerard, B. A., Creeephi, A., Thomas, G., Fayas, T. P., Rejana, K. P. and Varun, P. 2013. RAPD-derived SCAR marker associated with tall-type palm trait in coconut. *Sci. Hortic.* 150: 312–316.

Rallo, P., Dorado, G., and Martín, A. 2000. Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). *Theor. Appl. Genet.* 101: 984-989.

Rao, R., Hunter, D., Eyzaguirre, P. and Matthews, P. 2010. Ethnobotany and global diversity of taro. In: Ramanatha, R., Matthews, V., Ezyaguire, P. B., Hunter, D., (eds). The Global Diversity of Taro: Ethnobotany and Conservation; Bioversity International: Rome, Italy, pp 2-5.

Ratnaparkhe, M. B., Santra, D. K., Tullu, A. and Muehlbauer, F. J. 1998. Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with *Fusarium* wilt resistance gene in chickpea. *Theor. Appl. Genet.* 96: 348-353.

Reddy, P. M., Sarla, N. and Siddiq, E. A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128(1): 9-17.

Revill, P., Trinh, X., Dale, J. and Harding, R. 2005.Taro vein chlorosis virus: characterization and variability of a new *nucleor habdovirus. J. Gen. Virol.* 86: 491-499.

Ribeiro, R. A. and Lovato, M. B. 2007. Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia. Genet. Mol. Res.* 6:173-187.

Roder, M. S., Plaschke, J., Konig, S. U., Borner, A., Sorrells, M. E., Tanksley, S. D. and Ganal, M. W. 1995. Characterization of PCR-amplified microsatellite loci in wheat. *Mol. Gen. Genet.* 246: 327-333.

Rose warne, G. M., Singh, R. P., Huert a-Espino, J., William, H. M., Bouchet, S., Cloutier, S., Fadden, M. and H. Lagudah, E. S. 2006. Leaf tip necrosis, molecular markers and b1-proteasome subunits associated with the slow rusting resistance genes Lr46/Yr29. *Theor. Appl. Genet.* 112: 500-508.

Safo-Kantaka, O. 2004. *Colocasia esculenta* (L.) Schott. Record from Protabase. In: Grubben, G. J. H. Denton, O. A.PROTA (Plant Res. Tropical Afr / Ressources végétales de l'A friquetropicale). Wageningen, Netherlands: PROTA.

Saghai-Maroof, M. A., Biyashev, R. B., Yang, G. P, Zhang, Q. and Allard, R. W. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. *Proc. Natl. Acad.* Sci. 91:5466-5470.

Salimath, S. S., Oliviera, A. C., Godwin, I. D. and Bennetzen, J. L. 1995. Assessment of genome origin and genetic diversity in the genus Eleusine with DNA markers. *Genome* 38: 757-763.

Sar, S. A., Wayi, B. M. and Ghodake, R. D. 1998. Review of Research in Papua New Guinea for Sustainable Production of Taro (*Colocasia esculenta*). *Trinidad J. Trop. Agri.* 75(1): 134-138.

Sardos, J. L., Malapa, R., Bouchet, S. and Lebot, V. 2012. Genetic diversity of taro (*Colocasia esculenta* (L.) Schott) in Vanuatu (Oceania): an appraisal of the

distribution of allelic diversity (DAD) with SSR markers. *Genet. Resour. Crop Evol.* 59: 805-820.

Scott, K. D. and Playford, J. 1996. DNA extraction technique for PCR in rain forest plant species. *Biotech.* 20:974-979.

Senior, M. L. and Heun, M. 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. *Genome* 36: 884-889.

Senior, M. L., Murphy, J. P., Goodman, M. M. and Stuber, C. W. 1998. Utility of SSRs for Determining Genetic Similarities and Relationships in Maize Using an Agarose Gel System. *Crop Sci.* 38-41.

Sharma, K. K., Lavanya, M. and Anjaiah, V. 2000. A method for isolation and purification of peanut genomic DNA suitable for analytical applications. *Plant Mol. Biol. Rep.* 18:393a–393h.

Sharma, K., Mishra, A. K. and Misra, R. S. 2008.Analysis of AFLP Variation of Taro Population and Markers Associated with Leaf Blight Resistance Gene. *Acad. J. of Plant Sci.* 1 (3): 42-48.

