# IDENTIFICATION OF MOLECULAR MARKERS FOR RESISTANCE TO TARO LEAF BLIGHT IN *Colocasia esculenta* (L.) Schott

By

## ANJITHA NAIR U.M.

### (2013-09-116)

## THESIS

Submitted in partial fulfillment of the requirement for the degree of

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**Faculty of Agriculture**

**Kerala Agricultural University, Thrissur**



**Department of Plant Biotechnology**

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**COLLEGE OF AGRICULTURE**

**VELLAYANI, THIRUVANANTHAPURAM - 695 522**

**KERALA, INDIA**

**2018**

# DECLARATION

I hereby declare that this thesis entitled "Identification of molecular markers for resistance to taro leaf blight in *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: Sreekariyam

Date: 21.11.2018

**Anjitha Nair, U.M.**

2013-09-116

# भा.कृ.अनु.प- केंद्रीय कन्द फसल अनुसंधान संस्थान

(भारतीय कृषि अनुसंधान परिषद, कृषि और किसान कल्याण मंत्रालय, भारत सरकार)
श्रीकार्यम, तिरुवनन्तपुरम-695 017, केरल, भारत

## ICAR- CENTRAL TUBER CROPS RESEARCH INSTITUTE

(Indian Council of Agriculture Research, Ministry of Agriculture and Farmers Welfare, Govt. of India)
Sreekariyam, Thiruvananthapuram-695 017, Kerala, India

ISO 9001:2008

# CERTIFICATE

Certified that this thesis entitled **"Identification of molecular markers for resistance to taro leaf blight in *Colocasia esculenta* (L.) Schott"** is a record of research work done by **ANJITHA NAIR U. M. (2013-09-116)** under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.
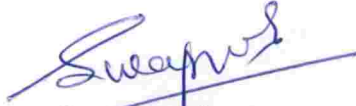
Place: Sreekariyam
Date: 21.11.2018

(Asha Devi A)
Principal Scientist (Genetics)
Division of Crop Improvement
ICAR-CTCRI, Sreekariyam
Thiruvananthapuram- 695017

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# CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Anjitha Nair U. M. (2013-09-116) a candidate for the degree of B.Sc.-M.Sc. (Integrated) Biotechnology, agree that the thesis entitled **"Identification of molecular markers for resistance to taro leaf blight in *Colocasia esculenta* (L.) Schott"** may be submitted by Ms. Anjitha Nair U.M. in partial fulfillment of the requirement for the degree.

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

**Dr. Swapna Alex**
(Member, Advisory Committee)
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani,
Thiruvananthapuram 695 522

**Dr. Veena S. S**
(Member, Advisory Committee)
Principal Scientist
Division of Crop Protection
ICAR-Central Tuber Crops Research
Institute
Sreekariyam,
Thiruvananthapuram-695017

**Dr. K. B. Soni**
(Member, Advisory Committee)
Professor
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

**Dr. Sabu K.K.**
(External Examiner)
Senior Scientist,
Biotechnology and Bioinformatics Division,
Jawaharlal Nehru Tropical Botanical
Garden and Research Institute,
(JNTBGRI), Palode,
Thiruvananthapuram-695562

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**Place:** *Sreekariyam*                                        *(ANJITHA NAIR U. M.)*

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| $^{\circ}$C | : | Degree Celsius |
| % | : | Percentage |
| μg | : | Microgram |
| μM | : | Micro molar |
| μl | : | Microliter |
| 2D | : | 2-Dimension |
| 3D | : | 3-Dimension |
| $A_{260}$ | : | Absorbance at 260nm |
| $A_{280}$ | : | Absorbance at 280nm |
| AFLP | : | Amplified fragment length polymorphism |
| $AgNO_3$ | : | Silver nitrate |
| ANOVA | : | Analysis of variance |
| AP – PCR | : | Arbitrarily primed - PCR |
| APS | : | Ammonium per sulfate |
| bp | : | Base pair |
| cm | : | Centimeter |
| CTAB | : | Cetyltrimethyl ammonium bromide |
| CTCRI | : | Central Tuber Crops Research Institute |
| DNA | : | Deoxyribo nucleic acid |
| dNTP | : | Deoxy nucleotide triphosphate |
| EDTA | : | Ethylene diamine tetra acetic acid |
| EST | : | Expressed sequence tag |
| et al. | : | And others |
| etc. | : | And so on; other people/ things |
| Fig. | : | Figure |

| | | |
|---|---|---|
| g | : | Gram |
| h | : | Hour |
| ha | : | Hectare |
| $HgCl_2$ | : | Mercuric chloride |
| ICAR | : | Indian Council of Agricultural Research |
| IITA | : | International Institute of Tropical Agriculture |
| ISSR | : | Inter simple sequence repeats |
| KAU | : | Kerela Agricultural University |
| kb | : | Kilo base pairs |
| kg | : | Kilogram |
| $L^{-1}$ | : | Per liter |
| Fig. | : | Figure |
| e.g. | : | For example, for instance |
| M | : | Molar |
| Max | : | Maximum |
| m | : | Metre |
| $MgCl_2$ | : | Magnesium chloride |
| $ms^{-1}$ | : | Metre per second |
| $m^2$ | : | Metre square |
| ml | : | Milli litre |
| mg | : | Milligram |
| M T | : | Million Tonnes |
| Min | : | Minimum |
| mM | : | Milli molar |
| mm | : | Millimeter |
| mt DNA | : | Mitochondrial DNA |
| ng | : | Nanogram |

| | | |
|---|---|---|
| nm | : | Nanometer |
| No. | : | Number |
| OD | : | Optical Density |
| PAGE | : | Poly acylamide gel electrophoresis |
| PCA | : | Principal component analysis |
| PCR | : | Polymerase chain reaction |
| pg | : | Picogram |
| pH | : | Power of hydrogen |
| PNG | : | Papua New Guinea |
| ppm | : | Part per million |
| psi | : | Pounds per square inch |
| PVP | : | Poly vinyl-pyrrolidine |
| RAPD | : | Random Amplified Polymorphic DNA |
| rDNA | : | Ribosomal DNA |
| RFLP | : | Restriction fragment length polymorphism |
| RNA | : | Ribonucleic acid |
| RNAase | : | Ribo nuclease |
| rpm | : | Revolutions per minute |
| sec | : | Second |
| SCAR | : | Sequence Characterized Amplified Regions |
| SNP | : | Single Nucleotide Polymorphism |
| SSLP | : | Simple Sequence Length Polymorphism |
| SSR | : | Simple Sequence Repeats |
| STR | : | Short Tandem Repeats |
| STS | : | Sequence tagged site |
| t | : | tonne |
| Taq | : | *Thermus aquaticus* |

| | | |
|---|---|---|
| TaroGen | : | Taro Genetic Resources: Conservation and Utilization |
| TBE | : | Tris-borate EDTA buffer |
| TDZ | : | Thidiazuron |
| TE | : | Tris EDTA Buffer |
| TEMED | : | N,N,N',N',-tetramethylethylenediamine |
| $T_m$ | : | Melting temperature |
| S | : | Second |
| TrisHCl | : | Tris (Hydroxy Methyl) aminomethane hydrochloride |
| U | : | Enzyme |
| UV | : | Ultraviolet |
| *viz.,* | : | Namely |
| v/v | : | Volume / volume |
| W | : | Watt |
| w/v | : | Weight / volume |
| t | : | Tonne |
| t ha$^{-1}$ | : | Tonne per hectare |
| Var. | : | Variety |
| Wt. | : | Weight |

# Introduction

# 1. INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott), a member of the monocotyledonous family Araceae, consisting of 100 genera and more than 1500 species, is one among the economically important crop valued for its edible corms, petioles and leaves (food and feed), mainly in Asia and Africa. In India, two types *viz.*, *Colocasia. esculenta* var. *esculenta* and *Colocasia esculenta* var. *antiquorum*are cultivated but their exact area and production are not known. Northern, Eastern and North-Eastern regions are the major taro producing states in India. There exists varying conclusion seeing the original home as well as its taxonomy and common names (Spier, 1951; Mehta, 1959; Coursey, 1968; Harian, 1975; Plucknett, 1976; Onwume, 1978; Kuruvilla and Singh, 1983; Jos and Sreekumari, 1990 and Sreekumari, 1993). It has versatile nutritive and traditional values (Oke, 1990; Bradburry and Holloway, 1998). Processed products of taro *viz.*, poi, chips, baby foods, alcohol, candy, etc. are popular. Its medicinal properties/values are accredited for treatment of tuberculosis, pulmonary congestion, ulcers, and fungal infection (Coates *et al.*, 1988).

Amongst various pathogens that invade taro, leaf blight caused by *Phytophthora colocasiae* Raciborski, is the most destructive one which was first reported from Java in 1900 by Raciborski. In India, its incidence was reported in the year 1913 (Butler and Kulkarni, 1913). Initial symptoms of the disease include small brown water soaked flecks on the leaf that enlarge to form dark brown lesions, often with a yellow margin. Secondary infections prompt fast demolition of the leaf, which may occur in 10-20 days or less in very susceptible varieties and inevitably causes corm rot during storage. The disease

significantly lowers the number of functional leaves and yield reductions to the magnitude of 50% (Trujillo and Aragaki, 1964; Trujillo, 1967; Thankappan, 1985; Jackson, 1999; Misra and Chowdhury, 1997). Inoculum in the form of spores spread by wind driven rain and dew to adjacent plants and nearby plantations. The disease can also be spread on planting material and the fungus has been reported as remaining alive on planting tops for about three weeks after harvest. This is the most likely source of pathogen to new countries and within a country, once established. Therefore, strict quarantine measures are required as a first line of defence (Jackson, 1999).

There are no popular varieties/hybrids released so far in India which has field tolerance/resistance to taro leaf blight except 'Muktakeshi'. Most of the high yielding varieties are as such susceptible and work carried out in this line is very scarce (Sriram *et al.*, 2001; Lakhanpaul *et al.*, 2003; Mishra *et al.*, 2008; Sharma *et al.*, 2008). As evident from the earlier reports the yield loss is up to 50% (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1999), so, there is an urgent need to develop or at least to identify a resistant source for further taro improvement programmes. Further, the resistant varieties developed, may lose their resistance due to developmental variations over the years (X-Q Li, 2009). Nevertheless, priority may be given in all breeding programmes of taro in such a way that, a variety/hybrid to be developed must be leaf blight resistant or at least tolerant, irrespective of the purpose for which it is bred.

Although conventional plant breeding had a significant impact on improving taro for resistance to important diseases, its nature of propagation, flowering behaviour (shyness in flowering), protogynous nature, time consuming process of making crosses and backcrosses, poor seed setting, germination protocols, selection of desired resistant progenies, etc. make it difficult to react adequately to the evolution of new virulent pathogens.

Availability of tightly linked molecular markers can now be used in marker-assisted selection (MAS) programs, especially for disease resistance gene, where it is possible to infer the gene by the marker without depending on the natural pathogen occurrence or waiting for its phenotypic expression.

Breeding for disease resistance is one of the principal efforts in many breeding programmes. As in natural farming the usage of fungicides is against the law, resistant cultivars are of great significance. To select for resistant genotypes/populations, screening assays are needed, that are often laborious, time consuming and require extensive knowledge on plant-pathogen interactions. To facilitate identification and/or introgression of resistance genes into adapted breeding material, molecular markers are the most powerful and essential tools (Whitkus *et al.*, 1994).

In view of the above, the present investigation entitled **"Identification of molecularmarkers for resistance to taro leaf blight in** *Colocasia esculenta* **(L.) Schott."** was undertaken with the following objectives -

1. To identify the molecular markers (RAPD, ISSR and SSR) associated with taro leaf blight resistance.
2. To characterize the identified markers (RAPD, ISSR and SSR) by sequencing of trait specific genes followed by BLAST analysis.
3. Assessing the genetic diversity existing in the resistant and susceptible populations studied.

# Review of Literature

# 2. REVIEW OF LITERATURE

Keeping in view the objectives of the current study on **"Identification of molecular markers for resistance to taro leaf blight in** *Colocasia esculenta* **(L.) Schott"**, the available literature on morphology of taro and molecular aspects of taro leaf blight has been reviewed and presented here with

## 2.1 TUBER CROPS

Tropical root and tuber crops are the third most important food crop after legumes and cereals either as staple or subsidiary food for about one-fifth of the world population (ICAR-CTCRI, 2016). It was only in the late $16^{th}$ century that the importance of these crops came to the limelight for the world. Their domestication started thousands of years ago in South-East Asia through West-Central Africa and Tropical Latin America. They include potato, cassava, sweet potato, yams, and aroids of different families. These crops get acclimatized very fast due to their high yield, resistance and earliness, adaptability to diverse soil and environmental conditions and suitability to various cropping systems with minimum inputs, in addition to dietary chart by the taste qualities (Leon, 1977). On an average, 836 million tons of tuber crops are produced per year, where Asia stands first, followed by Africa, Europe and America (Chandrasekara and Kumar, 2016).

## 2.2 AROIDS

Aroids belong to monocots in the family Araceae of the order Alismatales. The centers of origin are considered to be Tropical America and Tropical Asia. Some species could be found only in the Mediterranean and African regions. They are generally tropical and sub-tropical in nature and are distributed worldwide in various natural habitats *viz.*, swamps,

ponds, lakes, canals, and rivers to rice fields, climbers as well as epiphytes (Sulaiman and Mansor, 2005). They produce edible starchy storage corms (Dimbeshwar and Kangkan, 2014). Edible aroids mainly include taro (*Colocasia esculenta* (L.) Schott), tannia (*Xanthosoma sagittifolium* (L.) Schott), giant taro (*Alocasia macrorrhiza* (L.) Schott), swamp taro (*Cyrtosperma* spp. Griff.), elephant foot yam (*Amorphophalluspaeoniifolius* (Dennst.) Nicolson), etc.

## 2.3 TARO

Taro is one of the important tuber trim began in the Indo-Malayan area most likely in Eastern India and Bangladesh and is grown throughout many tropical and sub-tropical countries, Pacific Islands, parts of Asia, Africa, and other humid tropics. Edible aroids are mainly classified into two tribes and five genera including Colocasiodeae (*Alocasia, Colocasia,* and *Xanthosoma*) and Lasioideae (*Cyrtosperma* and *Amorphophallus*) (Plucknett, 1983).*Colocasia* is the most essential genus in the family with *esculenta* being the most financial species(Maga, 1992).

Taro has an incredible genetic diversity distributed all over the tropical regions (Coates *et al.,* 1988). Lebot and Aradhya (1991) reported eight variants which includes two commonly cultivated types *viz., Colocasia esculenta* var. *esculenta* Schott (dasheen types) and *Colocasia esculenta* var. *antiquorum*(L.) Schott (eddoe type). In India, including Andaman Islands, dasheen types are overwhelming in nature (Bose et al., 2003).

## 2.4 ORIGIN AND DISTRIBUTION

The probable center of origin of taro lies in the Indo-Malayan region particularly concentrated in Eastern India and Bangladesh (Yen and Wheeler, 1968 and Purseglove, 1972) and is domesticated throughout the

humid tropics of the world. Taro is cultivated in extensive pockets of Africa despite the fact that the season of its spread to this locale isn't known. Recently, it is gaining more economic importance in Cameroon, Nigeria, Ghana and Burkina Faso, where, it is grown on a large scale (Chair and Traore, 2016). Taro is an essential staple nourishment all throughout numerous Pacific Islands, parts of Africa, Asia and the Caribbean for its fleshy corms and nutritious leaves. Phylogenetic relationships and geographical differentiation of Asian taro, *Colocasia esculenta* (L.) Schott and related species were done by Random Amplified Polymorphic DNA and isozymes of 13 enzyme systems with exceptional enthusiasm in the accessions from Yunnan area of China, which as far as anyone knows served the optional focal point of taro expansion and dispersal into the mild Far East Asia. Similar studies with SSR (Noyer *et al.*, 2003) and AFLP markers have affirmed the presence of two particular gene pools in taro which evidently demonstrates that taro was tamed in Asia and also in the Pacific. However, the most recent study on genetic diversification and dispersal of taro using 11 microsatellite markers revealed that the most elevated genetic diversity and number of private alleles were seen in Asian accessions, primarily from India (Chair, *et al.*, 2016), proving that India is the main centre of origin for taro from where, it dispersed to various regions like West Africa, Madagascar, Costa Rica, etc.

## 2.5 TAXONOMY, CYTOLOGY AND MORPHOLOGY

Taro is an erect herbaceous perennial root crop widely cultivated in the tropical and subtropical world belonging to genus *Colocasia* in the family Araceae, consisting of 100 genera and more than 1500 species (Macharia and Muchugi, 2014). The two most widely cultivated taxonomic varieties include *Colocasia esculenta* var. *esculenta* commonly known as the "dasheen type" which has a large central corm with suckers and stolons

and the second is the "eddoe type" (*Colocasia esculenta* var. *antiquorum*), which has a small central corm and a large number of smaller cormels (Dai and Zhag, 2016 and Tumuhimbise and Ubalua, 2016). The available genotypes of taro are categorized into wild and cultivated types of which wild types are not used for food due to high concentration of calcium oxalate crystals (Quero-Garcia and Courtois, 2006). Most of the taros grown in Asia and Pacific regions are of dasheen type. In places where taro is grown primarily for leaves, *C. esculenta* var. *antiquorum* is preferred (Sullivan *et al.*, 1996).

Chromosome number of taro reported includes 2n = 22, 26, 28, 38 and 42. Most common chromosome numbers include, diploids 2n = 28 and triploids 3n = 42. Furthermore, plants with 3n = 42 are referred to as '*alowane*' (male and large plant) and those of 2n = 28 are referred to as '*alokine*' (female and short plant) by Solomon Island farmers (Jackson *etal.*, 1977; Onwueme, 1978; Kuruvilla and Singh, 1981; Wang, 1983; Lebotand Aradhya, 1991; Lee, 1999). Work done at ICAR-CTCRI, and other studies showed that Indian taro consisted of both diploids and triploids (Sreekumari, 1992, Nusaifa Beevi, 2009; Kuruvilla and Singh, 1981). Kuruvilla and Singh (1981) reported that clones collected from Meghalaya were diploids and triploids, whereas those collected from the plains of South India were diploids.

Taro is harvested after 5-12 months of growth for edible purpose (Mwenye, 2009). It develops to a height of 1-2 m comprising of a central corm, lying just underneath the soil surface, from which leaves grow positive photo tropically and roots grow positive geotropically, while corms, cormels and runners grow laterally (Ubalua and Ewa, 2016). Leaves are heart-shaped green or purple in colour together with long petioles, fibrous roots and cylindrical or frequently sporadic corm. The idea of blossoming, fruiting and seed development by wild or developed taro has not been completely examined (Matthews and Agoo, 2012).

Taro only occasionally flowers and when flowering occurs, the inflorescence comprises of a cylindrical spadix of flowers encased in a 12-15 cm spathe with female flowers at the base and the male flowers at the top of a spadix (Castro, 2006). Natural sexual reproduction is uncommon except if helped by plant physiology and present day reproducing innovations (Ivancic, 2004).

## 2.6 HABITAT AND REPRODUCTION

Taro is generally dispersed and cultivated in wet tropics of the world. In Ghana, its cultivation is constrained to river banks in extensive urban areas. It very well may be discovered growing for the most part in moist woods and wet regions in riparian habitats, along streams, riverbanks, canals secondary forests, marshes, roadsides, and close to deserted harvest fields. In Australia, taro is a natural weed attacking conduits and wetlands and supplanting local sea-going plants. It is recorded among the 200 most obtrusive plants in the area of Queensland and is likewise an issue in the seaside locale of New South Wales and along the conduits in Western Australia (Department of Primary Industries and Fisheries, Queensland, 2011).

Taro fits well into various agro ranger service frameworks and a few sorts are especially adjusted to unfavorable land and soil conditions, for example, poor waste. All things considered, taro is developed under escalated development as a starch trim (Jianchu *et al.*, 2001). Triploid taros are considered to have advanced from diploids by association of an unreduced gamete with a normal gamete. Isozyme analysis on Yunnan and Nepal collections showed autopolyploid nature of the triploids, enhancing triploid formation through an unreduced gamete (Tahara *et al.*, 1999). In India, only diploids were found in the plains of the Southern India, but in the hills of the North-Eastern part, both diploids and triploids were reported (Kuruvilla and Singh, 1981).

Propagation of taro is by suckers/corms/cormels. Most of the cultivars do not flower naturally, however it is known that gibberellic acid promotes flowering and techniques for pollinating and growing seedling population have been used in breeding programmes. Although reports reveal that taro produce viable seeds under natural conditions, the number of seeds per fruit varies from very few to thousands. Germination of taro seeds is affected by genotype, environmental factors, harvesting and storage conditions in addition to germination protocols (Shaw, 1975; Strauss *et al.,* 1979; Kuruvilla and Singh, 1980; Hanson and Imamuddin, 1983; Wilson, 1990; Ivancic, 1992; Singh *et al.,* 2001). In CTCRI, taro flowers and sets seeds, but flowering is irregular and genotype dependent.

## 2.7 NUTRITIONAL VALUE

Taro (is one of the most established cultivated crops developed for its edible corms and leaves. It serves as a staple for individuals around the world and is the fourteenth most expended vegetable around the world (Rao et al., 2010). All parts of the plant including corm, cormels, leaves, stalk, rhizome and flowers are palatable and contain bounteous starch (Bose et al., 2003). Its leaves contain larger amounts of protein and are likewise good source of carotene, potassium, calcium, iron, phosphorous, riboflavin, niacin, thiamine, nutrient A, nutrient C and dietary fiber (Bradburry and Holloway, 1998). Corms and leaves are additionally credited with restorative qualities and are utilized to reduce tuberculosis, ulcers, pneumonic clog and parasitic disease. Taro corm is a fantastic wellspring of sugar, the dominant part being starch of which 17-28% is amylase, and the rest of amylopectin (Oke, 1990).The size of starch grain is one-tenth that of potato and its digestibility has been estimated to be 98.8% because of which, it is suitable for people with digestive problems. 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).

## 2.8 ECONOMIC AND CULTURAL IMPORTANCE

Taro is a staple food crop in Fiji and parts of Africa. Tuber crops represent almost 50% of the Nation's calorie food of which about 40% is contributed by taro in Tonga. Similarly, in Samoa, prior to the spread of taro leaf blight disease, all the populations' dietary intake from tubers came from taro (CTA, 2003). It has now emerged as one of the principal export commodities presenting vast foreign exchange to some Pacific Islands.

Substantial amounts of taro are delivered in Asia and Pacific districts, with the corm being boiled, baked or fried and consumed with fish and coconut preparations. Most favorite and exceptionally Pacific approach to prepare taro is to cook it on hot stones in dugout earth stoves which is very regular in feasts and ceremonies. Younger taro leaves utilized as a fit for human consumption vegetable at some point of Melanesia and Polynesia in which they are generally boiled or covered with coconut cream, wrapped in banana or breadfruit leaves and cooked on warm stones. Taro can be processed and stored as chips and Poi.

Griffin (1982) has accentuated the advancement of taro silage and its use as animal feed particularly for swine, the functionality of taro alcohol as a fuel for far off islands and the capability of taro starch as a crude fabric in plastic fabricate and cosmetics. Moreover, taro flour and different products are utilized extensively for newborn child formulae in the United States and have framed a vital constituent of proprietary canned baby foods (Lee, 1999).

Taro has evolved with the cultures of people in Asia and Pacific region and has acquired considerable socio-cultural importance. It is considered a prestige crop and the crop of choice for royalty, gift-giving, traditional feasting and the fulfillment of social obligations. It features prominently in the folklore and other traditions of many cultures in Oceania and South-East Asia. Countries like Samoa and Tonga have prominent depictions of taro on their currencies. Moreover, in Hawaii, images of taro and taro farmers can be found throughout the islands, in murals, posters, original arts and other visuals, where its symbolic importance reflects its continuing role as a common food and common element in the agricultural landscape. The sociocultural attachment to taro means that taro itself has become a symbol of cultural identification, such that the people of Pacific Island origin continue to consume taro wherever they may live in the world. This is one of the means of maintaining links with their culture; consequently, this cultural attachment to taro has spawned a lucrative taro export market to ethnic Pacific Islanders living in Australia, New Zealand and western North America (Matthews, 1998; Onwueme, 1999).

## 2.9 MOLECULAR CHARACTERIZATION

The main advantages of using genetic / molecular markers and the potential value of linkage maps and direct plant breeding selection were first reported about eighty years ago (Crouch and Ortiz, 2004). It was not until the advent of DNA marker technology during the 1980s that countless obtuse genetic markers were created, to pursue the inheritance of important agronomic qualities and from that point forward DNA marker innovation has drastically upgraded the proficiency of plant breeding. DNA-based molecular markers have been a versatile tool and have found their own place in different fields such as taxonomy, plant breeding, genetic engineering and many others (Joshi *et al.*, 2011).

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Wide ranges of techniques are available to study the variation of DNA for characterizing taro germplasm. Development and use of molecular markers has led to tremendous progress in characterization and evaluation of plant genetic resources (Gupta and Varshney, 2000). Molecular data could be used in association with morphological analysis to reduce redundancy in collection and to maintain cost efficiency (Pissard *et al.*, 2008). Marker assisted selection is an advantageous method and it is very convenient in research field. Use of molecular markers saves time in lots of breeding programmes and aids in coming across more beneficial facts about the characteristic of the gene of interest and enables its use in genetic diversity assessment and quality control (Kithinji, 2011).

## 2.9.1 Molecular markers

Molecular markers are fragment(s) of DNA indicating mutations/variations that can be used to detect polymorphism between alleles of a gene for a particular sequence of DNA or different genotypes. Such fragments are linked to a definite location within the genome and may be detected by using certain molecular technology (Henry, 2012). Genes of agronomic and scientific importance can be isolated on the basis of their position on the genetic map (Thottappilly *et al.*, 2000). Recent years have witnessed a great interest towards molecular markers, revealing polymorphism at the DNA level. Sometimes the term "Smart Breeding" is used to describe marker supported breeding strategies.

A vast array of DNA based genetic markers has been discovered and new marker types are developed every year. Molecular marker is a sequence of DNA, which are located with a known position on the chromosome (Kumar, 1999), or a gene whose phenotypic expression is frequently easily

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discerned and used to detect an individual, or as a probe to mark a chromosome, nucleus, or locus (King and Stansfield, 1990; Schulmann, 2007). Markers exhibit polymorphism (Hartl and Clark, 1997) which make it possible to identify genetic differences between individual organisms/species (Collard *et al.*, 2005). Molecular markers such as Random Amplified Polymorphic DNA - RAPD (Williams *et al.*, 1990), Restriction Fragment Length polymorphism - RFLP (Grodzicker *et al.*, 1974), Inter Simple Sequence Repeats - ISSR (Zietkiewics *et al.*, 1994), Simple Sequence Repeats - SSR (Akkaya *et al.*, 1992), Single Nucleotide Polymorphism - SNP (Jordan and Humphries, 1994), Amplified Fragment Length Polymorphism - AFLP (Vos *et al.*, 1995), Sequence Characterized Amplified Regions - SCAR (Paran and Michealmore, 1993), Cleaved Amplified Polymorphic Sequence - CAPS (Akopyanz *et al.*, 1992), Sequence Tagged Site - STS (Olsen *et al.*, 1989) and Arbitrarily Primed Polymerase Chain Reaction AP-PCR (Welsh and McClelland, 1991), etc. are used to construct genetic map (Mohan *et al.*, 1997). Each marker has properties which differ from other markers in one or more properties. It is extremely difficult for a single genetic marker to possess all properties, hence, depending on the type of study to be undertaken a marker system can be identified that would fulfil at least a few of the characteristics.

2.9.1.1 Random Amplified Polymorphic DNA (RAPD)

RAPD is based on the PCR amplification of random DNA segments with primers of random nucleotide sequences. The primers bind to complementary DNA sequences and here two primers bind to the DNA sample in close enough position for successful PCR reaction. The amplified DNA products can then be visualized by gel electrophoresis (Williams, 1999; Gupta and Varshney, 2013). RAPD has been widely used in diverse plant species for assessment of genetic variation in populations and species, fingerprinting and study of phylogenetic relationships among species and

subspecies (Gupta, 1999). Nevertheless, disadvantages of RAPD markers are the fact that, it predominantly provides dominant markers, and is incapable to detect allelic differences in heterozygotes. Polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity (Dunn, 2005). Additionally, because of their random nature of amplification and short primer length, they are not ideal for genome mapping. Moreover, these markers do not exhibit dependable amplification patterns and differ with the experimental conditions.

Forty-four accessions of *Colocasia esculenta*, two *Xanthosoma* sps. and one *Colocasia gigantea* accessions were evaluated using RAPD primers. Of the 112 primers used seventy-three amplified, among which, thirty-two primers were highly informative as they amplified more than 5 bands. RAPDs showed high genetic diversity in taro accessions from Indonesia, were capable in distinguishing between Hawaiian accessions, and could separate triploid from diploid accessions. UPGMA cluster analysis separated the accessions into 3 main groups with *C. esculenta* divided into 5 subgroups. These primers will be useful for future genetic analysis and provide a genetic basis for selection of parents (Irwin *et al.*, 1998).

Taro germplasm accessions collected from different parts of India were subjected to RAPD analysis by Lakhanpaul *et al.* (2003) to assess the genetic diversity and to test the genetic basis of monotypic classification. Thirteen random decamer primers out of the 22 tested were used to analyze 32 taro accessions belonging to 28 morphotypes. Three out of thirteen primers showed 100% polymorphism (varied from 60 to 100). Similarity coefficient values ranged from 0.50 to 0.98. No two accessions analyzed showed a similarity coefficient value of one indicating their distinctness and high diversity. UPGMA analysis grouped genotypes into four clusters among which three accessions were placed as outliers. Clustering pattern

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did not show any strict relationship with geographical distribution, morphotype classification and genetic diversity. Presence of a very close gene-pool of the wild, weedy and cultivated forms with extreme levels of phenotypic and genotypic variation was suggested as the reason for high genetic diversity.

Ten taro accessions collected from different parts of India were subjected to RAPD analysis by Mishra *et al.* (2008) using eight random primers. Band sizes obtained from 0.4 to 2 kb and the number of scorable bands per primer ranged from 1 to 13 with an average of 9.75 bands per primer. One quarter of the primers analysed showed 100% polymorphism. High genetic diversity was revealed that ranged from 0.62 to 0.98. No two accessions analysed showed a similarity coefficient value of one thereby indicating their distinctness and diversity. Dendrogram obtained from UPGMA analysis grouped 10 accessions in two clusters. Clustering did not show any strict relationship with geographical distribution, morphotype classification and genotypic diversity. Forty-three loci or 84.31% were polymorphic. The presence of new recombination events by random and natural processes of mutation might be the reason for high diversity.

Geographical differentiation and phylogenetic relationships of Indian taro were analyzed by RAPD and isozyme of seven enzyme systems with specific reference to the Muktakeshi (resistant to taro leaf blight). RAPD markers showed higher values for genetic differentiation and lower coefficient of variation than those obtained from isozymes. It appears that when taro cultivation was introduced to a new area, only a small fraction of genetic variability in heterogeneous taro populations was transferred, possibly causing random differentiation among locally adapted taro populations. Selected primers will be useful for future genetic analysis and selection of parents while polymorphic markers identified in DNA fingerprinting will be helpful for screening a segregating population to

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The genetic diversity of taro accessions growing naturally in Andaman Islands were analyzed using morphological and DNA markers (Singh *et al.*, 2012). A total number of 491 amplified fragments were obtained of which 347 showed polymorphic banding patterns. The accessions were grouped into two major clusters with both RAPD and ISSR markers with 56 and 57% diversity, respectively. The reference genotypes were grouped into one cluster and Island population in other cluster. Both marker systems divided population into two sub clusters and showed correlation with morphological parameters. The diversity pattern observed showed rich genetic diversity and provided simple strategy for reducing repeatability of taro germplasm in gene banks.

Das *et al.* (2015) performed detailed karyotype, genome size and RAPD marker analysis to assess genetic diversity in taro. Amplification of genomic DNA in 10 genotypes using operon primers yielded 230 amplified fragments, ranging in size from 200 to 2500 bp, out of which 79 bands were polymorphic. A total of 8 RAPD bands were observed and primer wise polymorphism ranged from 16.66 to 47.36% with an average of 34.34%. Among the cultivars, the polymorphic percentage varied from 3.70%

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between DR-25 and Duradin and Telia and H-3 to 41.94% between Mothan and cv. Muktakeshi. Genetic similarity varied from 0.54 to 0.96, indicating wide genetic variability among the varieties. Similarity measures and cluster analysis generally reflected the expected trends in relationships of diploid and triplod taro varieties. Dendrogram obtained could be useful to choose the diverse parents for varietal improvement.

2.9.1.2 Inter simple sequence repeats (ISSR)

ISSR involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. This technique uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes. ISSRs are widely used in genetic diversity studies because it does not require genome sequence information for designing the primer, and are not proprietary, can be synthesized by anyone, their development costs are low, and the laboratory procedures can easily be applied to any plant species (Aga et al., 2005; Tesfaye et al., 2013; Zietkiewicz et al., 1994). They provide highly reproducible results and generate abundant polymorphisms. This technique can rapidly differentiate closely related individuals (Zietkiewicz et al., 1994) and have been successfully used to assess genetic diversity among closely related cultivars which were difficult to distinguish with other markers (Dagani et al., 2003; Salhi-Hannachi et al., 2005; Okpul et al., 2005). ISSR markers is considered to be rapid, robust and provide more informative data sets with less effort and cost than other dominant molecular marker techniques (Godwin et al., 1997; Salimath et al., 1995; Yang et al., 1996).

In a study on agro-morphological characterization, inter-simple sequence repeat (ISSR) markers were used to evaluate variability among

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selected Papua New Guinean (PNG) taro accessions. Twenty-three descriptors selected from the International Plant Genetic Resource Institute (IPGRI, 1999) descriptor list were combined with ISSR markers to characterize 13 taro accessions. Genotyping data were obtained *via* PCR with three ISSR primers [$(GA)_9AT$, $(GA)_9AC$ and $(ACC)_6Y$]. Initial evaluation of the agro-morphological variation indicated that the total variation was observed to be associated with corm flesh colour, corm cortex and petiole, stolon formation, corm shape, lamina orientation and lamina vein pattern. All genotypes were separated as distinct morphotypes. ISSR primers generated complex banding patterns, with primer $(GA)_9AC$ amplifying fewer but proportionately more (90%) polymorphic fragments. Fingerprinting with three ISSR primers enabled the identification of closely related genotypes and the separation of distantly related ones. There was not much correlation in between the agro-morphological and molecular data in the clusters generated (Okpul *et al.*, 2005).

A study was conducted to analyze the morphological and molecular characteristics of fourteen samples of *X. sagittifolium* and *C. esculenta* collected from Sao Paulo and Minas Gerais of Brazil (Maria *et al.*, 2017). Molecular analyses using eight ISSR revealed distinctive fingerprints for each of the species. The results with the 15 ISSR markers showed that only one primer did not have any amplification products (UBC 813), while other primers (UBC 2, 834, 845, 851, 858, 860, 864, 866) produced high resolution profiles which were selected for next stage. From these amplifications, 334 loci were obtained, with an average of 41.75 loci, varying between 22 (UBC 866) and 73 (UBC 2). Of these, 321 loci (96.11%) exhibited polymorphism and the rest 13 loci (3.89%) were monomorphic which showed an average of 26.52% genetic diversity among the species.

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## 2.9.1.3 Simple Sequence Repeats (SSRs)

During 1990s, Simple sequence repeats (SSRs) which are also known as microsatellites were discovered and provided a choice for many genetic researches since they are amenable to low, medium and high-throughput approaches. They are randomly tandem repeats of short nucleotide motifs (2 - 6 bp) (Dunn, 2005). SSRs are highly polymorphic sequences (Kalia, 2011) used to study the relationship between inherited traits within a species. They are often derived from noncoding/anonymous genomic regions, such as bacterial artificial chromosomes (BACs) and genomic survey sequences (GSSs). Therefore, development of these markers is expensive and laborious (Mir, 2013). This assay is easily detectable by gel electrophoresis for few to hundreds of samples, which could be inexpensive by researchers with limited resources. Polymorphism is based on the variation in the number of repeats in different genotypes (Ellegren, 2000). Since polymorphisms in longer penta-nucleotide and tetra repeats are easier to make a distinction in a variety of detection systems and longer repeats may be more robust (Koelling, 2002). In recent years, SSR markers can easily be developed *in silico* due to the availability of large-scale gene (expressed sequence tag) ESTs. Since EST sequencing projects have provided sequence data that is available in online databases and can be scanned for identification (Varshney and Graner, 2005). High degree of polymorphism as compared to RFLPs and RAPDs, their co-dominant nature and locus specificity, make them the markers of choice for a diversity of purposes. Therefore, SSRs have become a marker of choice for an array of applications in plants due to its extensive genome coverage and hyper variable nature. However, major drawback of SSRs is the cost and time needed for development. Characterization using simple sequence repeats (SSR) was undertaken for both *C. esculenta* and *Xanthosoma* (Godwin *et al.*, 2001; Mace and Godwin, 2002).

Khan Hu *et al.* (2009) designed forty-eight primer pairs from a microsatellite-enriched genomic library. Out of which 11 primer pairs have polymorphisms in 30 taro individuals collected from China. The results of which revealed two to six alleles per locus with the observed and expected heterozygosity levels ranging from 0 to 0.733 and 0.381 to 0.731, respectively which will be further useful for population ecology studies.

In a study, microsatellite containing sequences were isolated from enriched genomic libraries of *Colocasia esculenta* (L.) Schott. The sequencing of 269 clones yielded 77 inserts containing repeat motifs. The majorities (81.7%) were dinucleotide or trinucleotide repeats. The GT/CA repeat motif was the most common, accounting for 42%. From a total of 43 primer pairs designed, 41 produced markers within the expected size range, sixteen (39%) were polymorphic when screened against a restricted set of taro genotypes from Southeast Asia and Oceania, with an average of 3.2 alleles on each locus. These markers represent useful resource for taro germplasm management, genome mapping, and marker-assisted selection (Emma and Ian, 2002).

About 92 accessions of taro collected from various parts of South Africa were characterized using six simple sequence repeat (SSR) primers which exhibited a total of 52 scorable bands. Level of genetic diversity was observed to be high and three major clusters were identified in the cladogram. The accessions from the same locality did not always group together while some accessions collected from different provinces clustered together. The results suggested that germplasm has and is being exchanged between farmers from different regions, which is supported by anecdotal information from farmers. Furthermore, investigations on molecular level are required to give a better understanding of the genetic diversity within the local taro germplasm and how it contrasts with foreign germplasm (Jansen *et al.*, 2013).

Six microsatellite primers were deployed to analyze five populations of taro (consisting of 98 taro cultivars) from three different regions of East Africa covering Kenya, Tanzania and Uganda (Mercy *et al.*, 2014). Principal component analysis indicated variation but did not show any distinct structure. Population diversity estimate was relatively low with the highest being 0.27, for accessions sourced from Lake Vitoria Basin. Analysis of molecular variance (AMOVA) revealed most variation among individuals within population at 79%. Nei"s genetic distance revealed that relatedness is not based on geographical proximity. This study proposes for establishment of a regional collection that will be conserved and ensure a broad genetic base for available varieties and enable development of improved varieties through breeding programmes.

Diversity of 46 taro accessions collected from National Plant Genetic Resource Laboratory, Phillipiness were analyzed using fifteen sets of simple sequence repeats (SSR), among which 10 SSRs resulted in band amplification. Polymorphic Information Content (PIC) based on the banding pattern ranged from 0.69 to 0.96 indicating high genetic diversity. A dendrogram generated using NTSYS-pc formed 8 clusters and this revealed no association between geographic origin and genotypes of germplasm resources suggesting that the accessions even within location are diverse (Jhun *et al.*, 2016).

Palapala and Akwee (2016a) used six SSR primers to characterize 25 taro genotypes collected from different parts of Kenya. A total of 30 polymorphic alleles were generated. The number of alleles per locus ranged from 1 to 6 alleles, with an average of 3.0425 alleles across 18 loci. The PIC values ranged from 0.1875 to 0.5731 with an average of 0.4120 whereas, genetic diversity ranged from 0.25 to 0.6218 with genetic richness between 1.5 and 4.67. The frequency of the most common allele at each locus varied from 51.21% to 75%. Pair wise genetic dissimilarity co-efficient indicated

highest genetic distance between the Rift Valley and Nyanza populations (0.794). The closest allelic similarity was between Western and Nyanza (83.1%) populations while, the widest dissimilarity was between Rift Valley and Nyanza populations (45.2%). Being grouped into a distant cluster KK12 could be exploited as a probable parental for the development of variant taro varieties. In this study SSRs proved to be comprehensive source for the identification of genetically distant taro accessions as well as in the replica sorting of the phenotypically close germplasm.

Genetic structures of Kenyan and Tarogen taro germplasm (consisting of 50 cultivars) were studied using six SSR primers (Palapala and Akwee, 2016b). A total of 64 alleles were detected with sizes from 99 to 294 bp. Kenyan germplasm generated 30 alleles and averaged 5.0 alleles/locus while the Tarogen revealed 34 alleles with an average of 5.70 alleles/locus. Primers generated a higher PIC in Tarogen (0.6508) compared to Kenyan (0.6108) genotypes and genetic diversity index mean in Tarogen (0.6989) than Kenyan (0.6530) genotypes. Genetic diversity values ranged between 0.25 to 0.6218 and 0.25 to 0.06204 for Kenyan and Tarogen genotypes. Allele frequency ranged from 42.52 to 75% across the populations. Observed mean heterozygosity was lower than the expected mean genetic diversity indexes of 0.6530 and 0.6989 for Kenyan and Tarogen. Kenya recorded the highest mean genetic diversity (0.4735) and richness (3.04), allele frequency (0.62) and PIC (0.412) while Tarogen recorded 2.0, 0.4527, 0.5988 and 0.3917, respectively. All the evaluated primers displayed distinct polymorphisms among the taro accessions studied indicating their robust nature.

The determination of genetic diversity is core function towards understanding taro genetic resources for varietal identification to rationalize its collection and safeguarding the existing genetic diversity for taro germplasm conservation, management and for potential utilization for food security. Six SSR markers were used to genotype 50 cultivars collected

from Kenya and a taro genebank (SPC Tarogen) (Palapala and Akwee, 2016c). The average polymorphic loci were 87.88%. The highest Shannon information index was observed in the germplasm from Nyanza (1.04), Western (1.2) and Hawaii (1.11) and Malaysia (1.36). Only Malaysia and Thailand germplasm had allele unique to a single locus. Analysis of molecular variance (AMOVA) revealed that 70% of the variations found within individual taro accessions, 6% of variations among the taro populations and only 24% amongst individual taro genotypes. In total 50.06% and 51.82% of the variation was explained by the first three principal components of the taro germplasm. Some of the Kenyan taro cultivars clustered together with the Tarogen germplasm.

## 2.10 TARO LEAF BLIGHT

Leaf blight of taro caused by *Phytophthora colocasiae* Raciborski, is the most destructive disease of *Colocasia*. The occurrence of leaf blight has been reported from many countries. Butler and Kulkarni (1931) reported this for the first time in India. This disease is reported to have destroyed taro plantings in Papua New Guinea (Packard, 1975) and in American Samoa (Gurr, 1996). In India, leaf blight is reported to be a serious disease in many areas such as Kangra valley of Punjab (Luthra, 1938), Assam (Chowdhury, 1944), Bihar (Anonymous, 1950), Himachal Pradesh (Paharia and Mathur, 1961) and other states (Prasad, 1982; Thankappan, 1985; Misra, 1999). The geographical distribution of the disease is probably restricted to South-East Asia and Pacific Islands (Holliday, 1980).

Initial symptoms of the disease are small brown water-soaked flecks on the leaf that enlarge to form dark brown lesions, often with a yellow margin. Secondary infection leads to rapid destruction of the leaf which occurs in 10-20 days or less in susceptible varieties. The disease significantly reduces the number of functional leaves and can lead to yield reductions to the magnitude of 50% (Trujillo and Aragaki, 1964; Trujillo,

1967; Thankappan, 1985; Jackson, 1999; Misra and Chowdhury, 1997). Inoculum in the form of spores spread by wind driven rain and dew to adjacent plants and near-by plantations. The disease can also spread on taro planting material and the fungus has been reported as remaining alive on planting tops for about three weeks after harvest causing corm rot in storage (Jackson, 1999). Leaf blight is observed in severe form in areas having high relative humidity and frequent rainfall, whereas, warmer areas having little rainfall and relative humidity are comparably free from the disease. Trujillo (1965) found that blight epidemics occur when night and day temperatures ranged between 20-22 and 25-28°C, respectively, with a relative humidity of 65% during the day and 100% at night and accompanied by overcast rainy weather. Occasional sunlight with intermittent rain is more favourable for disease severity compared to prolonged cloudy weather with rainfall (Misra and Chowdhury, 1997).

Different varieties respond differentially against the *P. colocasiae* which is mainly due to the genetic make-up of taro which may favor the growth and spread of the pathogen or may resist and eliminate the spread of pathogen. Seeking out the resistant varieties from the taro accessions may help in eradicating the harmful effects of the disease. Metalaxyl- and mancozeb-based fungicides have been advocated to control disease. But waxy leaf surface and occurrence of disease during rainy season make fungicidal spray ineffective (Misra, 1999). Furthermore, the sprays are too costly to be afforded by small and marginal farmers, soil microorganism rapidly degrade Metalaxyl and release it into water and soil system and development of resistance against the fungicides is another major threat (Cohen, 1986). Thus, there is need to develop integrated management strategies to combat this disease using natural and environmental friendly mechanism. Perhaps, since most taros produced in India is consumed locally and never reaches the international market; its problems have been

relatively neglected (Jackson *et al.*, 1979; Gregory, 1983). In this view an attempt has been made to find out the markers linked to taro leaf blight.

## 2.11 MOLECULAR MARKER IDENTIFICATION AND ANALYSING TRAIT SPECIFIC GENES

Molecular markers are becoming essential tools in many plant breeding programs (Mohan *et al.*, 1997, Gupta *et al.*, 1999) and have several advantages over the traditional phenotypic markers that are difficult or time-consuming. These DNA markers are not influenced by environmental conditions and are detectable at all plant growth stages. Availability of tightly linked molecular markers can now be used specially for disease resistance gene where it is possible to infer the gene by the marker without depending on the natural pest or pathogen occurrence or waiting for its phenotypic expression. Moreover, molecular markers flanking disease resistance genes may be starting points for gene cloning and subsequently comprehension of their biological mechanisms (Martin *et al.*, 1993, Tanksley *et al.*, 1995).

Taro leaf blight resistance by breeding is an extremely cost-effective and environmentally acceptable approach. The success of breeding for resistance against TLB depends on the availability of genetic resources and the type of resistance they confer. Attempts were made at ICAR-CTCRI, Thiruvananthapuram, India (Pillai *et al.*, 1993) to develop TLB resistant taro lines through breeding. The maximum portion of resistant genotypes was obtained from variety „c-320" self (66%), followed by open pollinated progeny of „c-12" (33.33%), „c-78" (30%) and „Nadia local" (26.31%). Among the crosses, the maximum portion of resistant genotypes were obtained in „G2 × G16" (25%) followed by „Pig × G6" (23.8%). None of the tolerant parent bred were true for resistant genes. The appearance of resistance genotypes in the population resulting from crosses between two

partially susceptible genotypes was observed by Ivanicic *et al.*, 1995 which indicates that minor genes associated with partial resistance are involved.

Sriram *et al.* (2001) isolated glucan elicitors from *P. colocasiae* isolates. This PC-glucan elicitor could induce a hypersensitive reaction in the field tolerant cultivars like „Muktakeshi" and „Jankheri" while the induction of hypersensitive reaction was not induced or delayed in the susceptible variety „Telia". *In vitro* screening of the taro varieties for leaf blight resistance using PC-glucan elicitor has also been standardized.

Sharma *et al.* (2008a) employed Amplified Fragment Length Polymorphism (AFLP) analysis to analyze the geographical differentiation, phylogenetic relationships and to identify molecular markers.

# Material and Methods

# 3. MATERIALS AND METHODS

The present investigation **"Identification of molecular markers for resistance to taro leaf blight in taro (*Colocasia esculenta* (L.) Schott.)"** was undertaken with an objective to identify, characterize and validate the molecular markers (RAPD, ISSR and SSR) associated with taro leaf blight resistance. The study was undertaken at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala during 2017-2018. The details regarding the materials used and methods followed during the experiment were elucidated here.

## 3.1. SAMPLE COLLECTION

The experimental material for molecular characterization comprised of 36 genotypes of taro screened previously (18 genotypes each of resistant and susceptible ones), collected from the germplasm field maintained at the field genebank of ICAR-CTCRI, Sreekariyam.

Table 1.  List of 36 genotypes of taro selected for the study

| S. No. | Code | Genotype | S. No. | Code | Genotype |
|--------|------|----------|--------|------|----------|
| 1. | R1 | 450 | 19. | S1 | Sree Rashmi |
| 2. | R2 | IC012601 | 20. | S2 | 276 |
| 3. | R3 | TCR 429 | 21. | S3 | 557 |
| 4. | R4 | 723 | 22. | S4 | 628 |
| 5. | R5 | IC089624 | 23. | S5 | TCR 514 |
| 6. | R6 | IC122159 | 24. | S6 | VRS |
| 7. | R7 | 66 | 25. | S7 | Sonajuli (Bhu Sree) |
| 8. | R8 | 565 | 26. | S8 | Jhankri (Bhu Kripa) |
| 9. | R9 | E10 | 27. | S9 | Sree Pallavi |
| 10. | R10 | B4 | 28. | S10 | 22 |
| 11. | R11 | IC310104 | 29. | S11 | 485 |
| 12. | R12 | J14 | 30. | S12 | IC420620 |

table 1 cont…

| S. No. | Code | Genotype | S. No. | Code | Genotype |
|--------|------|----------|--------|------|----------|
| 13. | R13 | 370 | 31. | S13 | IC089583 |
| 14. | R14 | L12 | 32. | S14 | 553 |
| 15. | R15 | 203 | 33. | S15 | 85 |
| 16. | R16 | 679 | 34. | S16 | 621 |
| 17. | R17 | 84 | 35. | S17 | TCR 961 |
| 18. | R18 | Muktakeshi | 36. | S18 | B2 (SVP) |

From these 18 resistant genotypes, seven genotypes (E10, B4, IC310104, 370, 203, 679 and 84) were found to be resistant for the last four years under artificial screening.

## 3.2 DNA ISOLATION PROTOCOL

The CTAB extraction method developed by Doyle and Doyle (1987) and later modified by Sharma *et al.* (2008) was used for the isolation of DNA which is described below.

About 160mg of fresh taro leaf samples were collected during morning hours from the germplasm field and grounded into powdered form using liquid nitrogen in a pre-autoclaved mortar and pestle. 2ml of freshly prepared extraction buffer (Appendix I) was added to the powdered sample and the contents were homogenized before it was thawed. The mixture was then transferred into 2ml Eppendorf tubes. 5μl of proteinase K (10mg/ml) was added to these tubes and were incubated for two times, first at 37°C and again at 65°C respectively, for 30 min in a water bath with intermittent mixing. The tubes were then centrifuged at 12,000 rpm for 15 minutes and the supernatant thus obtained was transferred to fresh tubes. Equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed by thorough inversion. The tubes were allowed to stand for 5 minutes at room temperature for phase separation and

then centrifuged at 12,000 rpm for 15 minutes. The upper aqueous phase formed was transferred to fresh 2ml tubes (Eppendorf) using cut tips, to which equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed gently through inversion. After centrifugation at 12,000 rpm for 15 minutes, the resultant upper aqueous phase was transferred to 1.5ml tubes (Eppendorf). To this, equal volume of isopropanol was added and mixed gently till DNA threads were obtained, which were precipitated by centrifugation at 10,000 rpm for 10 min and then washed using 70% ethanol 2-3 times. The pellets formed were air dried to remove any traces of ethanol and was dissolved in 100µl TE buffer (APPENDIX II). About 5µl of RNase (10mg/ml) was added and incubated for 1 hour at 37°C. The DNA obtained finally by this method was stored in a $-20^0$C freezer till further use.

## 3.3 QUANTITY AND QUALITY ANALYSIS OF THE DNA

The extracted DNA was analyzed to confirm its quantity and quality using spectrophotometer and agarose gel electrophoresis.

### 3.3.1 QUANTIFICATION OF THE DNA

The quality and quantity of the isolated DNA was assessed by measuring the absorbance at 260nm and 280nm using a Nano spectrophotometer (NANODROP® ND-1000). The software was switched on by clicking on the icon. From the "user" folder for storage of data, the "nucleic acid" button was selected. The Module startup panel will come up on the screen. For the first step, the pedestals were cleaned and water sample was loaded in order to initialize the instrument. For preparing a report of all readings, the "recording" button was pressed. The report can log either for 12 or 32 measurements. Before doing "DNA", "RNA" or any other analysis, the

"blank" measurement was chosen. A blank (TE buffer) must be measured and stored before measuring the samples. 1µl of the TE buffer was placed on the pedestal and pressed "blank". The pedestal was cleaned using „KIM" wipes. A fresh sample was loaded on the pedestal and the concentration was measured by clicking the "measure" icon. The concentration of DNA, $A_{260}$ value, $A_{260}/A_{280}$ ratio will be displayed by the instrument. These measurements could be used to check the quantity and quality of DNA. "Exit" was pressed to exit the instrument.

The concentration of the DNA present in the sample was calculated by the formula: concentration $(\mu g^{-1})$ = absorbance at 260nm × 50 × dilution

Where, the value 50 gives the concentration of DNA in µg/ml of unit absorbance.

Dilution factor = total volume / volume of sample taken

The purity was determined by measuring the ratio of OD at 260nm/280nm.

### 3.3.2 AGAROSE GEL ELECTROPHORESIS

The isolated DNA was checked on 1% agarose gel following electrophoresis. 1g of agarose (Sigma Aldrich) was weighed and dissolved in 100ml of 1X TBE buffer by boiling. Once the agarose dissolves and the solution attains a bearable temperature, 1µl of ethidium bromide was added to it. After gentle and thorough mixing of ethidium bromide, the gel was poured on to the casting tray by placing a comb appropriately. The gel was then allowed to solidify for 20-30 minutes, after which, it was placed into the electrophoresis tank, in a manner such that the wells are in the vicinity of the cathode. Adequate quantity of 1X TBE (APPENDIX III) running buffer was poured into the tank so as to see that the wells were properly submerged. Finally, the comb was removed carefully without disrupting the wells. About

2μl of 1X loading dye (APPENDIX IV) and 3μl of DNA were mixed thoroughly using a micropipette. From which 5μl of the mixture was carefully added to each of the wells. After loading all the samples, the tray was closed properly, and a voltage of 100V was given. The gel was then allowed to run for 15-20 min, till the dye front reaches almost 2/3rd of the total gel length and the image was captured using the gel documentation system (G: Box Gel documentation system, M/s. Syngene).

## 3.4 MOLECULAR MARKERS

Three kinds of molecular markers *viz*., RAPD, ISSR and SSR were employed for the identification of resistance to leaf blight in taro germplasm selected.