Shaw, D. E. 1975. Illustrated notes on flowering, flowers, seed and germination in taro Colocasia esculenta. Papua New Guinea Department of Agriculture Stock and Fisheries Res. Bull. 13: 39-59.

Sheperd, K. W. and Mayo, G. M. E. 1972. Genes conferring specific plant disease resistance. *Sci.* 175: 375-380.

Shim, S. I. and Jorgensen, R. B. 2000. Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. *Theor. Appl. Genet.* 101:227-233.

Singh, D., Mace, E. S., Godwin, I. D., Mathur, P. N., Okpul, T., Taylor, M., Hunter, D., Kambuou, R., Rao, R. V. 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.

Somasundharam, S. T. and Kalaiselvam, M. 2011. Molecular tools for accessing genetic diversity. International Training Course on Mangroves and Biodiversity, Annamalai University, India, pp. 82-91.

Sreekumari, M. T. and Mathew, P. M. 1991. Distribution of diploid and triploid taro in India. *J. Root Crops.* 18(2): 132-133.

Srinivas, T., Nedunchezhiyan, M. and Misra, R. S. 2012. Marketing system of Aroids and Yams in India. In: *Technical Bulletian Series:53*. Akshara offset printing Press, Sreekariyam, Thiruvanathapuram. pp. 80.

Strauss, M. S., Michaud, J. D. and Arditti, J. 1979. Seed storage and germination and seedling proliferation in taro (*Colocasia esculenta* (L.) Schott). *Ann.of Bot.* 43:603- 612.

Sudupak, M. A., Bennetzen, J. L., Hulbert, S. H. 1993. Unequal exchange and meiotic instability of disease resistance genes in the Rp1 region of maize. *Genet.* 133: 119-125.

Tanimoto, T. and Matsumoto, T. 1986. Variation of morphological characters and isozyme patterns in Japanese cultivars of *Colocasia esculenta* Schott and *Colocasia gigantea* Hook. *Jpn. J. Plant Breed.* 36: 100-111.

Thankappan, M., 1985. Leaf blight of taro-a review. *J. Root Crps.* 11: 223-236.

Toktok, N. 2006. Methods of controlling taro leaf blight. In: Wet-Lowlands Mainland Programme, Sir Alkan Tololo Research Centre, Bubia, Morobe Province, Papua New Guinea.

Trujillo, E. E. and Aragaki, M. 1964. Taro blight and its control. *Hawaii Frm Sci.* 13: 11-13.

Trujillo, E. E. 1967. Diseases of the genus *Colocasia* in the Pacific Area and their control. In: *Proceeding of International Symposium of Tropical Root Crops*, Trinidad, Jamaica, pp. 13-18.

Tyagi, A. P, Taylor, M. and Deo, P. C. 2004. Seed germination and seedling development in taro (*Colocasia esculenta*). *S. Pacif. J. Nat. Sci.* 22: 61- 65.

USDA-ARS, 2012. Germplasm Resources Information Network (GRIN). Online Database. Beltsville, USA: National Germplasm Resources Laboratory. http://www.ars-grin.gov/.

Varshney, R. K., Sigmund, R., Boerner, A., Korzun, V., Stein, N., Sorrells, M., Langridge, P. and Graner, A. 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. *Plant Sci.* 168:195-202.

Vaughan, T. A. C., Russell, A., Hill, T. J., Frew and Gilpin, B. J. 1997. DNA markers for disease resistance breeding in peas (*Pisum sativum* L.) G.M. Proc. 50th N.Z. Plant Protection Conf. Copenhagen, Denmark pp. 314-315.

Vicente, M. C., Guzmán, F. A., Engels, J. and Rao, R.V. 2005. Genetic characterization and its use in decision making for the conservation of crop

germplasm. In. *The Role of Biotechnology*. Food and Agriculture Organization of the United Nations, Villa Gualino Rome, pp 121-128.

Vinutha, K. B. 2014. Genetic diversity analysis in taro (*Colocasia esculenta* (L.) Schott) of North East India. M.Sc thesis, Kerala Agricultural University, Thrissur, Kerala.