## 3.4.1 MOLECULAR CHARACTERIZATION USING RAPD

A set of 10 OPW series RAPD primers whose details are mentioned below (Table 2) were used in the present study. Composition of the PCR mixture for standardization of the template DNA and other components (Table 3) were taken in a PCR tubes for all the 36 genotypes of taro and subjected to PCR.

Table 2. Details of selected OPW series RAPD primers

| S. No. | Primer | Primer Sequence |
|--------|--------|-----------------|
| 1. | OPW1 | CTCAGTGTCC |
| 2. | OPW2 | ACCCCGCCAA |
| 3. | OPW5 | GGCGGATAAG |
| 4. | OPW6 | AGGCCCGATG |
| 5. | OPW8 | GACTGCCTCT |
| 6. | OPW12 | TGGGCAGAAG |
| 7. | OPW14 | CTGCTGAGCA |
| 8. | OPW16 | CAGCCTACCA |
| 9. | OPW17 | GTCCTGGGTT |
| 10. | OPW18 | TTCAGGGCAC |

Table 3. Composition of PCR reaction mix for RAPD primers

| Components | Stock concentration | Required concentration | Volume for one reaction (20µl) |
|-----------|---------------------|------------------------|--------------------------------|
| Buffer with 25mM $MgCl_2$ (Genei) | 10X | 1X | 2µl |
| $MgCl_2$ (Genei) | 25mM | 1.5mM | 1.2µl |
| dNTP (Genei) | 10mM | 0.2mM each | 0.4µl |
| Primer | 10µM | 0.6µM | 1.2µl |
| Template DNA | 10ng/µl | 30ng | 3µl |
| Taq DNA Polymerase (Genei) | 5U/µl | 1U | 0.2µl |
| $dH_2O$ | - | - | 12µl |
| Total | - | - | 20µl |

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PCR programming for RAPD primers was carried out in a Biorad thermal-cycler with steps of initial denaturation done at $94^0$C for 3 min followed by 40 cycles of denaturation at $94^0$C for 1 min, annealing at $32^0$C for 1 min and extension at $72^0$C for 1 min. Final extension was done at $72^0$C for 5 min and cooling at $4^0$C for $\infty$.

The PCR products thus obtained were resolved in a 2% agarose gel with 100bp and 1kb ladder in flanking wells so as to compare the size of amplicons. The gel was run at 100 V for 45 min. Once the run was completed, the images were captured in a gel documentation system for further analysis.

## 3.4.2 MOLECULAR CHARACTERIZATION USING ISSR

A set of 18 UBC ISSR primers whose details are mentioned below (Table 4) were used for the study. Composition of PCR mixture for standardization of the template DNA and other components (Table 5) were taken in a PCR tubes and the selected 36 genotypes of taro was subjected to PCR.

Table 4. Details of ISSR primers (UBC series)

| S. No. | Primers | Primer sequence (5'- 3') |
|--------|---------|--------------------------|
| 1. | UBC 809 | AGAGAGAGAGAGAGAGG |
| 2. | UBC 811 | ATATATATATATATATT |
| 3. | UBC 810 | GAGAGAGAGAGAGAGAT |
| 4. | UBC 814 | CTCTCTCTCTCTCTCTA |
| 5. | UBC 817 | CACACACACACACACAA |
| 6. | UBC 818 | CACACACACACACACAG |
| 7. | UBC 824 | TCTCTCTCTCTCTCTCG |
| 8. | UBC 825 | ACACACACACACACACT |
| 9. | UBC 827 | ACACACACACACACACG |
| 10. | UBC 836 | AGAGAGAGAGAGAGAGYA |
| 11. | UBC 841 | GAGAGAGAGAGAGAGAYC |
| 12. | UBC 847 | CACACACACACACACARC |
| 13. | UBC 848 | CACACACACACACACARG |
| 14. | UBC 857 | ACACACACACACACACYG |
| 15. | UBC 873 | GACAGACAGACAGACA |
| 16. | (AG)$_9$AC | AGAGAGAGAGAGAGAGAGAC |
| 17. | (AG)$_9$AT | AGAGAGAGAGAGAGAGAGAT |
| 18. | (ACC)$_6$ Y | ACCACCACCACCACCACCY |

Y = C or T

Table 5. Composition of PCR reaction mix for ISSR primers

| Components | Stock concentration | Required concentration | Volume for one reaction (20µl) |
|---|---|---|---|
| Buffer with 25mM MgCl$_2$ (Genei) | 10X | 1X | 2µl |
| MgCl$_2$ (Genei) | 50mM | 1mM | 0.4µl |
| dNTP (Genei) | 25mM | 0.2mM each | 0.16µl |
| Primer | 10µM | 0.25µM | 0.5µl |
| Template DNA | 10ng/µl | 40ng | 4µl |
| Taq DNA Polymerase (Genei) | 5U/µl | 1U | 0.2µl |
| dH$_2$O | - | - | 12.74µl |
| Total | - | - | 20µl |

PCR programming for ISSR primers was carried out in a Biorad thermal-cycler with steps of initial denaturation done at $94^0$C for 5 min followed by 35 cycles of denaturation at $94^0$C for 30 sec, annealing at $56.3^0$C for 1 min and extension at $72^0$C for 1 min. Final extension was done at $72^0$C for 10 min and cooling at $4^0$C for ∞.

The PCR products so obtained were analyzed in a 1.8% agarose gel using with 100bp and 1kb ladder, the images were captured in a gel documentation system for further analysis.

### 3.4.3 MOLECULAR CHARACTERIZATION USING SSR

Two of the most widely used SSR primer series *viz.*, Uq (Mace and Godwin, 2002) and Ce1 (Noyer *et al.*, 2004) series were selected for screening *viz.*,

Uq series: Uq 84-207, Uq 110-283, Uq 88B-94, Uq 97-256, Uq 201-302, Uq 115-71, Uq 132-147 and Uq 73-164

Ce1 series: Ce1 F12, Ce1 F04, Ce1 A08, Ce1 B03

**Gradient PCR for T$_a$ optimization**

According to the data obtained from the preliminary screening, standardization of the annealing temperatures using gradient PCR for the selected primer series were found necessary. Gradient PCR was carried out for the primers with a temperature range of $40^0$C-$60^0$C to obtain the most accurate annealing temperature giving specific bands with minimum errors.

Table 6. Details of selected SSR primers

| S. No. | SSR ID | Sequence |
|--------|--------|----------|
| 1. | Uq 84-207 | F-CCCATTGGAGAGATAGAGAGAC<br>R-AGGACAAAATAGCATCAGCAC |
| 2. | Uq 110-283 | F-GCCCAGTATATCTTGCATCTCC<br>R-AGCCACGACACTCAACTATC |
| 3. | Uq 88B-94 | F-CACACATACCCACATACACG<br>R-CCAGGCTCTAATGATGATGATG |
| 4. | Uq 97-256 | F-GTAATCTATTCAACCCCCCTTC<br>R-TCAACCTTCTCCATCAGTCC |
| 5. | Uq 201-302 | F-CTAAGGAGAGGAGATCCGAAC<br>R-CAAGACGATGCTGAACCAC |
| 6. | Uq 115-71 | F-CCCCTCTTTTGTAATAATCC<br>R-GTTTAAATGACTTGTTCTGC |
| 7. | Uq 132-147 | F-ACCCCGAAAAAGCCAATG<br>R-CTATCACTTGTTCCTCCTTCTC |
| 8. | Uq 73-164 | F-CGTCTAGCTTAGGACAACATGC<br>R-ATGCCAATGGAGGATGGCAG |
| 9. | Ce1 F12 | F-GATGCCTGTCCTTATGTTT<br>R-CTTAGCTTGTTCCCTAC |
| 10. | Ce1 F04 | F-ACGAGGGAAGAGTGTAAA<br>R-AGGGAATACAATGGCTC |
| 11. | Ce1 A08 | F-CATTGAGTGTTGGAAAAG<br>R- TGGGAAGTCATAATCTCA |
| 12. | Ce1 B03 | F-TTGCTTGGTGTGAATG<br>R-CTAGCTGTGTATGCAGTGT |

## 3.5.2 SSR ANALYSIS

A set of 12 SSR primers (table 6), were selected for characterization based on polymorphism and quality of electrophoretic patterns. SSR analysis was done for the selected 36 genotypes with the optimized annealing temperature. The amplicons were then resolved using 2.5% agarose gel.

## 3.5.3 SSR REACTION MIX FOR PCR STANDARDIZATION

Template DNA from 36 taro genotypes were subjected to PCR with pre fixed composition of PCR mixture (table 7).

Table 7. Composition of PCR reaction mix for SSR

| Components | Stock concentration | Required concentration | Volume for one reaction (20μl) |
|---|---|---|---|
| Taq buffer with 25mM MgCl$_2$ (Genei) | 10X | 1X | 2 |
| MgCl$_2$ (Genei) | 25mM | 1mM | 0.8 |
| dNTP (Genei) | 10 mM each | 0.3mM each | 0.6 |
| Primer (F) | 10μM | 0.25μM | 0.5 |
| Primer (R) | 10μM | 0.25μM | 0.5 |
| Template DNA | 10ng/μl | 20ng | 2 |
| Taq DNA Polymerase (Genei) | 5U/μl | 1U/reaction | 0.2 |
| SDW | - | - | 13.2 |
| Total | - | - | 20 |

PCR was carried out in a Biorad thermal-cycler which is programmed as below.

PCR programming for SSR primers was carried out in a Biorad thermal-cycler with steps of initial denaturation done at $94^0$C for 5 min followed by 35 cycles of denaturation at $94^0$C for 30 sec., annealing at $56^0$C for 1 min and extension at $72^0$C for 2 min. Final extension was done at $72^0$C for 10 min and cooling at $4^0$C for ∞.

The cycle was repeated for 35 times and the amplified PCR products were electrophoresed in 2.5% agarose gel using 100bp ladder and the gel image was captured in a gel documentation system (G:BOX, M/s Syngene).

## 3.6 ANALYSIS OF MOLECULAR MARKER DATA

### 3.6.1 BAND SCORING AND CLUSTER ANALYSIS

Scoring of bands and analysis of data including cluster analysis for molecular marker identification were computed by using NTSYS pc.

For diversity analysis, clear and reproducible bands obtained using RAPD, ISSR, SSR markers were selected for scoring. Binary scoring was followed by assigning "1" for presence of band and "0" for absence. The data matrix was created in excel format and used as input file for cluster analysis. This binary data thus obtained were 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 generates dendrogram, which group the genotypes on the basis of Nei genetic distance (Nei, 1979) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis. A dendrogram was constructed for grouping the 36 genotypes based on RAPD, ISSR, SSR marker data.

For identification of molecular markers (RAPD, ISSR and SSR) linked to leaf blight resistance, those bands which are repeated only in the resistant genotypes and not in susceptible ones was accounted for comparison and their

molecular weights was calculated using genetool software available with gel documentation system (G:BOX, M/s Syngene).

The scored molecular data was subjected to various measures of degrees of polymorphism *viz.*,

### 3.6.3 HETEROZYGOSITY

Heterozygosity (He) is a method to measure the degree of polymorphism. Its unbiased estimator and formula are well known (Nei and Roychoudhury, 1974). Heterozygosity is defined as the probability that a random population is heterozygous at a locus and is given in a randomly mating population by

$$H = 1 - \sum_i p_i^2$$

Where, p is the frequency of the ith allele in the population.

### 3.6.4. POLYMORPHISM INFORMATION CONTENT (PIC)

Polymorphism information content (PIC) is another measure of polymorphism (Botstein *et al.*, 1980) commonly used as a measure of polymorphism for a co-dominant marker locus used in linkage analysis

$$PIC = 1 - \sum_i p_i^2 - \sum_{i,j} p_i^2 p_j^2$$

Where, p is the frequency of the allele in a population.

### 3.6.6 AVERAGE NO. OF ALLELES PER LOCUS

The average no. of alleles per locus is calculated as

$$n = \left(\frac{1}{K}\right) \sum n_i$$

Where,

$n_i$ is the no. alleles per locus

k is the no. of loci

### 3.6.5. CORRELATION OF MORPHOLOGICAL AND MOLECULAR DATA

Mantel test was used to assess the correlation between the distance measures of the morphological and molecular characterization data.

### 3.7. GEL ELUTION

The specific band at 1270 bp produced by the ISSR primer UBC 811 for seven resistant lines out of the 18 selected, and which was not found in the susceptible genotypes was the target. PCR was done again with four resistant genotypes R2 (IC012601), R13 (370), R16 (679), R17 (84) as template DNA. Amplicons were run on 1.8% gel and specific bands for these genotypes were sliced under UV transilluminator using clean and sharp scalpel. Distinct bands obtained from these samples were collected in an eppendorf tube. The gel slices were weighed along with the eppendorf tube. Before that, weight of the empty eppendorf tube was recorded and the difference between gel slice containing eppendorf and empty eppendorf gave the weight of gel slices. Gel elution was done using QIAquick Gel Extraction Kit (Qiagen).

Using the QIAquick Gel Extraction Kit, added 3 volumes Buffer QG to 1 volume gel (100mg~ 100 µl). The maximum amount of gel per spin column

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was 400 mg. For >2% agarose gels, added 6 volumes Buffer QG. Incubated at 50°C for 10 minute or until the gel slice was completely dissolved. Vortexed the tube every 2 to 3 min. so as to help to dissolve gel. After the gel slice was dissolved completely, checked whether the color of the mixture was yellow, similar to the buffer QG. If the color of the mixture was orange or violet, added 10µl 3M sodium acetate pH 5.0, and mixed. The mixture will turn yellow. Added 1 volume isopropanol to the sample and mixed. Placed a QIAquick spin column in a 2ml collection tube provided or into a vacuum manifold. To bind DNA, applied the sample to the QIAquick column and centrifuged for 1 minute or applied vacuum to the manifold until all the samples have passed through column. Discarded flow through and placed the QIAquick column back into the same tube. For sample volumes of >800 µl, loaded and spinned/applied vacuum again. Added 500 µl Buffer QG to the QIAquick column and centrifuged for 1 minute/applied vacuum. Discarded flow-through and placed the QIAquick column back into the same tube. To wash, added 750 µl Buffer PE to QIAquick column and centrifuged for 1 minute. Discarded flow-through and placed the QIAquick column back into the same tube. Centrifuged the QIAquick column in the provided 2ml collection tube for 1 minute to remove residual wash buffer. Placed QIAquick column into a clean 1.5 ml micro-centrifuge tube. To the eluted DNA, added 50 µl buffer EB (10mM TrisHCl, pH 8.5) or water to the center of the QIAquick membrane and centrifuged the column for 1 minute. After the addition of Buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 minutes can increase the yield of purified DNA. Purified DNA was analyzed on a gel, by adding 1 volume of Loading Dye to 5 volumes of purified DNA. Mixed the solution by pipetting up and down before loading the gel. Gel image was documented and it was confirmed that eluted

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DNA band was present on the gel. Eluted samples were stored on -20$^0$C refrigerator for sequencing.

The eluted samples were run on 1.8% agarose gel. As the concentration of the 1270 bp band was less and the band size very high, it was re-amplified with the same primer and again checked on the gel. Of the two bands obtained here, the prominent one at 280 bp was used for sequencing.

## 3.8. DNA SEQUENCING

The PCR product of four genotypes was given to Agrigenome (a subsidiary of SciGenom Labs) for sequencing.

# Results

# 4. RESULT

The result of the present study titled "**Identification of molecular markers associated with taro leaf blight in *Colocasia esculenta* (L.) Schott**" carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2017-2018 are explained in this chapter.

## 4.1. MOLECULAR CHARACTERIZATION

The results of molecular characterization with respect to 36 taro genotypes collected from the germplasm maintained at ICAR - CTCRI using RAPD, ISSR and SSR primers are explained in this section. For this study, 18 resistant and 18 susceptible lines, previously screened at the Institute was used, the details of which are given in table 1.



Plate 1. General view of taro germplasm field
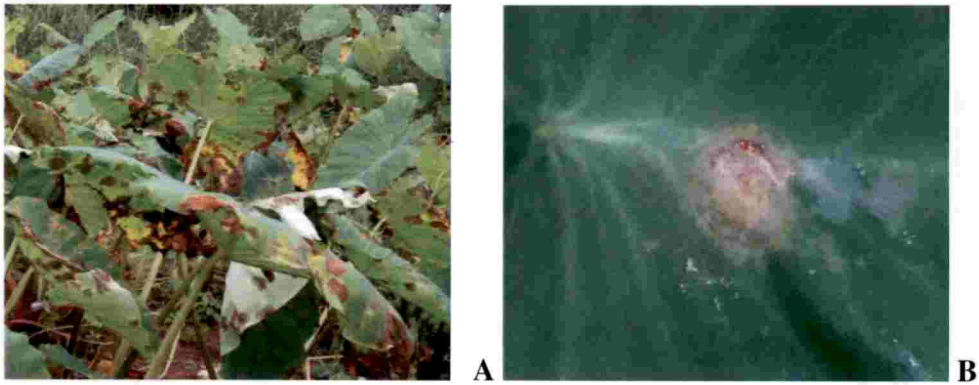
Plate 2 (A & B). Typical symptom of leaf blight infected taro plant (A) and enlarged leaf showing infection (B)

## 4.1.1 DNA ISOLATION PROTOCOL

DNA was isolated by employing the method standardized by Doyle and Doyle (1987) and modified by Sharma *et al.* (2008). This method gave good quality of DNA and revealed good absorbance values with purity ranging from 1.98 (485) - 2.39 (628). The spectrophotometric readings of DNA samples extracted from 36 taro genotypes are listed in the table 8.

Table 8. Yield and purity of DNA isolated following modified CTAB method
(Sharma *et al.*, 2008)

| S. No. | Code assigned | Genotype | DNA yield (ng/µl) | A260/A280 |
|--------|---------------|----------|-------------------|-----------|
| 1. | R1 | 450 | 1157.1 | 2.29 |
| 2. | R2 | IC012601 | 1836.7 | 2.16 |
| 3. | R3 | TCR 429 | 2014.4 | 2.26 |
| 4. | R4 | 723 | 1488.3 | 2.27 |
| 5. | R5 | IC089624 | 1847.7 | 2.19 |
| 6. | R6 | IC122159 | 635.2 | 2.32 |
| 7. | R7 | 66 | 897.6 | 2.22 |
| 8. | R8 | 565 | 2715.9 | 2.13 |
| 9. | R9 | E10 | 2635.2 | 2.22 |
| 10. | R10 | B4 | 1930.9 | 2.34 |
| 11. | R11 | IC310104 | 2133.5 | 2.15 |
| 12. | R12 | J14 | 1719.6 | 2.25 |
| 13. | R13 | 370 | 1802.6 | 2.19 |
| 14. | R14 | L12 | 4200.8 | 2.11 |
| 15. | R15 | 203 | 1365.9 | 2.22 |
| 16. | R16 | 679 | 1286.0 | 2.19 |
| 17. | R17 | 84 | 1341.2 | 2.09 |
| 18. | R18 | Muktakeshi | 394.8 | 2.08 |
| 19. | S1 | Sree Rashmi | 2081.9 | 2.17 |
| 20. | S2 | 276 | 1083.9 | 2.43 |
| 21. | S3 | 557 | 415.7 | 2.37 |
| 22. | S4 | 628 | 797.7 | 2.39 |

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table 8. cont..

| | | | | |
|---|---|---|---|---|
| 23. | S5 | TCR 514 | 864.9 | 2.35 |
| 24. | S6 | VRS | 278.2 | 2.19 |
| 25. | S7 | Sonajuli (Bhu Sree) | 915.9 | 2.36 |
| 26. | S8 | Jhankri (Bhu Kripa) | 812.2 | 2.30 |
| 27. | S9 | Sree Pallavi | 489.0 | 2.15 |
| 28. | S10 | 22 | 483.5 | 2.34 |
| 29. | S11 | 485 | 318.3 | 1.98 |
| 30. | S12 | IC420620 | 498.5 | 2.28 |
| 31. | S13 | IC089583 | 392.7 | 2.26 |
| 32. | S14 | 553 | 539.5 | 2.19 |
| 33. | S15 | 85 | 429.6 | 2.06 |
| 34. | S16 | 621 | 561.1 | 2.29 |
| 35. | S17 | TCR 961 | 684.1 | 2.23 |
| 36. | S18 | B2 (SVP) | 310.0 | 2.11 |
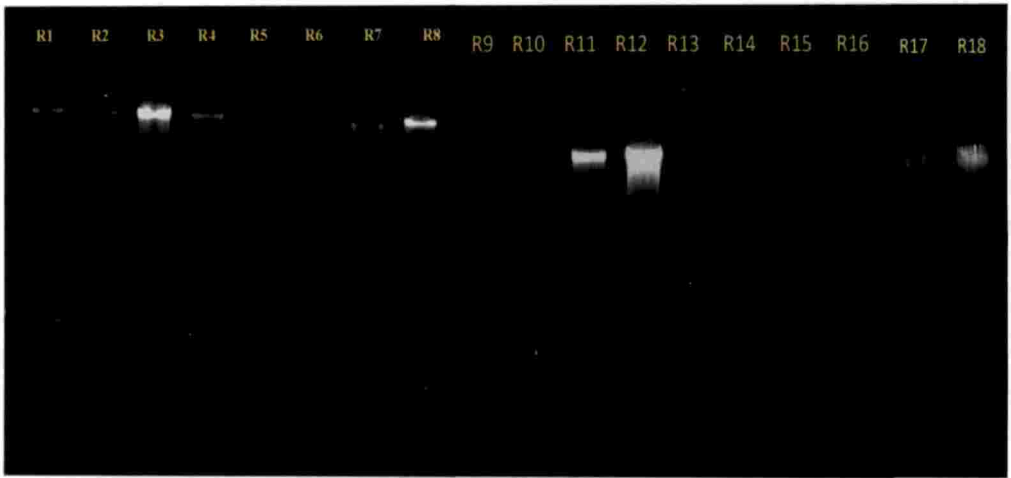
R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi
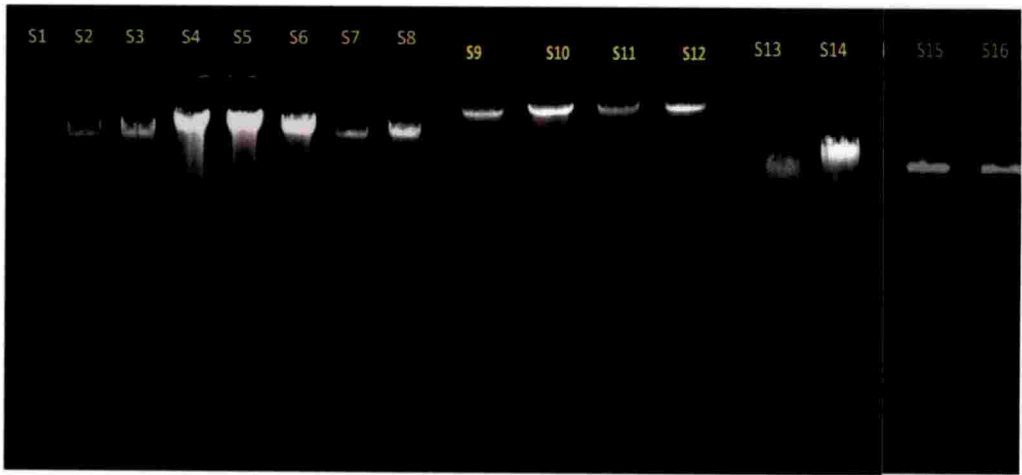


S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP)

Plate 3. DNA isolated using modified CTAB method (Sharma *et. al.*, 2008)

## 4.2. MOLECULAR MARKERS ASSOCIATED WITH TLB

For identifying molecular marker(s) linked to TLB, three marker systems were used *viz.,* RAPD, ISSR and SSR.

### 4.2.1 RAPD PRIMER SCREENING

A set of 10 RAPD markers were screened initially and of these, seven gave clear and consistent bands. These were OPW1, OPW2, OPW5, OPW6, OPW8, OPW12, and OPW16, which were used for the further studies.

### 4.2.1.1. PCR CONDITION

In the standardization of PCR conditions, annealing temperature for certain RAPD primers were changed, which gave better results. After standardization, PCR gave crisp and clear amplicons at an annealing temperature of $32^0$C (Plate 4 - 10). The bands were visualized using 2% agarose gels and presence and absence of bands were scored as '1' and '0', respectively.

### 4.2.1.2. ANALYSIS OF MOLECULAR DATA

The data was analyzed for various parameters estimated for the quantification of genetic variability using seven RAPD markers as given in table 9-10.

Plate 4. Agarose gel profile (2%) with the primer OPW1



Plate 5. Agarose gel profile (2%) with the primer OPW2

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp
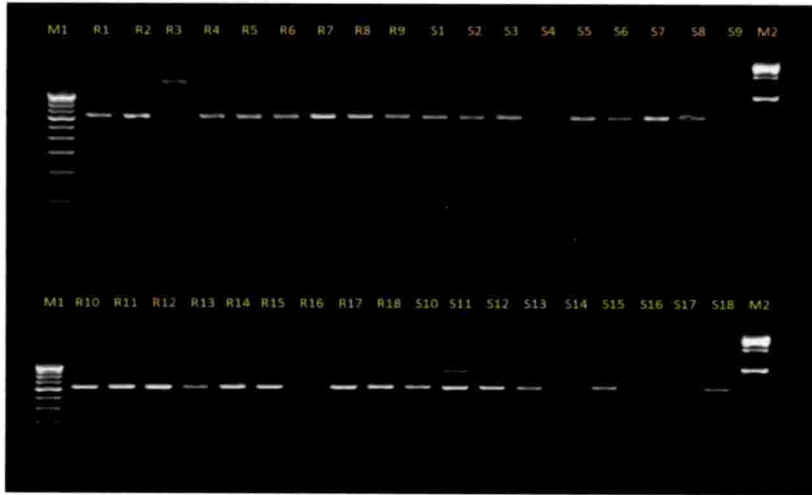
Plate 6. Agarose gel profile (2%) with the primer OPW5
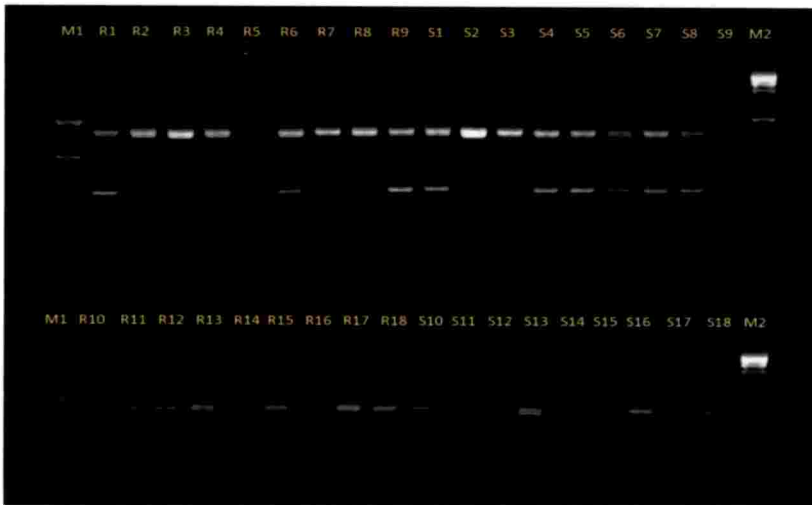


Plate 7. Agarose gel profile (2%) with the primer OPW6

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B-2 (SVP); M1: 100bp; M2: 1Kbp

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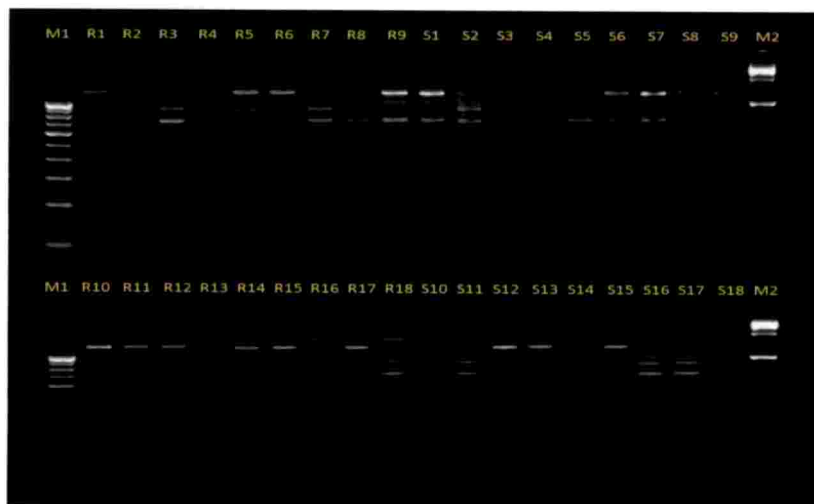
Plate 8. Agarose gel profile (2%) with the primer OPW8



Plate 9: Agarose gel profile (2%) with the primer OPW12

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159;
R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5:
TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree
Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203;
R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13:
IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1:
100bp; M2: 1Kbp

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Plate 10: Agarose gel profile (2%) with the primer OPW16

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp

Table 9. Percentage polymorphism shown by seven RAPD markers

| Primers | Total number of bands | Total number of polymorphic bands | Polymorphism (%) |
|---|---|---|---|
| OPW1 | 11 | 11 | 100.0 |
| OPW2 | 11 | 10 | 90.9 |
| OPW5 | 10 | 9 | 90.0 |
| OPW6 | 11 | 9 | 81.8 |
| OPW8 | 11 | 10 | 90.9 |
| OPW12 | 8 | 4 | 50.0 |
| OPW16 | 12 | 12 | 100.0 |
| Total | 74 | 65 | - |
| Mean | - | - | 86.22 |

Table 10. Diversity estimates for RAPD markers

| Primer | Ta (°C) | Average number of alleles per locus | Polymorphism Information Content (PIC) | Heterozygosity (He) |
|---|---|---|---|---|
| OPW1 | 32 | 7.6 | 0.885 | 0.89 |
| OPW2 | 32 | 8.0 | 0.886 | 0.89 |
| OPW5 | 32 | 4.0 | 0.841 | 0.82 |
| OPW6 | 32 | 6.7 | 0.878 | 0.88 |
| OPW8 | 32 | 8.2 | 0.888 | 0.89 |
| OPW12 | 32 | 5.1 | 0.804 | 0.82 |
| OPW16 | 32 | 7.1 | 0.615 | 0.66 |

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## 4.2.1.3. RAPD GEL ELECTROPHORESIS FOR PCR PRODUCT ANALYSIS

The PCR products obtained were resolved in 2% agarose gel for the identification of molecular markers associated with leaf blight resistance. Among the seven RAPD primers used, OPW1 was the best which produced 12 polymorphic bands followed by OPW16 with 11 polymorphic bands. Both these revealed 100% polymorphism. The lowest number of bands were recorded in OPW12 with 8 bands. The mean percentage polymorphism for the RAPD primers was 86.22 and the details are presented in Table 9.

For the primer OPW1, the resistant genotype 679 (R16) showed a unique band in the range of 1000 bp (Plate 4) whereas, for primer OPW16 resistant genotypes (R2, R3, R4, R16, R18) revealed a specific band in a range of 300 bp and the same band was noticed in only one susceptible line 276 (S2) (Plate 10) which infers that this band may play a role in resistance. For primer OPW5, the resistant genotype TCR 429 (R3) exhibited a different banding pattern (Plate 6).

The polymorphism of the RAPD primers studied ranged from 50 to 100%. OPW12 recorded the lowest polymorphism (50%) followed by OPW6 (81.8%). The observed heterozygosity values (He) ranged between 0.66 (OPW16) to 0.89 (OPW1, OPW2, OPW8). For most of the RAPD primers studied He was found to be >0.8.

The polymorphism Information Content (PIC) was highest for OPW8 (0.888) primer followed by OPW2 (0.886) and OPW1 (0.885) and least with OPW16 (0.615). Number of alleles per locus ranged from 4.0-8.2 with the maximum alleles been shown by OPW8 and the minimum by OPW5. The PIC, Number of alleles per locus and He values for the RAPD primers are given in table 10.

## 4.2.1.4. SIMILARITY INDEX

The similarity index values obtained for each pair wise comparison among the 36 taro accessions based on seven RAPD marker data is given in the fig 1. The similarity coefficient based on RAPD markers ranged from 0.47 to 0.91. Most of the similarity coefficients ranged between 0.55 to 0.80. Among the 36 taro accessions, the lowest similarity index (0.47) was observed between 557 (S3) and 370 (R13) and also between Sree Pallavi (S9) and 370 (R13) whereas, the highest similarity index (0.91) was observed between IC310104 (R11) and L-12 (R14). Amongst the susceptible lines, the highest similarity index (0.88) was shown between 22 (S10) and 621 (S16) as well as Bhu Sree (S7) and Bhu Kripa (S8).

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Similarity matrix using Jaccard's coefficient

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R2 | 0.69 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R3 | 0.57 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R4 | 0.68 | 0.82 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R5 | 0.72 | 0.65 | 0.69 | 0.66 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R6 | 0.81 | 0.72 | 0.62 | 0.65 | 0.66 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R7 | 0.68 | 0.72 | 0.70 | 0.73 | 0.69 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R8 | 0.72 | 0.73 | 0.66 | 0.72 | 0.76 | 0.77 | 0.77 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R9 | 0.81 | 0.69 | 0.62 | 0.68 | 0.66 | 0.84 | 0.70 | 0.69 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R10 | 0.61 | 0.70 | 0.64 | 0.66 | 0.59 | 0.72 | 0.61 | 0.68 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R11 | 0.59 | 0.58 | 0.57 | 0.59 | 0.55 | 0.65 | 0.59 | 0.64 | 0.70 | 0.82 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| R12 | 0.65 | 0.64 | 0.62 | 0.62 | 0.55 | 0.70 | 0.70 | 0.61 | 0.68 | 0.64 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| R13 | 0.49 | 0.66 | 0.65 | 0.62 | 0.55 | 0.62 | 0.65 | 0.58 | 0.65 | 0.66 | 0.65 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| R14 | 0.64 | 0.62 | 0.55 | 0.61 | 0.59 | 0.69 | 0.64 | 0.68 | 0.74 | 0.81 | 0.91 | 0.72 | 0.66 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| R15 | 0.57 | 0.66 | 0.62 | 0.59 | 0.55 | 0.70 | 0.62 | 0.66 | 0.68 | 0.82 | 0.78 | 0.65 | 0.76 | 0.82 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| R16 | 0.57 | 0.58 | 0.62 | 0.57 | 0.55 | 0.65 | 0.62 | 0.58 | 0.65 | 0.69 | 0.70 | 0.70 | 0.68 | 0.72 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| R17 | 0.57 | 0.61 | 0.59 | 0.65 | 0.50 | 0.68 | 0.68 | 0.64 | 0.70 | 0.77 | 0.76 | 0.73 | 0.70 | 0.80 | 0.84 | 0.65 | 1.00 | | | | | | | | | | | | | | | | | | | |
| R18 | 0.59 | 0.58 | 0.59 | 0.46 | 0.61 | 0.65 | 0.68 | 0.58 | 0.65 | 0.61 | 0.65 | 0.65 | 0.70 | 0.69 | 0.70 | 0.70 | 0.68 | 1.00 | | | | | | | | | | | | | | | | | | |
| S1 | 0.77 | 0.59 | 0.64 | 0.58 | 0.65 | 0.74 | 0.66 | 0.59 | 0.85 | 0.70 | 0.72 | 0.64 | 0.58 | 0.70 | 0.66 | 0.66 | 0.72 | 0.69 | 1.00 | | | | | | | | | | | | | | | | | |
| S2 | 0.64 | 0.68 | 0.69 | 0.61 | 0.68 | 0.69 | 0.64 | 0.70 | 0.64 | 0.68 | 0.61 | 0.55 | 0.58 | 0.57 | 0.66 | 0.50 | 0.61 | 0.69 | 0.73 | 1.00 | | | | | | | | | | | | | | | | |
| S3 | 0.77 | 0.68 | 0.55 | 0.72 | 0.73 | 0.69 | 0.66 | 0.70 | 0.72 | 0.62 | 0.55 | 0.58 | 0.47 | 0.59 | 0.50 | 0.55 | 0.58 | 0.50 | 0.70 | 0.59 | 1.00 | | | | | | | | | | | | | | | |
| S4 | 0.77 | 0.68 | 0.53 | 0.64 | 0.62 | 0.72 | 0.69 | 0.57 | 0.80 | 0.59 | 0.55 | 0.61 | 0.58 | 0.65 | 0.61 | 0.61 | 0.64 | 0.61 | 0.76 | 0.51 | 0.81 | 1.00 | | | | | | | | | | | | | | |
| S5 | 0.73 | 0.66 | 0.65 | 0.59 | 0.69 | 0.73 | 0.73 | 0.64 | 0.78 | 0.58 | 0.57 | 0.62 | 0.54 | 0.64 | 0.62 | 0.54 | 0.65 | 0.65 | 0.77 | 0.64 | 0.69 | 0.80 | 1.00 | | | | | | | | | | | | | |
| S6 | 0.69 | 0.59 | 0.64 | 0.58 | 0.65 | 0.74 | 0.74 | 0.65 | 0.74 | 0.62 | 0.61 | 0.64 | 0.58 | 0.70 | 0.64 | 0.64 | 0.64 | 0.64 | 0.70 | 0.57 | 0.62 | 0.78 | 0.77 | 1.00 | | | | | | | | | | | | |
| S7 | 0.78 | 0.69 | 0.62 | 0.65 | 0.69 | 0.73 | 0.68 | 0.66 | 0.86 | 0.66 | 0.62 | 0.62 | 0.59 | 0.69 | 0.59 | 0.62 | 0.65 | 0.62 | 0.82 | 0.58 | 0.74 | 0.85 | 0.81 | 0.77 | 1.00 | | | | | | | | | | | |
| S8 | 0.74 | 0.65 | 0.61 | 0.69 | 0.68 | 0.72 | 0.69 | 0.62 | 0.80 | 0.65 | 0.64 | 0.61 | 0.58 | 0.65 | 0.58 | 0.64 | 0.61 | 0.58 | 0.84 | 0.65 | 0.70 | 0.76 | 0.69 | 0.70 | 0.88 | 1.00 | | | | | | | | | | |
| S9 | 0.66 | 0.70 | 0.66 | 0.69 | 0.73 | 0.64 | 0.72 | 0.65 | 0.72 | 0.59 | 0.55 | 0.55 | 0.47 | 0.59 | 0.50 | 0.53 | 0.55 | 0.61 | 0.70 | 0.70 | 0.68 | 0.68 | 0.74 | 0.73 | 0.80 | 0.78 | 1.00 | | | | | | | | | |
| S10 | 0.65 | 0.64 | 0.65 | 0.68 | 0.61 | 0.70 | 0.76 | 0.69 | 0.73 | 0.72 | 0.76 | 0.86 | 0.70 | 0.77 | 0.68 | 0.70 | 0.81 | 0.62 | 0.72 | 0.55 | 0.64 | 0.64 | 0.68 | 0.66 | 0.70 | 0.69 | 0.61 | 1.00 | | | | | | | | |
| S11 | 0.54 | 0.61 | 0.54 | 0.65 | 0.58 | 0.59 | 0.65 | 0.61 | 0.65 | 0.64 | 0.73 | 0.70 | 0.76 | 0.72 | 0.68 | 0.76 | 0.73 | 0.70 | 0.64 | 0.55 | 0.55 | 0.61 | 0.54 | 0.61 | 0.59 | 0.61 | 0.55 | 0.73 | 1.00 | | | | | | | |
| S12 | 0.59 | 0.66 | 0.54 | 0.65 | 0.61 | 0.65 | 0.65 | 0.64 | 0.73 | 0.66 | 0.76 | 0.65 | 0.70 | 0.77 | 0.65 | 0.51 | 0.70 | 0.62 | 0.66 | 0.58 | 0.66 | 0.66 | 0.70 | 0.66 | 0.68 | 0.64 | 0.66 | 0.70 | 0.70 | 1.00 | | | | | | |
| S13 | 0.58 | 0.59 | 0.53 | 0.55 | 0.54 | 0.64 | 0.64 | 0.54 | 0.69 | 0.68 | 0.69 | 0.69 | 0.72 | 0.68 | 0.69 | 0.61 | 0.69 | 0.72 | 0.62 | 0.57 | 0.54 | 0.65 | 0.66 | 0.65 | 0.64 | 0.57 | 0.57 | 0.64 | 0.64 | 0.77 | 1.00 | | | | | |
| S14 | 0.59 | 0.64 | 0.59 | 0.59 | 0.50 | 0.70 | 0.70 | 0.64 | 0.68 | 0.69 | 0.70 | 0.84 | 0.70 | 0.69 | 0.70 | 0.68 | 0.78 | 0.62 | 0.64 | 0.50 | 0.58 | 0.64 | 0.65 | 0.64 | 0.65 | 0.58 | 0.50 | 0.86 | 0.62 | 0.65 | 0.74 | 1.00 | | | | |
| S15 | 0.58 | 0.57 | 0.53 | 0.58 | 0.59 | 0.55 | 0.61 | 0.57 | 0.66 | 0.57 | 0.61 | 0.66 | 0.74 | 0.62 | 0.58 | 0.61 | 0.64 | 0.64 | 0.59 | 0.46 | 0.57 | 0.62 | 0.64 | 0.59 | 0.61 | 0.57 | 0.54 | 0.72 | 0.72 | 0.74 | 0.76 | 0.66 | 1.00 | | | |
| S16 | 0.66 | 0.62 | 0.61 | 0.64 | 0.54 | 0.72 | 0.69 | 0.68 | 0.74 | 0.65 | 0.74 | 0.82 | 0.64 | 0.76 | 0.69 | 0.66 | 0.82 | 0.61 | 0.70 | 0.54 | 0.62 | 0.62 | 0.72 | 0.62 | 0.66 | 0.62 | 0.57 | 0.88 | 0.66 | 0.72 | 0.65 | 0.80 | 0.70 | 1.00 | | |
| S17 | 0.58 | 0.57 | 0.58 | 0.55 | 0.62 | 0.66 | 0.61 | 0.57 | 0.69 | 0.68 | 0.80 | 0.74 | 0.77 | 0.78 | 0.69 | 0.66 | 0.64 | 0.66 | 0.65 | 0.59 | 0.57 | 0.62 | 0.61 | 0.68 | 0.61 | 0.62 | 0.54 | 0.72 | 0.77 | 0.80 | 0.76 | 0.66 | 0.73 | 0.68 | 1.00 | |
| S18 | 0.58 | 0.62 | 0.66 | 0.58 | 0.54 | 0.58 | 0.66 | 0.57 | 0.61 | 0.59 | 0.66 | 0.66 | 0.72 | 0.68 | 0.69 | 0.64 | 0.66 | 0.80 | 0.59 | 0.59 | 0.51 | 0.59 | 0.61 | 0.62 | 0.55 | 0.49 | 0.51 | 0.64 | 0.72 | 0.61 | 0.68 | 0.64 | 0.62 | 0.65 | 0.70 | 1.00 |

Fig.1: Similarity coefficient based on Jaccard's coefficient for 36 taro genotypes with Seven RAPD markers

|      | R1   | R2   | R3   | R4   | R5   | R6   | R7   | R8   | R9   | R10  | R11  | R12  | R13  | R14  | R15  | R16  | R17  | R18  |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| **R1**  | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| **R2**  | 0.69 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| **R3**  | 0.57 | 0.72 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| **R4**  | 0.68 | 0.82 | 0.70 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| **R5**  | 0.72 | 0.65 | 0.69 | 0.66 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| **R6**  | 0.81 | 0.72 | 0.62 | 0.65 | 0.66 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |
| **R7**  | 0.68 | 0.72 | 0.70 | 0.73 | 0.69 | 0.70 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |
| **R8**  | 0.72 | 0.73 | 0.66 | 0.72 | 0.76 | 0.77 | 0.77 | 1.00 |      |      |      |      |      |      |      |      |      |      |
| **R9**  | 0.81 | 0.69 | 0.62 | 0.68 | 0.66 | 0.84 | 0.70 | 0.69 | 1.00 |      |      |      |      |      |      |      |      |      |
| **R10** | 0.61 | 0.70 | 0.64 | 0.66 | 0.59 | 0.72 | 0.61 | 0.68 | 0.72 | 1.00 |      |      |      |      |      |      |      |      |
| **R11** | 0.59 | 0.58 | 0.57 | 0.59 | 0.55 | 0.65 | 0.59 | 0.64 | 0.70 | 0.82 | 1.00 |      |      |      |      |      |      |      |
| **R12** | 0.65 | 0.64 | 0.62 | 0.62 | 0.55 | 0.70 | 0.70 | 0.61 | 0.68 | 0.64 | 0.76 | 1.00 |      |      |      |      |      |      |
| **R13** | 0.49 | 0.66 | 0.65 | 0.62 | 0.55 | 0.62 | 0.65 | 0.58 | 0.65 | 0.66 | 0.65 | 0.70 | 1.00 |      |      |      |      |      |
| **R14** | 0.64 | 0.62 | 0.55 | 0.61 | 0.59 | 0.69 | 0.64 | 0.68 | 0.74 | 0.81 | 0.91 | 0.72 | 0.66 | 1.00 |      |      |      |      |
| **R15** | 0.57 | 0.66 | 0.62 | 0.59 | 0.55 | 0.70 | 0.62 | 0.66 | 0.68 | 0.82 | 0.78 | 0.65 | 0.76 | 0.82 | 1.00 |      |      |      |
| **R16** | 0.57 | 0.58 | 0.62 | 0.57 | 0.55 | 0.65 | 0.62 | 0.58 | 0.65 | 0.69 | 0.70 | 0.70 | 0.68 | 0.72 | 0.70 | 1.00 |      |      |
| **R17** | 0.57 | 0.61 | 0.59 | 0.65 | 0.50 | 0.68 | 0.68 | 0.64 | 0.70 | 0.77 | 0.76 | 0.73 | 0.70 | 0.80 | 0.84 | 0.65 | 1.00 |      |
| **R18** | 0.59 | 0.58 | 0.59 | 0.46 | 0.61 | 0.65 | 0.68 | 0.58 | 0.65 | 0.61 | 0.65 | 0.65 | 0.70 | 0.69 | 0.70 | 0.70 | 0.68 | 1.00 |

Fig.1 (a). Similarity matrix based on Jaccard's coefficient for 18 resistant lines
based on seven RAPD markers

|     | R1   | R2   | R3   | R4   | R5   | R6   | R7   | R8   | R9   | R10  | R11  | R12  | R13  | R14  | R15  | R16  | R17  | R18  |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S1  | 0.77 | 0.59 | 0.64 | 0.58 | 0.65 | 0.74 | 0.66 | 0.59 | 0.85 | 0.70 | 0.72 | 0.64 | 0.58 | 0.70 | 0.66 | 0.66 | 0.72 | 0.69 |
| S2  | 0.64 | 0.68 | 0.69 | 0.61 | 0.68 | 0.69 | 0.64 | 0.70 | 0.64 | 0.68 | 0.61 | 0.55 | 0.58 | 0.57 | 0.66 | 0.50 | 0.61 | 0.69 |
| S3  | 0.77 | 0.68 | 0.55 | 0.72 | 0.73 | 0.69 | 0.66 | 0.70 | 0.72 | 0.62 | 0.55 | 0.58 | 0.47 | 0.59 | 0.50 | 0.55 | 0.58 | 0.50 |
| S4  | 0.77 | 0.68 | 0.53 | 0.64 | 0.62 | 0.72 | 0.69 | 0.57 | 0.80 | 0.59 | 0.55 | 0.61 | 0.58 | 0.65 | 0.61 | 0.61 | 0.64 | 0.61 |
| S5  | 0.73 | 0.66 | 0.65 | 0.59 | 0.69 | 0.73 | 0.73 | 0.64 | 0.78 | 0.58 | 0.57 | 0.62 | 0.54 | 0.64 | 0.62 | 0.54 | 0.65 | 0.65 |
| S6  | 0.69 | 0.59 | 0.64 | 0.58 | 0.65 | 0.74 | 0.74 | 0.65 | 0.74 | 0.62 | 0.61 | 0.64 | 0.58 | 0.70 | 0.64 | 0.64 | 0.64 | 0.64 |
| S7  | 0.78 | 0.69 | 0.62 | 0.65 | 0.69 | 0.73 | 0.68 | 0.66 | 0.86 | 0.66 | 0.62 | 0.62 | 0.59 | 0.69 | 0.59 | 0.62 | 0.65 | 0.62 |
| S8  | 0.74 | 0.65 | 0.61 | 0.69 | 0.68 | 0.72 | 0.69 | 0.62 | 0.80 | 0.65 | 0.64 | 0.61 | 0.58 | 0.65 | 0.58 | 0.64 | 0.61 | 0.58 |
| S9  | 0.66 | 0.70 | 0.66 | 0.69 | 0.73 | 0.64 | 0.72 | 0.65 | 0.72 | 0.59 | 0.55 | 0.55 | 0.47 | 0.59 | 0.50 | 0.53 | 0.55 | 0.61 |
| S10 | 0.65 | 0.64 | 0.65 | 0.68 | 0.61 | 0.70 | 0.76 | 0.69 | 0.73 | 0.72 | 0.76 | 0.86 | 0.70 | 0.77 | 0.68 | 0.70 | 0.81 | 0.62 |
| S11 | 0.54 | 0.61 | 0.54 | 0.65 | 0.58 | 0.59 | 0.65 | 0.61 | 0.65 | 0.64 | 0.73 | 0.70 | 0.76 | 0.72 | 0.68 | 0.76 | 0.73 | 0.70 |
| S12 | 0.59 | 0.66 | 0.54 | 0.65 | 0.61 | 0.65 | 0.65 | 0.64 | 0.73 | 0.66 | 0.76 | 0.65 | 0.70 | 0.77 | 0.65 | 0.51 | 0.70 | 0.62 |
| S13 | 0.58 | 0.59 | 0.53 | 0.55 | 0.54 | 0.64 | 0.64 | 0.54 | 0.69 | 0.68 | 0.69 | 0.69 | 0.72 | 0.68 | 0.69 | 0.61 | 0.69 | 0.72 |
| S14 | 0.59 | 0.64 | 0.59 | 0.59 | 0.50 | 0.70 | 0.70 | 0.64 | 0.68 | 0.69 | 0.70 | 0.84 | 0.70 | 0.69 | 0.70 | 0.68 | 0.78 | 0.62 |
| S15 | 0.58 | 0.57 | 0.53 | 0.58 | 0.59 | 0.55 | 0.61 | 0.57 | 0.66 | 0.57 | 0.61 | 0.66 | 0.74 | 0.62 | 0.58 | 0.61 | 0.64 | 0.64 |
| S16 | 0.66 | 0.62 | 0.61 | 0.64 | 0.54 | 0.72 | 0.69 | 0.68 | 0.74 | 0.65 | 0.74 | 0.82 | 0.64 | 0.76 | 0.69 | 0.66 | 0.82 | 0.61 |
| S17 | 0.58 | 0.57 | 0.58 | 0.55 | 0.62 | 0.66 | 0.61 | 0.57 | 0.69 | 0.68 | 0.80 | 0.74 | 0.77 | 0.78 | 0.69 | 0.66 | 0.64 | 0.66 |
| S18 | 0.58 | 0.62 | 0.66 | 0.58 | 0.54 | 0.58 | 0.66 | 0.57 | 0.61 | 0.59 | 0.66 | 0.66 | 0.72 | 0.68 | 0.69 | 0.64 | 0.66 | 0.80 |

Fig. 1 (b). Similarity coefficient based on Jaccard's coefficient for 18 resistant and 18 susceptible lines based on seven RAPD markers

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| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S1 | 1.00 | | | | | | | | | | | | | | | | | |
| S2 | 0.73 | 1.00 | | | | | | | | | | | | | | | | |
| S3 | 0.70 | 0.59 | 1.00 | | | | | | | | | | | | | | | |
| S4 | 0.76 | 0.51 | 0.81 | 1.00 | | | | | | | | | | | | | | |
| S5 | 0.77 | 0.64 | 0.69 | 0.80 | 1.00 | | | | | | | | | | | | | |
| S6 | 0.70 | 0.57 | 0.62 | 0.78 | 0.77 | 1.00 | | | | | | | | | | | | |
| S7 | 0.82 | 0.58 | 0.74 | 0.85 | 0.81 | 0.77 | 1.00 | | | | | | | | | | | |
| S8 | 0.84 | 0.65 | 0.70 | 0.76 | 0.69 | 0.70 | 0.88 | 1.00 | | | | | | | | | | |
| S9 | 0.70 | 0.70 | 0.68 | 0.68 | 0.74 | 0.73 | 0.80 | 0.78 | 1.00 | | | | | | | | | |
| S10 | 0.72 | 0.55 | 0.64 | 0.64 | 0.68 | 0.66 | 0.70 | 0.69 | 0.61 | 1.00 | | | | | | | | |
| S11 | 0.64 | 0.55 | 0.55 | 0.61 | 0.54 | 0.61 | 0.59 | 0.61 | 0.55 | 0.73 | 1.00 | | | | | | | |
| S12 | 0.66 | 0.58 | 0.66 | 0.66 | 0.70 | 0.66 | 0.68 | 0.64 | 0.66 | 0.70 | 0.70 | 1.00 | | | | | | |
| S13 | 0.62 | 0.57 | 0.54 | 0.65 | 0.66 | 0.65 | 0.64 | 0.57 | 0.57 | 0.64 | 0.64 | 0.77 | 1.00 | | | | | |
| S14 | 0.64 | 0.50 | 0.58 | 0.64 | 0.65 | 0.64 | 0.65 | 0.58 | 0.50 | 0.86 | 0.62 | 0.65 | 0.74 | 1.00 | | | | |
| S15 | 0.59 | 0.46 | 0.57 | 0.62 | 0.64 | 0.59 | 0.61 | 0.57 | 0.54 | 0.72 | 0.72 | 0.74 | 0.76 | 0.66 | 1.00 | | | |
| S16 | 0.70 | 0.54 | 0.62 | 0.62 | 0.72 | 0.62 | 0.66 | 0.62 | 0.57 | 0.88 | 0.66 | 0.72 | 0.65 | 0.80 | 0.70 | 1.00 | | |
| S17 | 0.65 | 0.59 | 0.57 | 0.62 | 0.61 | 0.68 | 0.61 | 0.62 | 0.54 | 0.72 | 0.77 | 0.80 | 0.76 | 0.66 | 0.73 | 0.68 | 1.00 | |
| S18 | 0.59 | 0.59 | 0.51 | 0.59 | 0.61 | 0.62 | 0.55 | 0.49 | 0.51 | 0.64 | 0.72 | 0.61 | 0.68 | 0.64 | 0.62 | 0.65 | 0.70 | 1.00 |

Fig. 1 (c). Similarity matrix based on Jaccard's coefficient for 18 susceptible lines based on seven RAPD markers

## 4.2.1.5. CLUTER ANALYSIS

A dendrogram was generated using UPGMA cluster analysis which grouped the 36 genotypes into two major clusters where, no separate cluster was observed between susceptible genotypes and resistant ones (Fig. 2). Cluster-I and Cluster-II comprising 18 genotypes each revealed 61% similarity. Cluster-I comprising each of nine resistant and nine susceptible genotypes was further sub divided into four sub-clusters. The first sub-cluster with three resistant and four susceptible lines included Bhu Sree (S7), Bhu Kripa (S8) and Sree Rashmi (S1) where, Bhu Sree and Bhu Kripa pooled together showing 88% similarity while, two susceptible lines, 557(S3) and 628 (S4) pooled

together revealing a similarity of 81%. The second sub-cluster with three susceptible lines including Sree Pallavi (S9) showed divergence with respect to Sree Pallavi. On the other hand, the third sub-cluster with six resistant lines, IC089624 (R5) showed divergence. The susceptible line, 276 (S2) formed an outlier.