Wang, J. K. 1983. Introduction. In: Wang, J. K. and Higa, S. (eds.). *Taro, a review of Colocasia esculenta, and its potentials*. Honolulu: University of Hawaii Press, pp 3-13.

Wang, Z., Weber, J. L., Zhong, G. and Tanksley, S. D. 1994. Survey of plant short tandem repeats. *Theor. Appl. Genet.* 88:1-6.

Weber, J. L. 1990. Mapping RFLP loci in maize using B-A translocations. *Genomics* 7:524-530.

Weber, J. L. and May, P. E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396.

Whitney, L. D., Bowers, F. A. I. and Takahashi, M. 1939. Taro varieties in Hawaii. Bulletin No. 84. Hawaii Agricultural Experiment Station of the University of Hawaii. pp. 86.

Wilson, J. E., Tjendana, T. S. and Wong, K. 1990. Taro Breeding. Agro-Facts. Ireta Publication No. 3/89. USP Alafua Campus, Apia, Western Samoa. pp. 9-20.

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

Wu, K. S. and Tanksley, S. D. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet.* 241:225-235.

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

Xu, J., Yang, Y., Pu, Y., Ayad, W. G. and Eyzaguirre, P. B. 2001. Genetic diversity in taro (*Colocasia esculenta* L. Schott, Araceae) in China: an ethnobotanical and genetic approach. *Econ. Bot.* 55:14-31.

Yalu, A., Singh, D. and Yadav, S. S. 2009. Taro Improvement and Development in Papua New Guinea – A Success Story. Asia Pacific Association of Agricultural Research Institutions (APAARI), c/FAO Regional Office for Asia and the Pacific, Bangkok, Thailand.

Yamamoto, T. K., Mochida, T., Imai, Y. Z. Shi, I. Ogiwara and T. Hayashi. 2002. Microsatellite markers in peach [*Prunu spersica*(L.) Batsch] derived from an enriched DNA and genomic libraries. *Mol. Ecol. Notes* 2:298-301.

Yen, D. E. 1995. The development of Sahul agriculture with Australia as bystander. *Antiquity* 69: 831-847.

Yen, D. E. and Wheeler, J. M. 1968. Introduction of taro into the Pacific: the indications of the chromosome numbers. *Ethnology* 7: 259-267.

Yi, Y., Li, R., Xu, H., Wu, X. Li, S., Zhang, J. and Yin, Y. 2013. Identification of SRAP and RGA markers linked to powdery mildew (*Blumeria graminis*) resistance gene *PmZB90* in common wheat. *Afr. J. Plant Sci.* 7(3):454-459.

Zentmyer, G. A. 1988. Origin and distribution of four species of *Phytophthora*. *Trans. Br. Mycol. Soc.* 91(3): 367-378.

Zeven, A. C. 1980. Polyploidy and domestication: the origin and survival of polyploids in cytotype mixtures. In: Lewis, W. H. (ed.). *Polyploidy: Biological Relevance*. Plenum Press, New York, USA, pp. 385–408.

Zhang, W., Zhang, L., Qiao, L., Wu, J., Zhao, G., Jing, R., Lv, W. and Jia, J. Cloning and haplotype analysis of TaSTE, which is associated with plant height in bread wheat (*Triticum aestivum L.) Mol. Breed.* 31(1) 47-56.

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

APPENDICES

8. APPENDIX

APPENDIX 1

CTAB EXTRACTION BUFFER (CTAB METHOD)

Composition	Concentration
Tris HCl (pH: 8)	100 mM
EDTA	25 mM
NaCl	1.5 M
CTAB	2.5%
β- mercaptoethanol	2%
PVP	1%

APPENDIX II

TE Buffer (10 X)

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

APPENDIX III

TBE BUFFER (10X)

Composition	Concentration

Tris base	107 g
Boric acid	55 g
0.5 M EDTA	40 ml

The solution is made up to 1000ml.

APPENDIX 1V

40% ACRYLAMIDE SOLUTION

Composition	Concentration
Acrylamide	38 g
Bisacrylamide	2 g

Dissolve 38 g acrylamide in 40 ml solution and then dissolve 2 g bis acrylamide and make up to 100 ml.