In the Cluster-II, each of nine resistant and nine susceptible genotypes were included which is further sub-divided into four sub-clusters. The first sub-cluster showing 72% similarity included six resistant and three susceptible lines wherein a resistant line, IC310104 (R11) and a susceptible line, L12 (R14) revealed 91% similarity. Only one resistant line, 679 (R16) formed an outlier and was found to be divergent. The second sub-cluster consisted of only one resistant line 370 (R13) while the remaining were susceptible ones with genotype 85 showing divergence and was susceptible. The third sub-cluster contained only two genotypes - resistant variety Muktakeshi (R18) and a susceptible genotype B2 (SVP) (S18) which revealed 80% similarity. Except Muktakeshi, all other released varieties of taro *viz.*, Sree Rashmi (S1), Sree Pallavi (S9), Bhu Sree and Bhu Kripa were included in the Cluster I. Of the 7 resistant lines which showed consistency with respect to resistance in the pot studies, identified earlier, only E-10 grouped in the Cluster-I and remaining six lines were grouped in the Cluster II. Bhu Sree, Bhu Kripa and Sree Rashmi existed in the same sub-cluster-I and which showed a similarity of 83%.
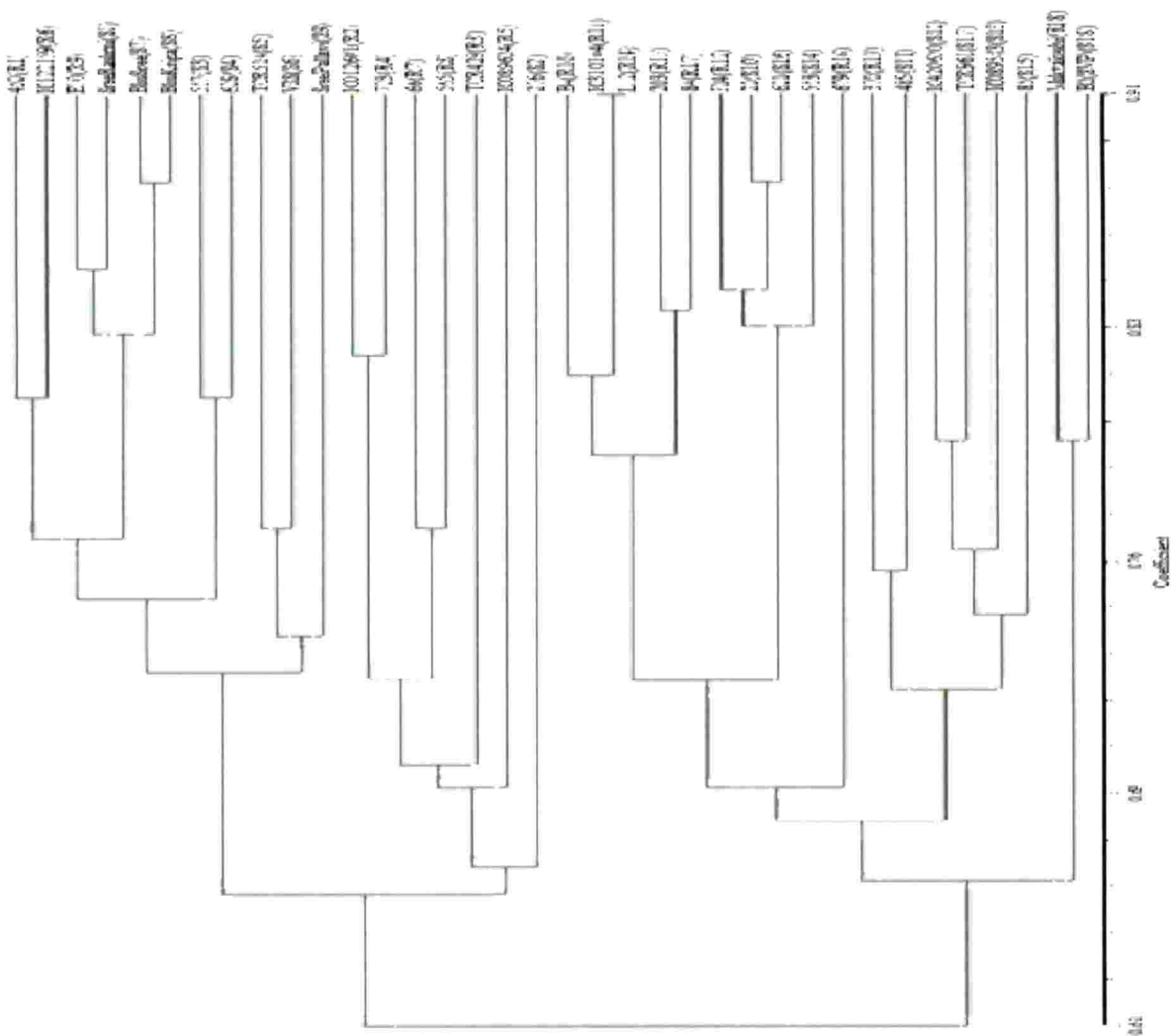
Fig. 2. Dendrogram showing the clustering pattern with seven RAPD markers

Table 11. Distribution of genotypes into different clusters with seven RAPD primers

| Cluster | | Number of accessions | Genotypes |
|---|---|---|---|
| 1 | a | 8 | 450, IC122159, E10, Sree Rashmi, Bhu Sree, 557, Bhu Kripa, 628 |
| | b | 3 | TCR 514, VRS, Sree Pallavi |
| | c | 6 | IC012601, 723, 66, 565, TCR 429, IC089624 |
| | Outlier | | 276 |
| 2 | a | 9 | B-4, IC310104, L12, 203, 84, J14, 22, 621, 553 |
| | Outlier | | 679 |
| | b | 6 | 370, 485, IC420620, TCR 961, IC089583, 85 |
| | c | 2 | Muktakeshi, B2 (SVP) |

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## 4.2.2. ISSR PRIMER SCREENING

A total of 19 ISSR primers were screened of which 14 were used for further studies based on clear and consistent bands shown by them. The selected primers included, UBC 809, UBC 810, UBC 811, UBC 817, UBC 818, UBC 824, UBC 825, UBC 827, UBC 836, UBC 841, UBC 847, $(GA)_9AC$, $(GA)_9AT$,) and $(ACC)_9$ Y), whereas, UBC 871, UBC 14, UBC 848, UBC 857 and UBC 473 were eliminated due of their poor amplification profile.

### 4.2.2.1. PCR CONDITIONS

PCR conditions already standardized in the Molecular Biology Lab at the Division of Crop Improvement, ICAR-CTCRI was followed to obtain crisp and clear amplicons with an annealing temperature of $56.3^0C$ (Plate 11 - 22). The bands produced by these selected primers were visualized using 1.8% agarose gels and presence and absence of bands were scored as '1' and '0', respectively.

### 4.2.2.2. ANALYSIS OF MOLECULAR DATA

The data was analyzed for various parameters estimated for the quantification of genetic variability using seven RAPD markers as given in table 12-13.

The gel images of some selected ISSR primers for TLB screening are as follows:

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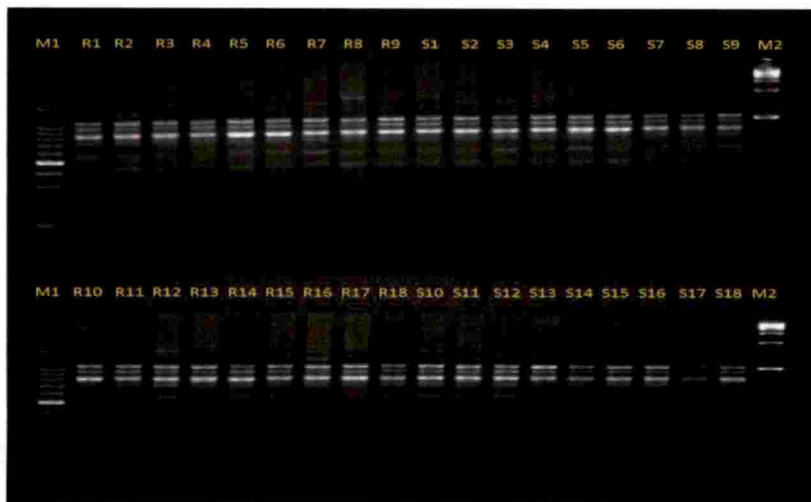
Plate 11. Agarose gel profile (1.8%) with the primer (ACC)$_9$Y
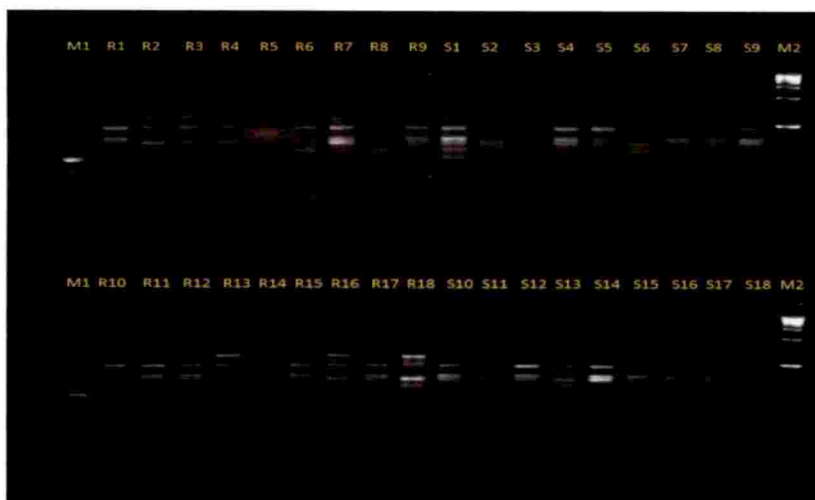


Plate 12. Agarose gel profile (1.8%) with the primer (GA)$_9$AC

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp
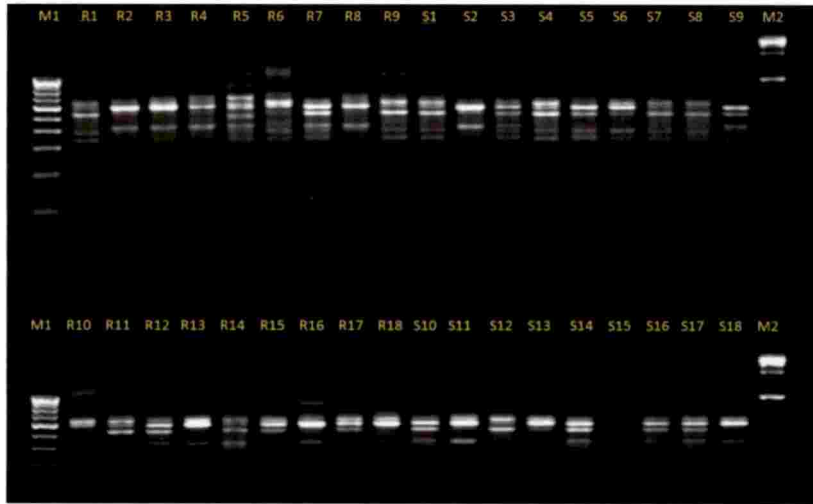
Plate 13: Agarose gel profile (1.8%) with the primer (GA)$_9$AT
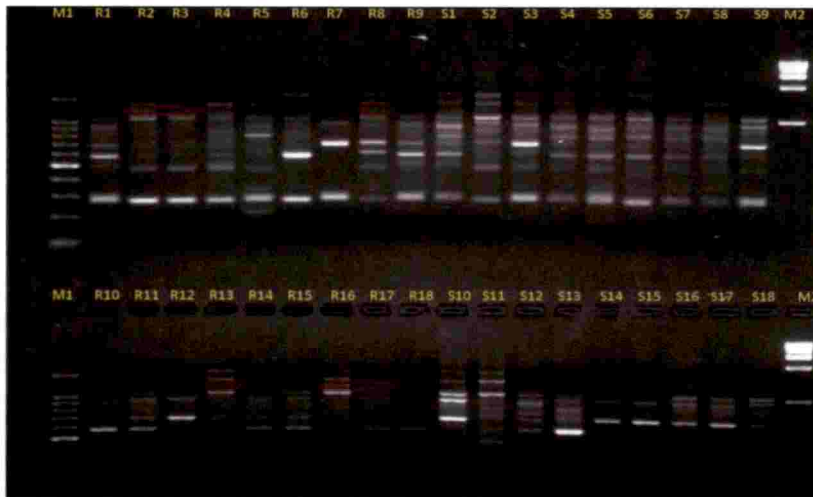


Plate 14: Agarose gel profile (1.8%) with the primer UBC 811

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp

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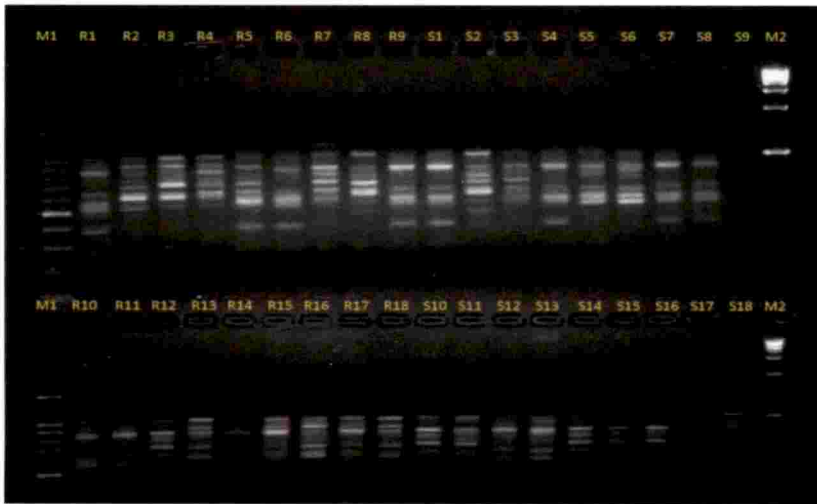
Plate 15: Agarose gel profile (1.8%) with the primer UBC 810
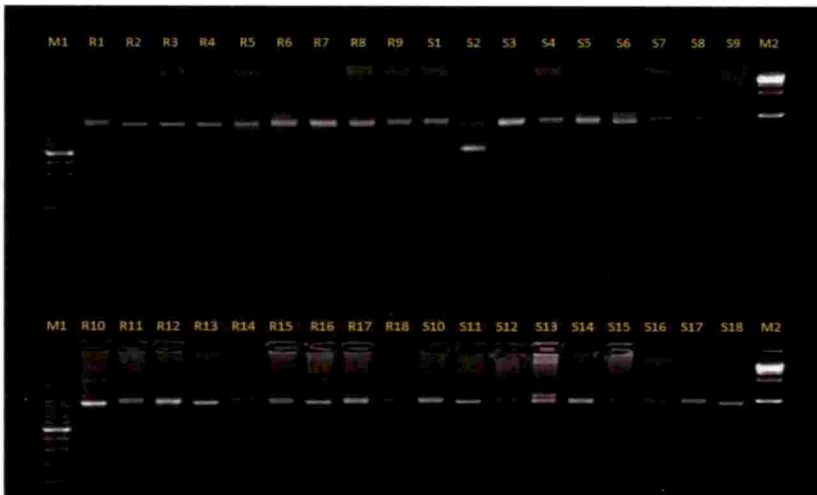


Plate 16: Agarose gel profile (1.8%) with the primer UBC 818

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E-10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp
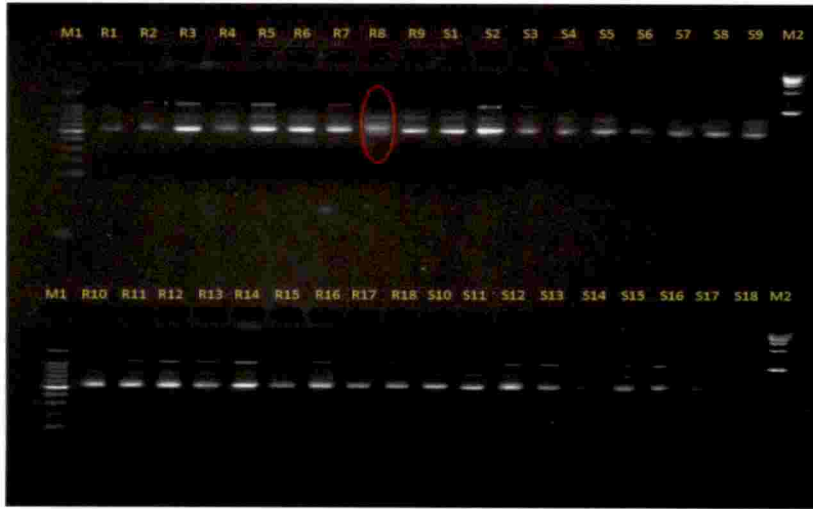
Plate 17: Agarose gel profile (1.8%) with the primer UBC 824
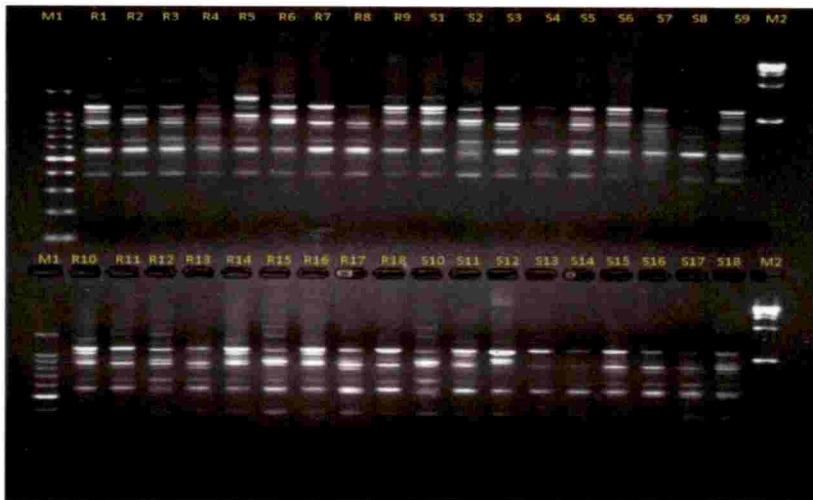


Plate 18: Agarose gel profile (1.8%) with the primer UBC 825

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B-4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp
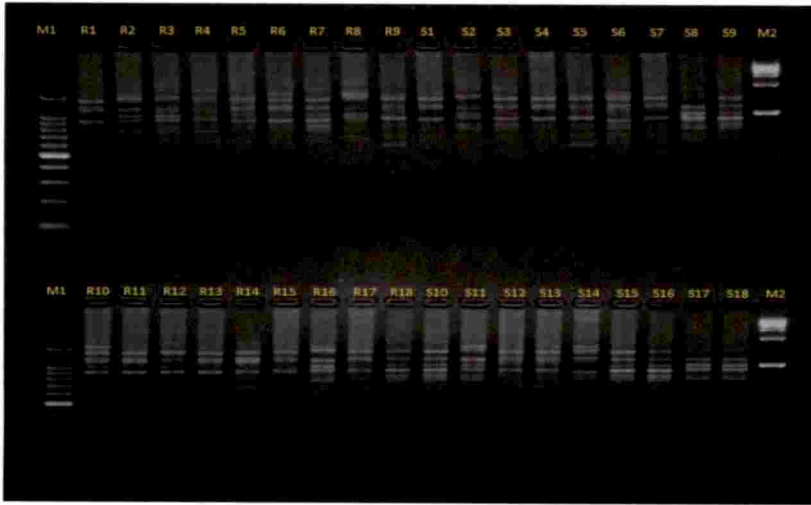
Plate 19: Agarose gel profile (1.8%) with the primer UBC 827
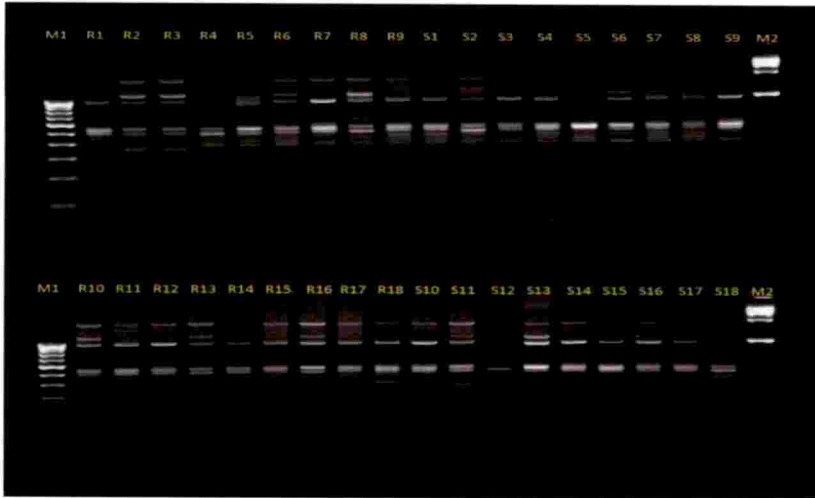


Plate 20: Agarose gel profile (1.8%) with the primer UBC 836

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159;
R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5:
TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree
Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203;
R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13:
IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1:
100bp; M2: 1Kbp
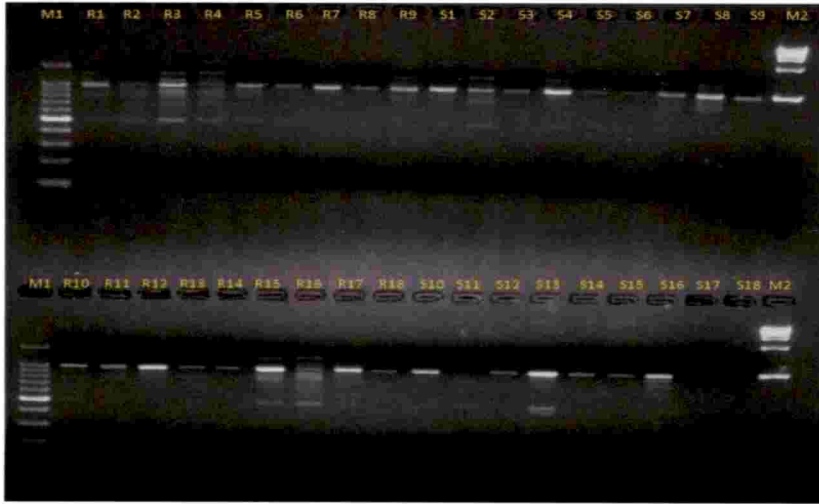
Plate 21: Agarose gel profile (1.8%) with the primer UBC 841
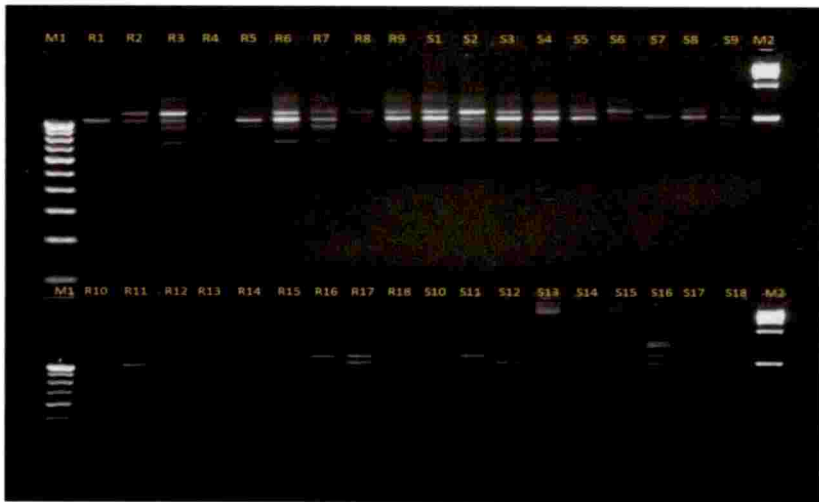


Plate 22: Agarose gel profile (1.8%) with the primer UBC 847

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1: 100bp; M2: 1Kbp

## 4.2.2.3. ISSR GEL ELECTROPHORESIS FOR PCR PRODUCT ANALYSIS

The PCR products were resolved in 1.8% agarose gel for the identification of markers associated with leaf blight resistance. Out of 14 primers, the primer (UBC 811) gave an extra band for 7 resistant genotypes IC012601, IC089624, TCR 429, 679, 370, 84 and 565 in 1270 bp and it included three resistant lines, 679, 370 and 84 which showed resistance for the last four years under artificial screening (Plate 14). For primer UBC 836, a resistant genotype IC089624 expressed a unique band at 1000 bp (Plate 20). The primer $(GA)_9AC$ also produced an unique band for resistant genotype, IC089624 at 800 bp (Plate 12). For primer, UBC 824, a resistant line, 565 showed a different banding pattern (Plate 17).

The total number of bands per ISSR primer ranged from 5 (UBC847) to 10 (UBC 818). The lowest number of bands was recorded for UBC847 (5) followed by $(GA)_9AT$, UBC 817, $(GA)_9AC$ and UBC 841 with seven bands each. Primer UBC 818 which produced highest number of bands also recorded the highest number of polymorphic bands (10) followed by UBC 809, UBC 810 and UBC 811 with nine bands each. Number of alleles per locus ranged from 2.38-6.13 with the maximum alleles shown UBC 811 and the minimum showed by UBC 817.

The polymorphism of the ISSR primers studied ranged from 60 to 100% with UBC 827 recording the lowest (60%) while 12 primers recorded the highest values (100%). Average percentage of polymorphism for ISSR primer was 95.7%.

The observed heterozygosity value (He) ranged between 0.75 (UBC 827) to 0.87 (UBC 809, UBC 818 and UBC 811). For most of the ISSR primers the He values were found to be > 0.8.

The polymorphism Information content (PIC) of the primers were highest for UBC 818 (0.862) followed by UBC 811 (0.861) and UBC 809 (0.857). The primers, UBC 827 (0.710) recorded the lowest PIC content of <0.8. Number of alleles per locus ranged from 2.38-6.13 with the maximum alleles shown by UBC 811 and the minimum by UBC 817. The PIC, Number of alleles and He values of the ISSR primers studied are given in Table 13. Percent polymorphism of the ISSR primers studied are given in table 12.