APPENDIX V

BIND SILANE

Composition	Concentration
Absolute alcohol	95%
γ-methacryloxypropyl-methoxy silane	1 µl
0.5% acetic acid	2.5 ml

The solution is then made up to 100ml.

APPENDIX VI

FIXER

Composition	Concentration
Acetic acid	200 g
Distilled water	1800 ml

APPENDIX VII

SILVER STAIN

2 g silver nitrate dissolved in 2000 ml distilled water with thorough mixing. Added 3 ml formaldehyde to this solution to make up the stain.

APPENDIX VIII

DEVELOPER

60 g sodium carbonate dissolved in 2000 ml distilled water. Store the solution at 20^{0}C. Just before use, add 400 µl chilled sodium thiosulphate (10 mg/ml) and 3 ml formaldehyde.

APPENDIX IX

NaOH

40 g NaOH dissolved in 2000 ml distilled water.

ABSTRACT

GENETIC DIVERSITY ANALYSIS AND IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH LEAF BLIGHT RESISTANCE IN TARO (*Colocasia esculenta* (L.) Schott)

ASWATHY ANAND A.

(2010-09-106)

Abstract of the 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

2015

7. ABSTRACT

The project titled "Genetic diversity analysis and identification of molecular markers associated with leaf blight resistance in taro (*Colocasia esculenta* (L.) Schott)" was carried out at the Division of Crop improvement, ICAR-CTCRI, Sreekaryam, Thiruvananthapuram, during 2014-15. Thirty Kerala accessions were employed to study the genetic diversity and 12 genotypes comprising 6 susceptible and 6 resistant which were screened in the fields of ICAR-CTCRI was taken for the study.

DNA was isolated from all the 30 varieties by a method standardized by Doyle and Doyle, (1987) and later by Sharma *et al.*, (2008) which was slightly modified by Vinutha, K. B. (2014). A purity level of 1.7-2.0 was achieved by most of the genotypes.

PCR conditions already standardized in the molecular biology lab of Crop Improvement lab of ICAR-CTCRI were used to amplify the SSR markers. After preliminary screening, 5 primers were eliminated from the study because of its poor amplification profile. 10 primers out of the 15 screened were utilized for PCR. The PCR products were made to run in 6% acrylamide gel (denaturing urea gel) and the bands were studied after staining with silver nitrate. PIC and cluster analysis was done to analyze its diversity.

By calculating polymorphic information content, the highest heterozygosity was obtained for the primer uq 97-256 with a value of 0.871 and the lowest was obtained for the primer uq 84-207 with a value of 0.694. Number of alleles per locus ranged from 1.9-4.56 with the maximum alleles shown by uq 97-256 and the minimum showed by uq 84-207 and uq 201-302.

The first cluster comprised of the accessions from C-406 to TCR 450. The second cluster consists of C-218 to C-384 and the third large cluster groups most of the genotypes ranging from TCR 424 to TCR-296 and ADS2014-20 has formed an

173755

outlier. The third cluster is further subdivided into four sub clusters and the second sub cluster consists of major number of genoytpes and has an outlier C-273.

In the next part of the work, 12 sets of DNA was isolated comprising 6 susceptible and 6 resistant varieties each, to identify molecular markers associated with TLB. 13 ISSR primers were tried to identify the same. The product was amplified at 56^0C. The PCR products were resolved in 2% agarose Out of the 13 primers, 5 of them gave an extra band for resistant varieties. UBC 825 and $(AG)_9AC$ gave an extra band in all the resistant varieties in 685 bp and 808 bp regions, respectively. The primers UBC 827, UBC 817 and UBC 808 gave bands in 5 resistant varieties in the region, 598, 675 and 667 bp, respectively. The dendrogram analysis using UPMGA clustered resistant and susceptible varieties into two different clusters which gave a more promising result. But the SSR primers employed for the same study using the primers Cel A08, Cel F04, Cel CO3, Cel F12, Cel H12, uq 97- 256, uq 201- 302, uq 73-164, uq 115-71 and uq 88-94 failed to show any specific bands in the expected product size. However, more number of accessions showing different levels of tolerance and susceptibility to TLB as well as more number of markers could lead to the identification of promising markers associated with TLB resistance.