Table 12. Percent polymorphism shown by 36 taro lines with fourteen ISSR primers

| S. No. | Primers | Total no. of Bands | Total no. of polymorphic bands | Percent polymorphism (%) |
|--------|---------|--------------------|--------------------------------|---------------------------|
| 1. | (GA)$_9$AC | 7 | 7 | 100 |
| 2. | (ACC)$_9$ Y | 8 | 8 | 100 |
| 3. | (GA)$_9$AT | 7 | 7 | 100 |
| 4. | UBC 809 | 9 | 9 | 100 |
| 5. | UBC 810 | 9 | 9 | 100 |
| 6. | UBC 811 | 9 | 9 | 100 |
| 7. | UBC 817 | 7 | 7 | 100 |
| 8. | UBC 818 | 10 | 8 | 80 |
| 9. | UBC 824 | 8 | 8 | 100 |
| 10. | UBC 825 | 8 | 8 | 100 |
| 11. | UBC 827 | 6 | 4 | 60 |
| 12. | UBC 836 | 8 | 8 | 100 |
| 13. | UBC 841 | 7 | 7 | 100 |
| 14. | UBC 847 | 5 | 5 | 100 |
| Total | | 108 | 105 | - |
| Mean | | - | - | 95.7 |

Table 13. Estimates of diversity for 14 ISSR primers

| S. No. | Primer | Avg. no. of alleles per locus | Polymorphism Information Content (PIC) | Heterozygosity (He) |
|--------|--------|-------------------------------|----------------------------------------|---------------------|
| 1. | (ACC)9Y | 5.05 | 0.836 | 0.85 |
| 2. | (GA)9AC | 3.83 | 0.808 | 0.83 |
| 3. | (GA)9AT | 3.88 | 0.787 | 0.81 |
| 4. | UBC809 | 5.55 | 0.856 | 0.87 |
| 5. | UBC810 | 4.61 | 0.829 | 0.84 |
| 6. | UBC811 | 6.13 | 0.861 | 0.87 |
| 7. | UBC817 | 2.38 | 0.808 | 0.83 |
| 8. | UBC818 | 6.00 | 0.862 | 0.87 |
| 9. | UBC824 | 1.94 | 0.766 | 0.79 |
| 10. | UBC825 | 4.61 | 0.825 | 0.84 |
| 11. | UBC827 | 4.33 | 0.709 | 0.75 |
| 12. | UBC836 | 5.25 | 0.800 | 0.82 |
| 13. | UBC841 | 2.72 | 0.791 | 0.81 |
| 14. | UBC847 | 3.63 | 0.756 | 0.78 |

## 4.2.2.4. SIMILARITY INDEX

The similarity index values obtained for each pair wise comparison among the 36 taro genotypes based on 14 ISSR marker data ranged from 0.50 to 0.88 (Fig. 3). Most of the similarity coefficient values ranged between 0.61 to 0.79. Among the 36 taro accessions, the lowest similarity index (0.50) was observed between Sree Rashmi (S1) and B2 (SVP) (18), whereas, the highest (0.88) was between Sree Rashmi and E-10 (R9).

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| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R2 | 0.74 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R3 | 0.63 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R4 | 0.67 | 0.80 | 0.74 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R5 | 0.65 | 0.76 | 0.72 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R6 | 0.76 | 0.72 | 0.72 | 0.72 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R7 | 0.65 | 0.74 | 0.72 | 0.72 | 0.74 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R8 | 0.63 | 0.71 | 0.63 | 0.63 | 0.61 | 0.71 | 0.71 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R9 | 0.75 | 0.70 | 0.64 | 0.70 | 0.68 | 0.77 | 0.75 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R10 | 0.68 | 0.70 | 0.62 | 0.61 | 0.62 | 0.70 | 0.61 | 0.57 | 0.65 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R11 | 0.70 | 0.64 | 0.62 | 0.70 | 0.70 | 0.66 | 0.64 | 0.57 | 0.65 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| R12 | 0.62 | 0.72 | 0.72 | 0.72 | 0.68 | 0.66 | 0.72 | 0.53 | 0.63 | 0.71 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| R13 | 0.64 | 0.66 | 0.72 | 0.62 | 0.62 | 0.64 | 0.64 | 0.62 | 0.60 | 0.71 | 0.72 | 0.74 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| R14 | 0.75 | 0.66 | 0.68 | 0.66 | 0.66 | 0.72 | 0.72 | 0.66 | 0.63 | 0.65 | 0.69 | 0.72 | 0.69 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| R15 | 0.65 | 0.71 | 0.72 | 0.72 | 0.65 | 0.63 | 0.74 | 0.67 | 0.68 | 0.70 | 0.79 | 0.86 | 0.75 | 0.75 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| R16 | 0.61 | 0.67 | 0.67 | 0.71 | 0.61 | 0.65 | 0.67 | 0.67 | 0.64 | 0.68 | 0.70 | 0.75 | 0.70 | 0.68 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| R17 | 0.66 | 0.66 | 0.62 | 0.62 | 0.61 | 0.62 | 0.64 | 0.64 | 0.60 | 0.63 | 0.74 | 0.76 | 0.69 | 0.72 | 0.81 | 0.77 | 1.00 | | | | | | | | | | | | | | | | | | | |
| R18 | 0.60 | 0.63 | 0.58 | 0.63 | 0.58 | 0.63 | 0.61 | 0.60 | 0.53 | 0.66 | 0.62 | 0.68 | 0.64 | 0.68 | 0.65 | 0.63 | 0.62 | 1.00 | | | | | | | | | | | | | | | | | | |
| S1 | 0.69 | 0.69 | 0.63 | 0.71 | 0.69 | 0.72 | 0.71 | 0.67 | 0.88 | 0.57 | 0.64 | 0.62 | 0.61 | 0.66 | 0.65 | 0.67 | 0.62 | 0.60 | 1.00 | | | | | | | | | | | | | | | | | |
| S2 | 0.66 | 0.72 | 0.70 | 0.73 | 0.68 | 0.70 | 0.77 | 0.75 | 0.76 | 0.60 | 0.60 | 0.65 | 0.60 | 0.65 | 0.70 | 0.77 | 0.61 | 0.61 | 0.75 | 1.00 | | | | | | | | | | | | | | | | |
| S3 | 0.65 | 0.74 | 0.65 | 0.72 | 0.65 | 0.71 | 0.72 | 0.71 | 0.77 | 0.61 | 0.61 | 0.62 | 0.61 | 0.62 | 0.65 | 0.71 | 0.66 | 0.58 | 0.72 | 0.83 | 1.00 | | | | | | | | | | | | | | | |
| S4 | 0.66 | 0.62 | 0.61 | 0.66 | 0.66 | 0.70 | 0.72 | 0.77 | 0.78 | 0.50 | 0.60 | 0.54 | 0.58 | 0.61 | 0.59 | 0.64 | 0.60 | 0.53 | 0.81 | 0.74 | 0.68 | 1.00 | | | | | | | | | | | | | | |
| S5 | 0.72 | 0.68 | 0.62 | 0.72 | 0.62 | 0.73 | 0.66 | 0.72 | 0.82 | 0.60 | 0.61 | 0.58 | 0.56 | 0.67 | 0.61 | 0.64 | 0.56 | 0.59 | 0.79 | 0.78 | 0.73 | 0.80 | 1.00 | | | | | | | | | | | | | |
| S6 | 0.71 | 0.69 | 0.65 | 0.74 | 0.67 | 0.72 | 0.72 | 0.74 | 0.75 | 0.64 | 0.68 | 0.62 | 0.62 | 0.70 | 0.67 | 0.72 | 0.68 | 0.60 | 0.76 | 0.75 | 0.76 | 0.73 | 0.79 | 1.00 | | | | | | | | | | | | |
| S7 | 0.72 | 0.63 | 0.63 | 0.63 | 0.60 | 0.67 | 0.63 | 0.72 | 0.79 | 0.57 | 0.61 | 0.55 | 0.59 | 0.66 | 0.61 | 0.63 | 0.59 | 0.52 | 0.76 | 0.75 | 0.74 | 0.79 | 0.83 | 0.74 | 1.00 | | | | | | | | | | | |
| S8 | 0.63 | 0.61 | 0.56 | 0.67 | 0.65 | 0.65 | 0.60 | 0.71 | 0.75 | 0.55 | 0.62 | 0.53 | 0.53 | 0.62 | 0.56 | 0.61 | 0.59 | 0.58 | 0.76 | 0.72 | 0.69 | 0.81 | 0.81 | 0.76 | 0.82 | 1.00 | | | | | | | | | | |
| S9 | 0.65 | 0.65 | 0.63 | 0.63 | 0.65 | 0.63 | 0.67 | 0.67 | 0.68 | 0.61 | 0.68 | 0.64 | 0.62 | 0.70 | 0.67 | 0.69 | 0.64 | 0.58 | 0.63 | 0.72 | 0.74 | 0.70 | 0.72 | 0.78 | 0.74 | 0.74 | 1.00 | | | | | | | | | |
| S10 | 0.59 | 0.70 | 0.68 | 0.70 | 0.62 | 0.64 | 0.70 | 0.66 | 0.60 | 0.67 | 0.67 | 0.74 | 0.71 | 0.63 | 0.73 | 0.73 | 0.72 | 0.70 | 0.66 | 0.72 | 0.66 | 0.60 | 0.58 | 0.66 | 0.57 | 0.57 | 0.70 | 1.00 | | | | | | | | |
| S11 | 0.65 | 0.71 | 0.67 | 0.74 | 0.65 | 0.69 | 0.63 | 0.72 | 0.64 | 0.68 | 0.66 | 0.70 | 0.64 | 0.62 | 0.69 | 0.76 | 0.70 | 0.67 | 0.67 | 0.73 | 0.67 | 0.68 | 0.62 | 0.74 | 0.60 | 0.65 | 0.67 | 0.79 | 1.00 | | | | | | | |
| S12 | 0.66 | 0.72 | 0.66 | 0.73 | 0.68 | 0.68 | 0.72 | 0.68 | 0.67 | 0.65 | 0.67 | 0.71 | 0.63 | 0.74 | 0.72 | 0.73 | 0.74 | 0.64 | 0.70 | 0.76 | 0.72 | 0.69 | 0.63 | 0.72 | 0.66 | 0.58 | 0.72 | 0.80 | 0.79 | 1.00 | | | | | | |
| S13 | 0.58 | 0.61 | 0.65 | 0.65 | 0.63 | 0.63 | 0.63 | 0.63 | 0.57 | 0.64 | 0.64 | 0.68 | 0.66 | 0.62 | 0.67 | 0.69 | 0.70 | 0.60 | 0.58 | 0.66 | 0.61 | 0.64 | 0.62 | 0.63 | 0.61 | 0.60 | 0.63 | 0.70 | 0.76 | 0.75 | 1.00 | | | | | |
| S14 | 0.61 | 0.62 | 0.59 | 0.62 | 0.61 | 0.61 | 0.70 | 0.66 | 0.63 | 0.65 | 0.61 | 0.65 | 0.65 | 0.67 | 0.68 | 0.62 | 0.65 | 0.64 | 0.62 | 0.67 | 0.61 | 0.65 | 0.61 | 0.68 | 0.57 | 0.57 | 0.64 | 0.72 | 0.70 | 0.72 | 0.73 | 1.00 | | | | |
| S15 | 0.59 | 0.57 | 0.55 | 0.61 | 0.59 | 0.55 | 0.61 | 0.61 | 0.56 | 0.61 | 0.60 | 0.67 | 0.61 | 0.65 | 0.70 | 0.62 | 0.69 | 0.62 | 0.57 | 0.63 | 0.61 | 0.54 | 0.58 | 0.62 | 0.53 | 0.51 | 0.59 | 0.63 | 0.66 | 0.72 | 0.75 | 0.80 | 1.00 | | | |
| S16 | 0.59 | 0.66 | 0.64 | 0.66 | 0.62 | 0.62 | 0.66 | 0.68 | 0.60 | 0.56 | 0.58 | 0.65 | 0.61 | 0.65 | 0.68 | 0.64 | 0.65 | 0.59 | 0.62 | 0.72 | 0.66 | 0.67 | 0.63 | 0.72 | 0.61 | 0.64 | 0.70 | 0.72 | 0.77 | 0.76 | 0.77 | 0.78 | 0.80 | 1.00 | | |
| S17 | 0.62 | 0.64 | 0.59 | 0.62 | 0.66 | 0.68 | 0.62 | 0.61 | 0.61 | 0.65 | 0.61 | 0.63 | 0.60 | 0.65 | 0.62 | 0.53 | 0.61 | 0.62 | 0.62 | 0.61 | 0.59 | 0.60 | 0.63 | 0.64 | 0.57 | 0.61 | 0.66 | 0.67 | 0.68 | 0.72 | 0.73 | 0.82 | 0.76 | 0.82 | 1.00 | |
| S18 | 0.54 | 0.58 | 0.58 | 0.56 | 0.58 | 0.60 | 0.58 | 0.63 | 0.53 | 0.62 | 0.57 | 0.59 | 0.59 | 0.64 | 0.61 | 0.58 | 0.64 | 0.52 | 0.50 | 0.53 | 0.60 | 0.53 | 0.53 | 0.67 | 0.56 | 0.56 | 0.65 | 0.64 | 0.65 | 0.68 | 0.71 | 0.72 | 0.66 | 0.72 | 0.73 | 1.00 |

Fig.3 : Similarity coefficient based on Jaccard's coefficient for 36 taro genotypes with 14 ISSR markers

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 1.00 | | | | | | | | | | | | | | | | | |
| R2 | 0.74 | 1.00 | | | | | | | | | | | | | | | | |
| R3 | 0.63 | 0.76 | 1.00 | | | | | | | | | | | | | | | |
| R4 | 0.67 | 0.80 | 0.74 | 1.00 | | | | | | | | | | | | | | |
| R5 | 0.65 | 0.76 | 0.72 | 0.72 | 1.00 | | | | | | | | | | | | | |
| R6 | 0.76 | 0.72 | 0.72 | 0.72 | 0.72 | 1.00 | | | | | | | | | | | | |
| R7 | 0.65 | 0.74 | 0.72 | 0.72 | 0.74 | 0.76 | 1.00 | | | | | | | | | | | |
| R8 | 0.69 | 0.71 | 0.69 | 0.69 | 0.61 | 0.71 | 0.71 | 1.00 | | | | | | | | | | |
| R9 | 0.75 | 0.70 | 0.64 | 0.70 | 0.68 | 0.77 | 0.75 | 0.72 | 1.00 | | | | | | | | | |
| R10 | 0.68 | 0.70 | 0.62 | 0.61 | 0.62 | 0.70 | 0.61 | 0.57 | 0.65 | 1.00 | | | | | | | | |
| R11 | 0.70 | 0.64 | 0.62 | 0.70 | 0.70 | 0.66 | 0.64 | 0.57 | 0.65 | 0.78 | 1.00 | | | | | | | |
| R12 | 0.62 | 0.72 | 0.72 | 0.72 | 0.68 | 0.66 | 0.72 | 0.59 | 0.63 | 0.71 | 0.78 | 1.00 | | | | | | |
| R13 | 0.64 | 0.66 | 0.72 | 0.62 | 0.62 | 0.64 | 0.64 | 0.62 | 0.60 | 0.71 | 0.72 | 0.74 | 1.00 | | | | | |
| R14 | 0.75 | 0.66 | 0.68 | 0.66 | 0.66 | 0.72 | 0.72 | 0.66 | 0.69 | 0.65 | 0.69 | 0.72 | 0.69 | 1.00 | | | | |
| R15 | 0.65 | 0.71 | 0.72 | 0.72 | 0.65 | 0.69 | 0.74 | 0.67 | 0.68 | 0.70 | 0.79 | 0.86 | 0.75 | 0.75 | 1.00 | | | |
| R16 | 0.61 | 0.67 | 0.67 | 0.71 | 0.61 | 0.65 | 0.67 | 0.67 | 0.64 | 0.68 | 0.70 | 0.75 | 0.70 | 0.68 | 0.76 | 1.00 | | |
| R17 | 0.66 | 0.66 | 0.62 | 0.62 | 0.61 | 0.62 | 0.64 | 0.64 | 0.60 | 0.69 | 0.74 | 0.76 | 0.69 | 0.72 | 0.81 | 0.77 | 1.00 | |
| R18 | 0.60 | 0.63 | 0.58 | 0.63 | 0.58 | 0.63 | 0.61 | 0.60 | 0.59 | 0.66 | 0.62 | 0.68 | 0.64 | 0.68 | 0.65 | 0.69 | 0.62 | 1.00 |

Fig. 3 (a). Similarity matrix based on Jaccard's coefficient for 18 resistant lines based on 14 ISSR markers

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| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 0.69 | 0.69 | 0.63 | 0.71 | 0.69 | 0.72 | 0.71 | 0.67 | **0.88** | 0.57 | 0.64 | 0.62 | 0.61 | 0.66 | 0.65 | 0.67 | 0.62 | 0.60 |
| S2 | 0.66 | 0.72 | 0.70 | 0.73 | 0.68 | 0.70 | 0.77 | 0.75 | 0.76 | 0.60 | 0.60 | 0.65 | 0.60 | 0.65 | 0.70 | 0.77 | 0.61 | 0.61 |
| S3 | 0.65 | 0.74 | 0.65 | 0.72 | 0.65 | 0.71 | 0.72 | 0.71 | 0.77 | 0.61 | 0.61 | 0.62 | 0.61 | 0.62 | 0.65 | 0.71 | 0.66 | 0.58 |
| S4 | 0.66 | 0.62 | 0.61 | 0.66 | 0.66 | 0.70 | 0.72 | 0.77 | 0.78 | 0.50 | 0.60 | 0.54 | 0.58 | 0.61 | 0.59 | 0.64 | 0.60 | 0.53 |
| S5 | 0.72 | 0.68 | 0.62 | 0.72 | 0.62 | 0.73 | 0.68 | 0.72 | 0.82 | 0.60 | 0.61 | 0.58 | 0.56 | 0.67 | 0.61 | 0.64 | 0.56 | 0.59 |
| S6 | 0.71 | 0.69 | 0.65 | 0.74 | 0.67 | 0.72 | 0.72 | 0.74 | 0.75 | 0.64 | 0.68 | 0.62 | 0.62 | 0.70 | 0.67 | 0.72 | 0.68 | 0.60 |
| S7 | 0.72 | 0.63 | 0.63 | 0.63 | 0.60 | 0.67 | 0.63 | 0.72 | 0.79 | 0.57 | 0.61 | 0.55 | 0.59 | 0.66 | 0.61 | 0.63 | 0.59 | 0.52 |
| S8 | 0.69 | 0.61 | 0.56 | 0.67 | 0.65 | 0.65 | 0.60 | 0.71 | 0.75 | 0.55 | 0.62 | 0.53 | 0.53 | 0.62 | 0.56 | 0.61 | 0.59 | 0.58 |
| S9 | 0.65 | 0.65 | 0.63 | 0.69 | 0.65 | 0.69 | 0.67 | 0.67 | 0.68 | 0.61 | 0.68 | 0.64 | 0.62 | 0.70 | 0.67 | 0.69 | 0.64 | 0.58 |
| S10 | 0.59 | 0.70 | 0.68 | 0.70 | 0.62 | 0.64 | 0.70 | 0.66 | 0.60 | 0.67 | 0.67 | 0.74 | 0.71 | 0.69 | 0.73 | 0.79 | 0.72 | 0.70 |
| S11 | 0.65 | 0.71 | 0.67 | 0.74 | 0.65 | 0.69 | 0.69 | 0.72 | 0.64 | 0.68 | 0.66 | 0.70 | 0.64 | 0.62 | 0.69 | 0.76 | 0.70 | 0.67 |
| S12 | 0.66 | 0.72 | 0.66 | 0.73 | 0.68 | 0.68 | 0.72 | 0.68 | 0.67 | 0.65 | 0.67 | 0.71 | 0.63 | 0.74 | 0.72 | 0.73 | 0.74 | 0.64 |
| S13 | 0.58 | 0.61 | 0.65 | 0.65 | 0.63 | 0.63 | 0.63 | 0.69 | 0.57 | 0.64 | 0.64 | 0.68 | 0.66 | 0.62 | 0.67 | 0.69 | 0.70 | 0.60 |
| S14 | 0.61 | 0.62 | 0.59 | 0.62 | 0.61 | 0.61 | 0.70 | 0.66 | 0.63 | 0.65 | 0.61 | 0.65 | 0.65 | 0.67 | 0.68 | 0.62 | 0.65 | 0.64 |
| S15 | 0.59 | 0.57 | 0.55 | 0.61 | 0.59 | 0.55 | 0.61 | 0.61 | 0.56 | 0.61 | 0.60 | 0.67 | 0.61 | 0.65 | 0.70 | 0.62 | 0.69 | 0.62 |
| S16 | 0.59 | 0.66 | 0.64 | 0.66 | 0.62 | 0.62 | 0.66 | 0.68 | 0.60 | 0.56 | 0.58 | 0.65 | 0.61 | 0.65 | 0.68 | 0.64 | 0.65 | 0.59 |
| S17 | 0.62 | 0.64 | 0.59 | 0.62 | 0.66 | 0.68 | 0.62 | 0.61 | 0.61 | 0.65 | 0.61 | 0.63 | 0.60 | 0.65 | 0.62 | 0.53 | 0.61 | 0.62 |
| S18 | 0.54 | 0.58 | 0.58 | 0.56 | 0.58 | 0.60 | 0.58 | 0.63 | 0.53 | 0.62 | 0.57 | 0.59 | 0.59 | 0.64 | 0.61 | 0.58 | 0.64 | 0.52 |

Fig. 3 (b). Similarity coefficient based on Jaccard's coefficient for 18 resistant and 18 susceptible lines based on 14 ISSR markers

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|     | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 |
|-----|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| S1  | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S2  | 0.75 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S3  | 0.72 | 0.83 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S4  | 0.81 | 0.74 | 0.68 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S5  | 0.79 | 0.78 | 0.73 | 0.80 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S6  | 0.76 | 0.75 | 0.76 | 0.79 | 0.79 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |
| S7  | 0.76 | 0.75 | 0.74 | 0.79 | 0.83 | 0.74 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |
| S8  | 0.76 | 0.72 | 0.69 | 0.81 | 0.81 | 0.76 | 0.82 | 1.00 |      |      |      |      |      |      |      |      |      |      |
| S9  | 0.69 | 0.72 | 0.74 | 0.70 | 0.72 | 0.78 | 0.74 | 0.74 | 1.00 |      |      |      |      |      |      |      |      |      |
| S10 | 0.66 | 0.72 | 0.66 | 0.60 | 0.58 | 0.66 | 0.57 | 0.57 | 0.70 | 1.00 |      |      |      |      |      |      |      |      |
| S11 | 0.67 | 0.73 | 0.67 | 0.68 | 0.62 | 0.74 | 0.60 | 0.65 | 0.67 | 0.79 | 1.00 |      |      |      |      |      |      |      |
| S12 | 0.70 | 0.76 | 0.72 | 0.69 | 0.69 | 0.72 | 0.66 | 0.68 | 0.72 | 0.80 | 0.79 | 1.00 |      |      |      |      |      |      |
| S13 | 0.58 | 0.66 | 0.61 | 0.64 | 0.62 | 0.69 | 0.61 | 0.60 | 0.69 | 0.70 | 0.76 | 0.75 | 1.00 |      |      |      |      |      |
| S14 | 0.62 | 0.67 | 0.61 | 0.65 | 0.61 | 0.68 | 0.57 | 0.57 | 0.64 | 0.72 | 0.70 | 0.72 | 0.73 | 1.00 |      |      |      |      |
| S15 | 0.57 | 0.63 | 0.61 | 0.54 | 0.58 | 0.62 | 0.53 | 0.51 | 0.59 | 0.69 | 0.66 | 0.72 | 0.75 | 0.80 | 1.00 |      |      |      |
| S16 | 0.62 | 0.72 | 0.66 | 0.67 | 0.63 | 0.72 | 0.61 | 0.64 | 0.70 | 0.72 | 0.77 | 0.76 | 0.77 | 0.78 | 0.80 | 1.00 |      |      |
| S17 | 0.62 | 0.61 | 0.59 | 0.60 | 0.63 | 0.64 | 0.57 | 0.61 | 0.66 | 0.67 | 0.68 | 0.72 | 0.73 | 0.82 | 0.76 | 0.82 | 1.00 |      |
| S18 | 0.50 | 0.59 | 0.60 | 0.53 | 0.53 | 0.67 | 0.56 | 0.56 | 0.65 | 0.64 | 0.65 | 0.68 | 0.71 | 0.72 | 0.66 | 0.72 | 0.73 | 1.00 |

Fig. 3 (c). Similarity matrix based on Jaccard's coefficient for 18 susceptible lines based on 14 ISSR markers

## 4.2.2.5. CLUSTER ANALYSIS

A dendrogram generated using UPGMA cluster analysis separated the 36 taro genotypes into two major clusters where, the susceptible genotypes clustered together with the resistant genotypes (Fig. 4). Cluster-I and II have four sub-clusters each. Cluster-I comprised 12 resistant lines and seven susceptible lines and was divided into four sub-clusters. The first sub-cluster included three resistant genotypes wherein L12 (R14) showed divergence. The

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second sub-cluster included four resistant genotypes wherein TCR 429 (R3) and IC089624 (R5) showed divergence. Third sub-cluster consisted of two resistant and two susceptible genotypes, where, a resistant genotype 565 (R8) showed divergence. In the last sub-cluster only one resistant genotype E10 (R9) was included while, the rest were susceptible ones including the variety Sree Rashmi (S1) which grouped together with E10 (R9) and revealed 88% similarity. All the four susceptible varieties are included in this sub-cluster and expressed 74% similarity.

With respect to Cluster-II which comprised eight resistant and nine susceptible lines is also subdivided into four sub-clusters. The first sub-cluster with six resistant genotypes showed 72% similarity having a resistant line, B4 (R10) showing divergence. Genotypes 203 (R15) and J14 (R12) which are resistant showed 81% similarity. In the second subcluster, with three susceptible and only one resistant genotype, 679 (R16) are noticed. Muktakeshi (R18) formed as an outlier. So it can't be considered as a cluster and also it is divergent. In the third subcluster only susceptible genotypes existed showing a similarity of 71% with genotype B2 (SVP) (S18) showing divergence.
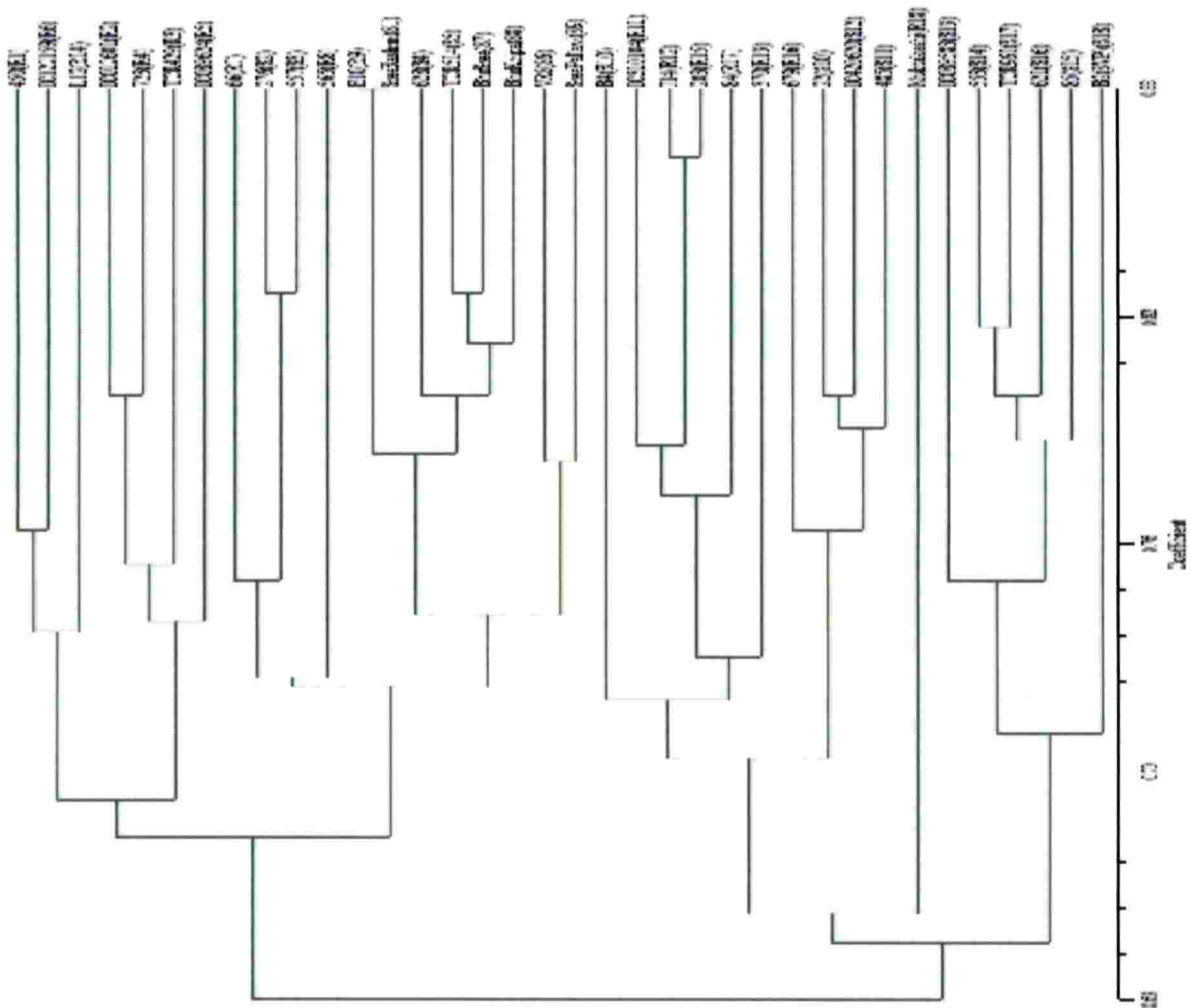
Fig. 4. Dendrogram showing the clustering pattern with seven ISSR markers

Table 14. Distribution of genotypes into different clusters with ISSR primers

| Cluster | | Number of accessions | Genotypes |
|---|---|---|---|
| 1 | a | 3 | 450, IC122159, L12 |
| | b | 4 | L-14, 723, TCR 429, IC089624 |
| | c | 4 | 276, 66, 557, 565 |
| | d | 8 | E10, Sree Rashmi, 628, TCR 514, Bhu Sree, Bhu Kripa, VRS, Sree Pallavi |
| 2 | a | 6 | B4, IC310104, J14, 203, 84, 370 |
| | b | 4 | 679, 22, 485, IC420620 |
| | Outlier | | Muktakeshi |
| | d | 6 | 553, IC-089583, TCR 961, B2SVP, 621, 85 |

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## 4.2.2.6. SEQUENCE DATA

For the ISSR primer UBC 811, seven resistant genotypes showed a specific band of 1270 bp size as shown in plate 14, which was completely absent in the 18 susceptible genotypes studied. Hence, this was eluted using QIAquick Gel Extraction kit. Eluted DNA was checked on 1.0 % agarose gel for its presence. As the size of the band was very high and concentration was less, it was re-amplified with the same primer. For re-amplification, only four genotypes (IC012601- R2; 370 – R13; 679 – R16 and 84 – R17) were selected based on the band intensity. This product was then checked in agarose gel, which gave two bands of which one was very prominent at approximately 280 bp (Fig. 5 ). This band was isolated and sequenced. Sequence data showed that the size ranged from 242 bp, 252 bp, 247 bp and 252 bp, respectively (Table 15).



Fig. 5. Reamplification of eluted samples, which gave a prominent at
approximately 280bp

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Table 15. List of genotypes used for sequencing and their sequence details

| Genotype details | Sequence obtained | Size (bp) |
|---|---|---|
| R2- IC012601 | AAAGGCTTATCCTCAGTTATTGAGGATCCTAGACTAGAATT AGGCAAAATACTAGTTCGACCAATGCGCTGCCACACGTTAG CAAAACATCATTCGTAATACCACAAGTTATTGAGACGATCT TTTGAAGAAGATAGCCTGTCGCTCGAAATCGCTTTTTGATCA CTACACTGGAAACTCTTTCGGGCCAACCATCATCATTCAGG GAGTTCACTGCCCCGCTATTGTGTCTCTCTCTCTCTCATCA CCC | 242 |
| R13 - 370 | AAAGGCTTATCCTCAGTTATTGAGGATCCTAGACTAGAATTA GGCAAAATACTAGTTCGACCAATGCGCTGCCACACGTTAGC AAAACATCATTCGTAATACCACAAGTTATTGAGACGATCTTT TGAAGAAGATAGCCTGTCGCTCGAAATCGCTTTTTGATCACT ACACTGGAAACTCTTTCGGGCCAACCATCATCATTCAGGGA GTTCACTGCCCCGCTATTGTGTCTCTCTCTCTCTCATCACC C | 252 |
| R16 - 679 | GTAGCAAATCACTCAGTAATTGAGGATCGTAGACTAAAATT GGTAAATACGTAATTCGACCAAAGCGCTGCCACACATTAGC AAAACATCATTCGTAAAAACACAAGTTATTGAGAGGATCTT TTGAAGAAGATAGCCAGTCGCTCGAAATCGCTTTTTGATCAT AACACTGGAAACTCTTTCGGGCCATCGATCATCGTTCAGGG AGTTCACTGCCCCGCTATCTGTCTCTCTCTCTCTCATCA | 247 |
| R17 - 84 | GATGGTGTAGCATCAGTTGTTGATGATCCTAGGCTAGATATT AGTCTAAATACGTATATTCGACCAAAGCGCTGCCACACATT AGCAAAACATCATTCGTAAAACCACAAGTTATTGAGACGAT CTTTTGAACAAGATAGCCAGTCGCTCGAAATCGTAGTTTGAT CATAACACTGGAAACTCTTTCGGGCCAACCATCCTCATTCAG GGAGTTCACTGCCCCCTATCTGTCTCTCTCTTCTCCTCCTCAC C | 252 |

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## 4.2.2.7. BLAST ANALYSIS

The sequences obtained as above, were used for similarity search in BLASTn and 100% identity and 8% query cover with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), mRNA was obtained for the DNA sequence from R13 (370) (Fig. 6). The following is the sequence which showed similarity with the above mRNA - TTTGAAGAAGATAGCCT (17 bp).

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A



PREDICTED: Arabidopsis lyrata subsp. lyrata disease resistance protein RML1B (LOC9323997), mRNA

Sequence ID: XM_002886333.2  Length: 3470  Number of Matches: 1

Range 1: 3081 to 3097 GenBank Graphics    ▼ Next Match  ▲ Previous Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 31.9 bits(34) | 6.5 | 17/17(100%) | 0/17(0%) | Plus/Plus |

```
Query  64    TTTGAAGAAGATAGCCT  80
             |||||||||||||||||
Sbjct  3081  TTTGAAGAAGATAGCCT  3097
```

B

**PREDICTED: Arabidopsis lyrata subsp. lyrata disease resistance protein RML1B (LOC9323997), mRNA**
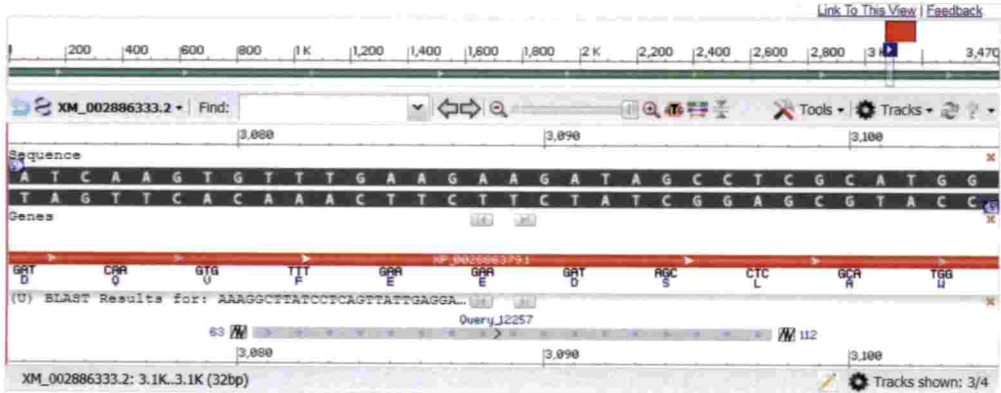
Fig. 6: A - Similarity obtained from the raw sequence data (252 bp) for the genotype 370 (R13) using ISSR marker UBC811 by BLASTn analysis; B - prediction showing similarity with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), C - mRNA and its graphic representation

## 4.2.3. SSR MARKER SCREENING

Of the 12 SSR markers screened initially, nine were finally selected for studies based on clear and consistent bands obtained. The selected ones included Ce1 F04, Ce1 B03, Ce1 F12, Uq 97-256, Uq 201-302, Uq 73-164, Uq 84-207, Uq 110-283 and Uq 132-147.

### 4.2.3.1. PCR CONDITIONS

PCR conditions already standardized in the Molecular Biology Lab at the Division of Crop Improvement, ICAR-CTCRI was followed to obtain crisp and clear amplicons with an annealing temperature of $56^0$C. The annealing temperatures standardized earlier was used in these experiments too. The gels were resolved in 2.5% agarose gel for better resolution of the PCR products. The bands were marked as present (1) or absent (0) and this binary data was used for generating the similarity matrix as well as the dendrogram (Plate 23 - 31).

### 4.2.3.2. ANALYSIS OF MOLECULAR DATA

The data was analyzed for various diversity parameters estimated for the quantification of genetic variability using nine SSR markers as given in table 16-17.

The gel images of some selected SSR primers for TLB screening are as follows:

Plate 23. Agarose gel profile (2.5%) with the primer Ce1F12
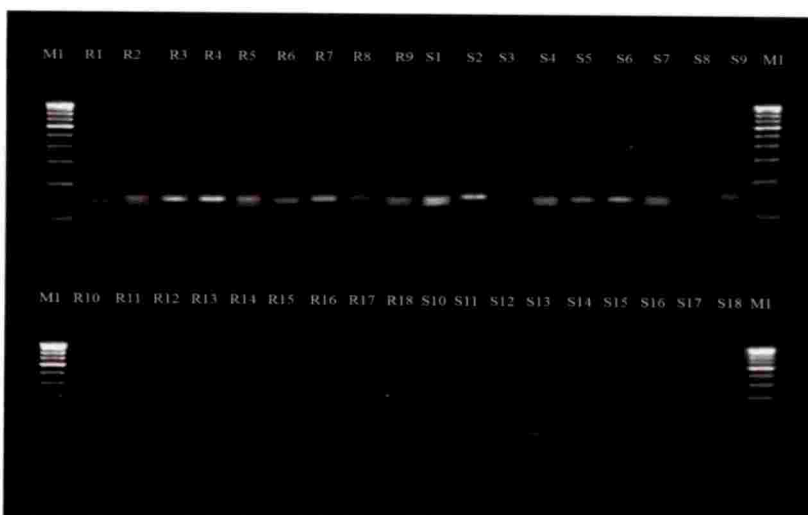


Plate 24. Agarose gel profile (2.5%) with the primer Ce1F04

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1-100bp
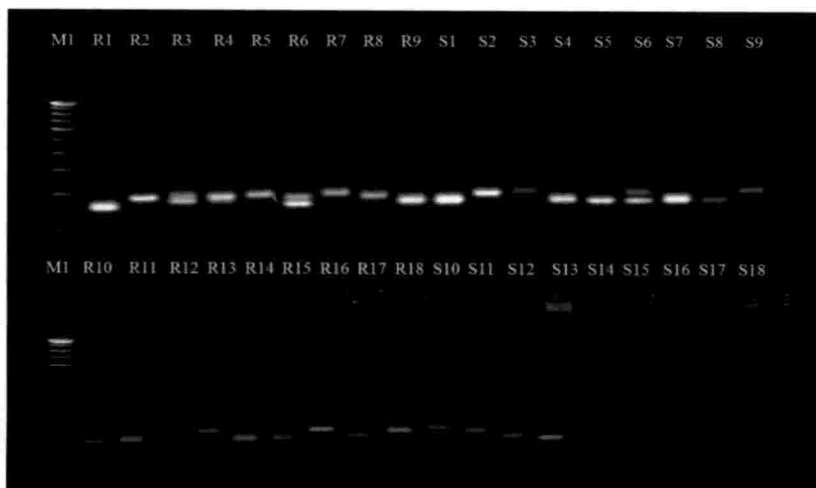
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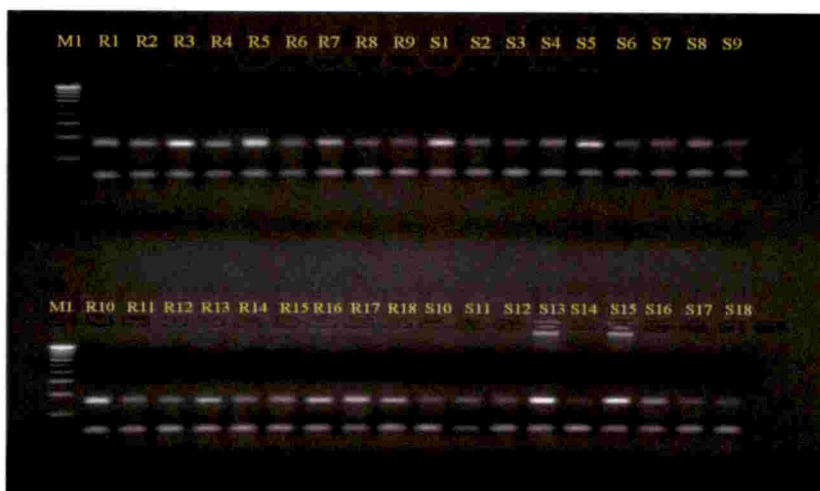Plate 25. Agarose gel profile (2.5%) with the primer Ce1B03



Plate 26. Agarose gel profile (2.5%) with the primer Uq 73-164

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159; R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5: TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203; R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13: IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B-2 (SVP); M1-100bp
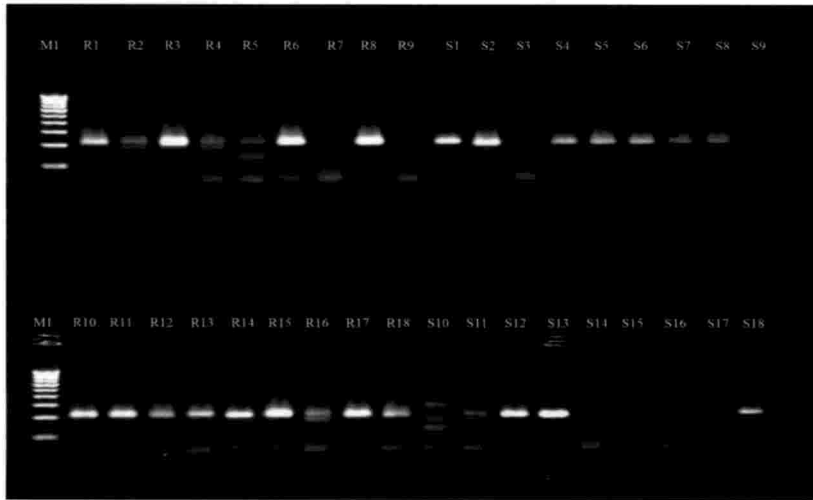
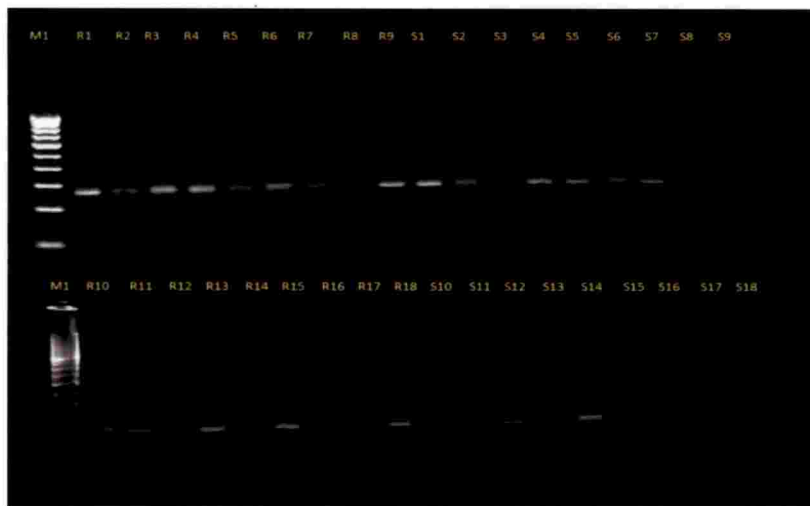Plate 27. Agarose gel profile (2.5%) with the primer Uq 84-207



Plate 28. Agarose gel profile (2.5%) with the primer Uq 110-283

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159;
R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5:
TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree
Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203;
R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13:
IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B2 (SVP); M1-
100bp
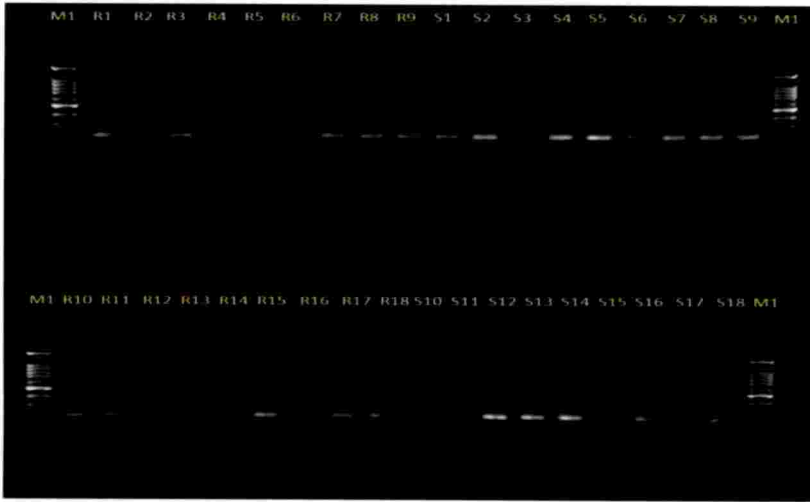
Plate 29.  Agarose gel profile (2.5%) with the primer Uq 201-302
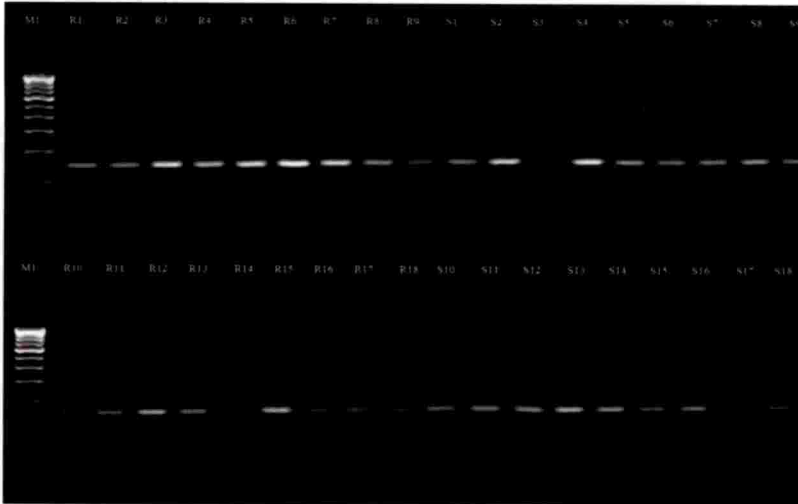


Plate 30.  Agarose gel profile (2.5%) with the primer Uq 132-147

R1: 450; R2: IC012601; R3: TCR 429; R4: 723; R5: IC089624; R6: IC122159;
    R7: 66; R8: 565; R9: E10; S1: Sree Rashmi; S2: 276; S3: 557; S4: 628; S5:
TCR 514; S6: VRS; S7: Sonajuli (Bhu Sree); S8: Jhankri (Bhu Kripa); S9: Sree
Pallavi; R10: B4; R11: IC310104; R12: J14; R13: 370; R14: L12; R15: 203;
R16: 679; R17: 84; R18: Muktakeshi; S10: 22; S11: 485; S12: IC420620; S13:
IC089583; S14: 553; S15: 85; S16: 621; S17: TCR 961; S18: B-2 (SVP); M1-
100bp

## 4.2.3.3. SSR GEL ELECTROPHORESIS

The PCR products were resolved in 2.5% agarose gel for the identification of markers associated with leaf blight resistance. However, no specific bands were obtained for the SSR markers studied.

The total number of bands per SSR primer ranged from 2 (Uq 110-283) to 11 (Uq 97-256). Least number of bands were recorded with Uq 110-283 (2) followed by Uq 73-164, Uq 84-207, Ce1 B03, Ce1 F04 and Ce1 F12 with seven bands each whereas, Uq 97-256 recorded the highest number of polymorphic bands (11).

The polymorphism of the SSR primers studied ranged from 33.33 to 100% where Uq 73-164 recorded the lowest polymorphism (33.33%) followed by Uq 201-302 (50%). Ce1 F12, Uq 97-256 showed 100% polymorphism. Average polymorphism percentage of SSR primers was 71.29%.

The observed heterozygosity value (He) ranged between 0.33 (Uq 84-207) to 0.74 (Uq 132-147 and Uq 201-302). For most of the SSR primers studied He was found to be less than 0.8.

The polymorphism Information content (PIC) of the primers were highest (0.69) for Uq 132-147and Uq 201-302 followed by Uq 97-256 (0.61) and Uq 73-164 (0.59). The primer, Uq 84-207 (0.30), recorded the lowest PIC content. Number of alleles per locus ranged from 1.08-6.22 with the maximum alleles shown in Uq 97-256 and the minimum showed by Ce1 B03. The PIC, Number of alleles value and He values of the SSR primers studied are presented in table 17.

Table 16. Percent polymorphism obtained with nine SSR primers

| Sl. No. | Primers | Total number of bands | Total number of polymorphic bands | Percent polymorphism (%) |
|---|---|---|---|---|
| 1. | Uq 73-164 | 3 | 1 | 33.33 |
| 2. | Uq 84-207 | 3 | 2 | 66.66 |
| 3. | Uq 97-256 | 11 | 11 | 100 |
| 4. | Uq 110-283 | 2 | 1 | 50 |
| 5. | Uq 132-147 | 4 | 3 | 75 |
| 6. | Uq 201-302 | 4 | 2 | 50 |
| 7. | Ce1 B03 | 3 | 3 | 100 |
| 8. | Ce1 F12 | 3 | 3 | 100 |
| 9. | Ce1 F04 | 3 | 2 | 66.66 |
| Total | | 36 | 28 | - |
| Mean | | - | - | 71.29 |

Table 17. Diversity estimates for nine SSR markers

| S. No. | Primer | Ta (°C) | Average number of alleles per locus | Polymorphism Information Content | Heterozygosity (He) |
|---|---|---|---|---|---|
| 1. | Uq 73-164 | 56 | 2.8 | 0.59 | 0.66 |
| 2. | Uq 84-207 | 56 | 1.25 | 0.30 | 0.33 |
| 3. | Uq 97-256 | 56 | 6.22 | 0.61 | 0.66 |
| 4. | Uq 110-283 | 56 | 1.6 | 0.36 | 0.47 |
| 5. | Uq 132-147 | 56 | 3.25 | 0.69 | 0.74 |
| 6. | Uq 201-302 | 56 | 3.5 | 0.69 | 0.74 |
| 7. | Ce1 B03 | 56 | 1.08 | 0.48 | 0.57 |
| 8. | Ce1 F12 | 56 | 1.63 | 0.44 | 0.54 |
| 9. | Ce1 F04 | 56 | 1.75 | 0.50 | 0.57 |

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## 4.2.3.4. SIMILARITY INDEX

The similarity index values obtained for each pair wise comparison among the 36 taro genotypes based on nine SSR marker data is given in the fig.7. The similarity coefficient values ranged from 0.49 to 0.89 concentrating between 0.56 to 0.86. Among the 36 taro accessions, the lowest similarity index (0.49) was observed between 485 (S11) and 450 (R1), whereas, the highest (0.89) was between 679 (R16) and TCR 961 (S17).

|  | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 1.00 | | | | | | | | | | | | | | | | | |
| R2 | 0.70 | 1.00 | | | | | | | | | | | | | | | | |
| R3 | 0.59 | 0.78 | 1.00 | | | | | | | | | | | | | | | |
| R4 | 0.59 | 0.78 | 0.73 | 1.00 | | | | | | | | | | | | | | |
| R5 | 0.59 | 0.84 | 0.73 | 0.73 | 1.00 | | | | | | | | | | | | | |
| R6 | 0.57 | 0.65 | 0.65 | 0.65 | 0.76 | 1.00 | | | | | | | | | | | | |
| R7 | 0.73 | 0.65 | 0.65 | 0.76 | 0.65 | 0.62 | 1.00 | | | | | | | | | | | |
| R8 | 0.65 | 0.73 | 0.73 | 0.78 | 0.73 | 0.70 | 0.86 | 1.00 | | | | | | | | | | |
| R9 | 0.73 | 0.65 | 0.54 | 0.65 | 0.65 | 0.57 | 0.78 | 0.70 | 1.00 | | | | | | | | | |
| R10 | 0.81 | 0.68 | 0.73 | 0.62 | 0.62 | 0.65 | 0.81 | 0.73 | 0.70 | 1.00 | | | | | | | | |
| R11 | 0.65 | 0.62 | 0.68 | 0.62 | 0.57 | 0.54 | 0.81 | 0.73 | 0.76 | 0.78 | 1.00 | | | | | | | |
| R12 | 0.51 | 0.49 | 0.54 | 0.65 | 0.54 | 0.51 | 0.62 | 0.70 | 0.62 | 0.54 | 0.65 | 1.00 | | | | | | |
| R13 | 0.54 | 0.68 | 0.68 | 0.68 | 0.73 | 0.65 | 0.76 | 0.84 | 0.70 | 0.68 | 0.84 | 0.76 | 1.00 | | | | | |
| R14 | 0.68 | 0.65 | 0.65 | 0.70 | 0.65 | 0.57 | 0.78 | 0.70 | 0.73 | 0.70 | 0.81 | 0.73 | 0.81 | 1.00 | | | | |
| R15 | 0.62 | 0.65 | 0.70 | 0.59 | 0.65 | 0.68 | 0.73 | 0.76 | 0.73 | 0.76 | 0.81 | 0.68 | 0.81 | 0.73 | 1.00 | | | |
| R16 | 0.54 | 0.78 | 0.73 | 0.68 | 0.78 | 0.59 | 0.65 | 0.62 | 0.65 | 0.62 | 0.62 | 0.43 | 0.68 | 0.65 | 0.70 | 1.00 | | |
| R17 | 0.59 | 0.73 | 0.73 | 0.73 | 0.68 | 0.59 | 0.81 | 0.84 | 0.76 | 0.73 | 0.89 | 0.70 | 0.95 | 0.86 | 0.81 | 0.73 | 1.00 | |
| R18 | 0.54 | 0.62 | 0.73 | 0.68 | 0.62 | 0.59 | 0.76 | 0.73 | 0.65 | 0.73 | 0.68 | 0.59 | 0.73 | 0.70 | 0.70 | 0.78 | 0.78 | 1.00 |

Fig.7 (a). Similarity matrix based on Jaccard's coefficient for 18 resistant lines based on nine SSR markers

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| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R2 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R3 | 0.59 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R4 | 0.59 | 0.78 | 0.73 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R5 | 0.59 | 0.84 | 0.73 | 0.73 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R6 | 0.57 | 0.65 | 0.65 | 0.65 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R7 | 0.73 | 0.65 | 0.65 | 0.76 | 0.65 | 0.62 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R8 | 0.65 | 0.73 | 0.73 | 0.78 | 0.73 | 0.70 | 0.86 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R9 | 0.73 | 0.65 | 0.54 | 0.65 | 0.65 | 0.57 | 0.78 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R10 | 0.81 | 0.68 | 0.73 | 0.62 | 0.62 | 0.65 | 0.81 | 0.73 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| R11 | 0.65 | 0.62 | 0.68 | 0.62 | 0.57 | 0.54 | 0.81 | 0.73 | 0.76 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| R12 | 0.51 | 0.49 | 0.54 | 0.65 | 0.54 | 0.51 | 0.62 | 0.70 | 0.62 | 0.54 | 0.65 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| R13 | 0.54 | 0.68 | 0.68 | 0.68 | 0.73 | 0.65 | 0.76 | 0.84 | 0.70 | 0.68 | 0.84 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| R14 | 0.68 | 0.65 | 0.65 | 0.70 | 0.65 | 0.57 | 0.78 | 0.70 | 0.73 | 0.70 | 0.81 | 0.73 | 0.81 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| R15 | 0.62 | 0.65 | 0.70 | 0.59 | 0.65 | 0.68 | 0.73 | 0.76 | 0.73 | 0.76 | 0.81 | 0.68 | 0.81 | 0.73 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| R16 | 0.54 | 0.78 | 0.73 | 0.68 | 0.78 | 0.59 | 0.65 | 0.62 | 0.65 | 0.62 | 0.62 | 0.43 | 0.68 | 0.65 | 0.70 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| R17 | 0.59 | 0.73 | 0.73 | 0.73 | 0.68 | 0.59 | 0.81 | 0.84 | 0.76 | 0.73 | 0.89 | 0.70 | 0.95 | 0.86 | 0.81 | 0.73 | 1.00 | | | | | | | | | | | | | | | | | | | |
| R18 | 0.54 | 0.62 | 0.73 | 0.68 | 0.62 | 0.59 | 0.76 | 0.73 | 0.65 | 0.73 | 0.68 | 0.59 | 0.73 | 0.70 | 0.70 | 0.78 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | |
| S1 | 0.65 | 0.73 | 0.78 | 0.62 | 0.68 | 0.70 | 0.70 | 0.73 | 0.65 | 0.78 | 0.73 | 0.49 | 0.73 | 0.70 | 0.81 | 0.78 | 0.78 | 0.84 | 1.00 | | | | | | | | | | | | | | | | | |
| S2 | 0.65 | 0.73 | 0.78 | 0.73 | 0.73 | 0.70 | 0.86 | 0.78 | 0.70 | 0.78 | 0.73 | 0.54 | 0.73 | 0.76 | 0.81 | 0.78 | 0.78 | 0.84 | 0.84 | 1.00 | | | | | | | | | | | | | | | | |
| S3 | 0.62 | 0.76 | 0.81 | 0.65 | 0.70 | 0.62 | 0.73 | 0.81 | 0.68 | 0.70 | 0.76 | 0.62 | 0.76 | 0.68 | 0.68 | 0.65 | 0.81 | 0.65 | 0.70 | 0.76 | 1.00 | | | | | | | | | | | | | | | |
| S4 | 0.68 | 0.65 | 0.59 | 0.65 | 0.65 | 0.57 | 0.68 | 0.76 | 0.62 | 0.59 | 0.70 | 0.73 | 0.76 | 0.68 | 0.73 | 0.59 | 0.70 | 0.59 | 0.65 | 0.65 | 0.68 | 1.00 | | | | | | | | | | | | | | |
| S5 | 0.62 | 0.65 | 0.81 | 0.54 | 0.65 | 0.62 | 0.73 | 0.70 | 0.68 | 0.76 | 0.76 | 0.57 | 0.70 | 0.73 | 0.73 | 0.70 | 0.76 | 0.70 | 0.76 | 0.81 | 0.89 | 0.57 | 1.00 | | | | | | | | | | | | | |
| S6 | 0.68 | 0.65 | 0.70 | 0.70 | 0.54 | 0.62 | 0.78 | 0.76 | 0.62 | 0.65 | 0.70 | 0.62 | 0.65 | 0.73 | 0.73 | 0.65 | 0.70 | 0.70 | 0.76 | 0.81 | 0.73 | 0.84 | 0.73 | 1.00 | | | | | | | | | | | | |
| S7 | 0.68 | 0.70 | 0.76 | 0.54 | 0.65 | 0.68 | 0.62 | 0.70 | 0.68 | 0.70 | 0.70 | 0.57 | 0.76 | 0.68 | 0.73 | 0.70 | 0.81 | 0.70 | 0.81 | 0.70 | 0.84 | 0.57 | 0.84 | 0.62 | 1.00 | | | | | | | | | | | |
| S8 | 0.62 | 0.65 | 0.70 | 0.54 | 0.65 | 0.68 | 0.62 | 0.65 | 0.68 | 0.70 | 0.70 | 0.62 | 0.81 | 0.68 | 0.78 | 0.65 | 0.76 | 0.65 | 0.76 | 0.70 | 0.73 | 0.62 | 0.78 | 0.62 | 0.84 | 1.00 | | | | | | | | | | |
| S9 | 0.65 | 0.62 | 0.73 | 0.51 | 0.68 | 0.70 | 0.65 | 0.68 | 0.59 | 0.78 | 0.62 | 0.59 | 0.68 | 0.59 | 0.76 | 0.62 | 0.62 | 0.62 | 0.68 | 0.73 | 0.70 | 0.59 | 0.81 | 0.59 | 0.70 | 0.81 | 1.00 | | | | | | | | | |
| S10 | 0.51 | 0.70 | 0.70 | 0.76 | 0.76 | 0.62 | 0.78 | 0.81 | 0.62 | 0.70 | 0.70 | 0.62 | 0.81 | 0.73 | 0.73 | 0.81 | 0.81 | 0.86 | 0.76 | 0.81 | 0.68 | 0.68 | 0.68 | 0.68 | 0.62 | 0.62 | 0.65 | 1.00 | | | | | | | | |
| S11 | 0.49 | 0.62 | 0.57 | 0.57 | 0.73 | 0.54 | 0.59 | 0.62 | 0.65 | 0.57 | 0.68 | 0.65 | 0.73 | 0.59 | 0.65 | 0.73 | 0.68 | 0.68 | 0.57 | 0.57 | 0.65 | 0.70 | 0.65 | 0.54 | 0.59 | 0.65 | 0.68 | 0.76 | 1.00 | | | | | | | |
| S12 | 0.57 | 0.70 | 0.70 | 0.65 | 0.81 | 0.62 | 0.68 | 0.70 | 0.68 | 0.70 | 0.65 | 0.62 | 0.76 | 0.62 | 0.73 | 0.81 | 0.70 | 0.76 | 0.70 | 0.76 | 0.68 | 0.73 | 0.68 | 0.62 | 0.62 | 0.68 | 0.76 | 0.84 | 0.81 | 1.00 | | | | | | |
| S13 | 0.65 | 0.73 | 0.73 | 0.68 | 0.73 | 0.54 | 0.81 | 0.78 | 0.76 | 0.73 | 0.78 | 0.65 | 0.84 | 0.81 | 0.76 | 0.78 | 0.89 | 0.84 | 0.73 | 0.78 | 0.76 | 0.65 | 0.76 | 0.65 | 0.76 | 0.70 | 0.68 | 0.81 | 0.78 | 0.76 | 1.00 | | | | | |
| S14 | 0.51 | 0.59 | 0.70 | 0.65 | 0.54 | 0.62 | 0.78 | 0.76 | 0.68 | 0.70 | 0.81 | 0.68 | 0.81 | 0.78 | 0.78 | 0.70 | 0.86 | 0.86 | 0.81 | 0.81 | 0.73 | 0.62 | 0.78 | 0.78 | 0.73 | 0.73 | 0.65 | 0.78 | 0.65 | 0.68 | 0.81 | 1.00 | | | | |
| S15 | 0.59 | 0.62 | 0.73 | 0.73 | 0.57 | 0.59 | 0.70 | 0.73 | 0.65 | 0.68 | 0.78 | 0.81 | 0.78 | 0.86 | 0.76 | 0.62 | 0.84 | 0.73 | 0.68 | 0.73 | 0.70 | 0.70 | 0.70 | 0.76 | 0.70 | 0.65 | 0.62 | 0.70 | 0.57 | 0.65 | 0.73 | 0.81 | 1.00 | | | |
| S16 | 0.57 | 0.59 | 0.65 | 0.65 | 0.65 | 0.68 | 0.73 | 0.81 | 0.73 | 0.76 | 0.76 | 0.78 | 0.86 | 0.68 | 0.84 | 0.59 | 0.81 | 0.76 | 0.70 | 0.70 | 0.68 | 0.73 | 0.62 | 0.62 | 0.68 | 0.73 | 0.70 | 0.78 | 0.70 | 0.78 | 0.76 | 0.73 | 0.76 | 1.00 | | |
| S17 | 0.62 | 0.70 | 0.76 | 0.76 | 0.65 | 0.62 | 0.84 | 0.86 | 0.73 | 0.81 | 0.86 | 0.73 | 0.86 | 0.78 | 0.84 | 0.70 | 0.92 | 0.81 | 0.76 | 0.81 | 0.78 | 0.73 | 0.73 | 0.73 | 0.73 | 0.68 | 0.70 | 0.84 | 0.70 | 0.78 | 0.86 | 0.84 | 0.86 | 0.89 | 1.00 | |
| S18 | 0.57 | 0.70 | 0.65 | 0.65 | 0.70 | 0.57 | 0.73 | 0.70 | 0.62 | 0.70 | 0.70 | 0.62 | 0.76 | 0.73 | 0.73 | 0.81 | 0.81 | 0.81 | 0.76 | 0.76 | 0.73 | 0.68 | 0.73 | 0.68 | 0.73 | 0.68 | 0.65 | 0.84 | 0.81 | 0.73 | 0.86 | 0.78 | 0.65 | 0.68 | 0.78 | 1.00 |

Fig.7 : Similarity coefficient based on Jaccard's coefficient for 36 taro genotypes with nine SSR markers

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 0.65 | 0.73 | 0.78 | 0.62 | 0.68 | 0.70 | 0.70 | 0.73 | 0.65 | 0.78 | 0.73 | **0.49** | 0.73 | 0.70 | 0.81 | 0.78 | 0.78 | 0.84 |
| S2 | 0.65 | 0.73 | 0.78 | 0.73 | 0.73 | 0.70 | 0.86 | 0.78 | 0.70 | 0.78 | 0.73 | 0.54 | 0.73 | 0.76 | 0.81 | 0.78 | 0.78 | 0.84 |
| S3 | 0.62 | 0.76 | 0.81 | 0.65 | 0.70 | 0.62 | 0.73 | 0.81 | 0.68 | 0.70 | 0.76 | 0.62 | 0.76 | 0.68 | 0.68 | 0.65 | 0.81 | 0.65 |
| S4 | 0.68 | 0.65 | 0.59 | 0.65 | 0.65 | 0.57 | 0.68 | 0.76 | 0.62 | 0.59 | 0.70 | 0.73 | 0.76 | 0.68 | 0.73 | 0.59 | 0.70 | 0.59 |
| S5 | 0.62 | 0.65 | 0.81 | 0.54 | 0.65 | 0.62 | 0.73 | 0.70 | 0.68 | 0.76 | 0.76 | 0.57 | 0.70 | 0.73 | 0.73 | 0.70 | 0.76 | 0.70 |
| S6 | 0.68 | 0.65 | 0.70 | 0.70 | 0.54 | 0.62 | 0.78 | 0.76 | 0.62 | 0.65 | 0.70 | 0.62 | 0.65 | 0.73 | 0.73 | 0.65 | 0.70 | 0.70 |
| S7 | 0.68 | 0.70 | 0.76 | 0.54 | 0.65 | 0.68 | 0.62 | 0.70 | 0.68 | 0.70 | 0.70 | 0.57 | 0.76 | 0.68 | 0.73 | 0.70 | 0.81 | 0.70 |
| S8 | 0.62 | 0.65 | 0.70 | 0.54 | 0.65 | 0.68 | 0.62 | 0.65 | 0.68 | 0.70 | 0.70 | 0.62 | 0.81 | 0.68 | 0.78 | 0.65 | 0.76 | 0.65 |
| S9 | 0.65 | 0.62 | 0.73 | 0.51 | 0.68 | 0.70 | 0.65 | 0.68 | 0.59 | 0.78 | 0.62 | 0.59 | 0.68 | 0.59 | 0.76 | 0.62 | 0.62 | 0.62 |
| S10 | 0.51 | 0.70 | 0.70 | 0.76 | 0.76 | 0.62 | 0.78 | 0.81 | 0.62 | 0.70 | 0.70 | 0.62 | 0.81 | 0.73 | 0.73 | 0.81 | 0.81 | 0.86 |
| S11 | **0.49** | 0.62 | 0.57 | 0.57 | 0.73 | 0.54 | 0.59 | 0.62 | 0.65 | 0.57 | 0.68 | 0.65 | 0.73 | 0.59 | 0.65 | 0.73 | 0.68 | 0.68 |
| S12 | 0.57 | 0.70 | 0.70 | 0.65 | 0.81 | 0.62 | 0.68 | 0.70 | 0.68 | 0.70 | 0.65 | 0.62 | 0.76 | 0.62 | 0.73 | 0.81 | 0.70 | 0.76 |
| S13 | 0.65 | 0.73 | 0.73 | 0.68 | 0.73 | 0.54 | 0.81 | 0.78 | 0.76 | 0.73 | 0.78 | 0.65 | 0.84 | 0.81 | 0.76 | 0.78 | 0.89 | 0.84 |
| S14 | 0.51 | 0.59 | 0.70 | 0.65 | 0.54 | 0.62 | 0.78 | 0.76 | 0.68 | 0.70 | 0.81 | 0.68 | 0.81 | 0.78 | 0.78 | 0.70 | 0.86 | 0.86 |
| S15 | 0.59 | 0.62 | 0.73 | 0.73 | 0.57 | 0.59 | 0.70 | 0.73 | 0.65 | 0.68 | 0.78 | 0.81 | 0.78 | 0.86 | 0.76 | 0.62 | 0.84 | 0.73 |
| S16 | 0.57 | 0.59 | 0.65 | 0.65 | 0.65 | 0.68 | 0.73 | 0.81 | 0.73 | 0.76 | 0.76 | 0.78 | 0.86 | 0.68 | 0.84 | 0.59 | 0.81 | 0.76 |
| S17 | 0.62 | 0.70 | 0.76 | 0.76 | 0.65 | 0.62 | 0.84 | 0.86 | 0.73 | 0.81 | 0.86 | 0.73 | 0.86 | 0.78 | 0.84 | 0.70 | 0.92 | 0.81 |
| S18 | 0.57 | 0.70 | 0.65 | 0.65 | 0.70 | 0.57 | 0.73 | 0.70 | 0.62 | 0.70 | 0.70 | 0.62 | 0.76 | 0.73 | 0.73 | 0.81 | 0.81 | 0.81 |

Fig. 7 (b). Similarity coefficient based on Jaccard's coefficient for 18 resistant
and 18 susceptible lines based on nine SSR markers

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 | R13 | R14 | R15 | R16 | R17 | R18 |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S1 | 1.00 | | | | | | | | | | | | | | | | | |
| S2 | 0.84 | 1.00 | | | | | | | | | | | | | | | | |
| S3 | 0.70 | 0.76 | 1.00 | | | | | | | | | | | | | | | |
| S4 | 0.65 | 0.65 | 0.68 | 1.00 | | | | | | | | | | | | | | |
| S5 | 0.76 | 0.81 | 0.89 | 0.57 | 1.00 | | | | | | | | | | | | | |
| S6 | 0.76 | 0.81 | 0.73 | 0.84 | 0.73 | 1.00 | | | | | | | | | | | | |
| S7 | 0.81 | 0.70 | 0.84 | 0.57 | 0.84 | 0.62 | 1.00 | | | | | | | | | | | |
| S8 | 0.76 | 0.70 | 0.73 | 0.62 | 0.78 | 0.62 | 0.84 | 1.00 | | | | | | | | | | |
| S9 | 0.68 | 0.73 | 0.70 | 0.59 | 0.81 | 0.59 | 0.70 | 0.81 | 1.00 | | | | | | | | | |
| S10 | 0.76 | 0.81 | 0.68 | 0.68 | 0.68 | 0.68 | 0.62 | 0.62 | 0.65 | 1.00 | | | | | | | | |
| S11 | 0.57 | 0.57 | 0.65 | 0.70 | 0.65 | 0.54 | 0.59 | 0.65 | 0.68 | 0.76 | 1.00 | | | | | | | |
| S12 | 0.70 | 0.76 | 0.68 | 0.73 | 0.68 | 0.62 | 0.62 | 0.68 | 0.76 | 0.84 | 0.81 | 1.00 | | | | | | |
| S13 | 0.73 | 0.78 | 0.76 | 0.65 | 0.76 | 0.65 | 0.76 | 0.70 | 0.68 | 0.81 | 0.78 | 0.76 | 1.00 | | | | | |
| S14 | 0.81 | 0.81 | 0.73 | 0.62 | 0.78 | 0.78 | 0.73 | 0.73 | 0.65 | 0.78 | 0.65 | 0.68 | 0.81 | 1.00 | | | | |
| S15 | 0.68 | 0.73 | 0.70 | 0.70 | 0.70 | 0.76 | 0.70 | 0.65 | 0.62 | 0.70 | 0.57 | 0.65 | 0.73 | 0.81 | 1.00 | | | |
| S16 | 0.70 | 0.70 | 0.68 | 0.73 | 0.62 | 0.62 | 0.68 | 0.73 | 0.70 | 0.78 | 0.70 | 0.78 | 0.76 | 0.73 | 0.76 | 1.00 | | |
| S17 | 0.76 | 0.81 | 0.78 | 0.73 | 0.73 | 0.73 | 0.73 | 0.68 | 0.70 | 0.84 | 0.70 | 0.78 | 0.86 | 0.84 | 0.86 | 0.89 | 1.00 | |
| S18 | 0.76 | 0.76 | 0.73 | 0.68 | 0.73 | 0.68 | 0.73 | 0.68 | 0.65 | 0.84 | 0.81 | 0.73 | 0.86 | 0.78 | 0.65 | 0.68 | 0.78 | 1.00 |

Fig.7 (c). Similarity matrix based on Jaccard's coefficient for 18 susceptible lines based on nine SSR markers

## 4.2.3.5. CLUSTER ANALYSIS

A dendrogram generated using UPGMA cluster analysis separated the 36 taro genotypes into five major clusters and a highly divergent resistant genotype. Here the susceptible ones clustered together with the resistant genotypes as in RAPD and ISSR (Fig. 8). Cluster-I comprised three resistant genotypes (Table 18). Second cluster consisted of six genotypes of which, five were susceptible (including three varieties viz., Bhu Sree (S7), Bhu Kripa (S8) and Sree Pallavi (S9)) and only one resistant genotype, TCR 429 (R3). The third cluster consisted the maximum of 20 genotypes of which nine were

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resistant including Muktakeshi (R18) and eleven susceptible genotypes including the variety, Sree Rashmi (S1). Accession 370 (R13) and 84 (R17) revealed 95% similarity and both were resistant ones. The fourth cluster comprised of only two genotypes, both susceptible. Fifth cluster contained of four genotypes and all were resistant. J14 (R12), a resistant genotype was found to be highly divergent than the remaining ones and formed as an outlier showing a similarity of only 62% with the rest.

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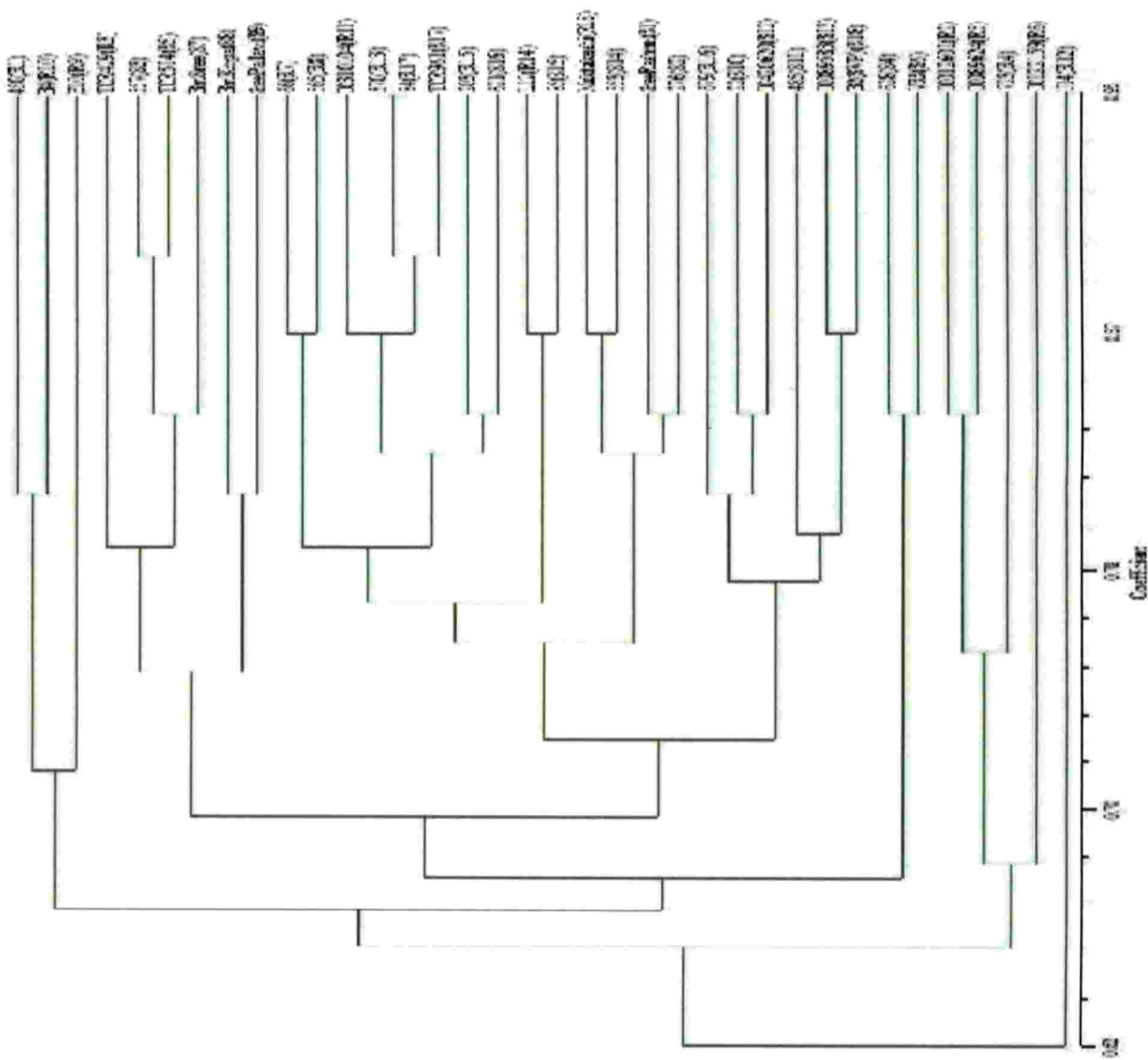
Fig. 8. Dendrogram showing the distribution of clusters with SSR markers

Table 18. Details of accessions grouping under various clusters

| Cluster | | No. of accessions | Genotypes |
|---|---|---|---|
| 1. | | 3 | 450, B4, E10 |
| 2. | a | 4 | TCR 429, 557, TCR 514, Bhu Sree |
| | b | 2 | Bhu Kripa, Sree Pallavi |
| 3. | a | 7 | 66, 565, IC310104, 370, 84, TCR 961, 203 |
| | b | 2 | 621, L12 |
| | c | 4 | Muktakeshi, 553, Sree Rashmi, 276 |
| | d | 3 | 679, 22, IC420620 |
| | e | 3 | 485, IC089583, B2(SVP) |
| 4. | | 2 | 628, VRS |
| 5. | | 4 | IC012601, IC089624, 723, IC122159 |
| Outlier | | | J14 |

## 4.3 CORRELATION BETWEEN Mantel's test using NTSYS pc. ver. 2.2

Mantel's test was done for all the three marker systems to find if any correlation existed between the three systems. All the three systems were compared with each other and it established that no correlation was there between the three molecular marker systems used for the study. This is reasonable as we did not get a trait specific marker in all the three marker systems. Hence in these three only the genetic diversity is reflected. If p value is less than 0.05, the result is significant. Here all the values are above that. Hence, there is no correlation between the different marker systems studied. The results of the same are shown below:

**RAPD vs. ISSR**

Matrix correlation:              $r = 0.43105$

Prob. random Z < obs. Z:    $p = 1.0000$

**RAPD vs. SSR**

Matrix correlation:              $r = 0.17271$

Prob. random Z < obs. Z:    $p = 0.9982$

**ISSR vs. SSR**

Matrix correlation:              $r = 0.09405$

Prob. random Z < obs. Z:    $p = 0.9360$

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# Discussion

# 5. DISCUSSION

Taro (*Colocasia esculenta* (L.) Schott), a member of the monocotyledonous family Araceae is an important staple food crop grown throughout many Pacific Island countries, parts of Africa, Asia and the Caribbean for its fleshy corms, petioles and nutritious leaves (Butler and Kulkarni, 1913; Plucknett, 1976; Kuruvilla and Singh, 1981; Bose *et al.*, 2003). Ethno-botanical evidences suggest that taro is originated in South Central Asia, probably in India or the Malay Peninsula and gradually spread worldwide by pioneers. However, the most recent study on genetic diversification and dispersal of taro using 11 microsatellite markers revealed that the highest genetic diversity and number of private alleles were observed in Asian accessions, mainly from India (Chair, *et. al.*, 2016), proving that India is the main centre of origin for taro from where, it dispersed to various regions like West Africa, Madagascar, Costa Rica, etc. Hence, a high degree of genetic diversity is expected in India.

Taro leaf blight (TLB) caused by *Phytophthora colocasiae* Raciborski has become a major limiting factor in all taro producing countries including India causing yield loss upto 50% (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1999). Resistant cultivars offer the best sustainable management strategy against taro leaf blight. Development and employment of molecular in general and use of molecular markers linked to useful traits in particular had made tremendous progress in recent years (Sharma *et al.*, 2008).

## 5.1 MOLECULAR CHARACTERISATION

### 5.1.1 DNA ISOLATION PROTOCOL

Modified CTAB protocol developed by Sharma *et al.,* 2008 (some modifications to remove polysaccharides) was employed for extraction of pure DNA in appreciable concentrations. The concentration of PVP and β – mercaptoethanol were altered in order to remove the secondary metabolites which are useful to prevent oxidation of secondary metabolites in the disrupted samples (Prittila *et al.,* 2001; Warude *et al.,* 2003), avoiding the browning of the sample, thus increasing the yield and quality of the DNA which is considered to be pure if the ratio of absorbance between the 260nm to that at 280nm is in the range 1.8-2.0 (Weising *et al.,* 2005). The DNA extracted in the study by employing the method developed by Sharma *et al.* (2008) was considered best as its A260/A280 ratio ranged from 1.98 - 2.3. Though the data shows that there could be slight RNA contamination from the OD values, upon checking the same using agarose gel showed that no RNA contamination was there. Hence, this DNA was used for further molecular studies. Unopened or just opened young leaves was preferred over others due to the low concentrations of polysaccharides, polyphenols and other secondary metabolites making it easy for DNA isolation (Debo *et al.,* 1993; Zhang and Steward, 2000).

### 5.1.2. PCR STANDARDIZATION AND PRIMER SCREENING

Three marker types *viz.,* RAPD, ISSR and SSR were employed for identification of molecular markers resistance to taro leaf blight. PCR conditions already standardized at the Molecular Biology Lab of ICAR-CTCRI were utilized for PCR amplification. 40ng/µl DNA for ISSR, RAPD and SSR reaction mixture was also standardized for one reaction. Higher concentrations

of DNA contain more amount of polyphenols which may interact with Taq polymerase and can hinder the amplification process. According to Ahmed, *et al*. (2009), too much of template DNA may reduce PCR efficiency due to presence of contaminants in DNA preparations. 1mM $MgCl_2$ was used in the study which was found to be optimum for good amplification profile. Increased $Mg^{+2}$ concentrations enhance Taq activity up to a certain level, above which it may act as a depressant (Kramer *et al*., 2004). Development of an ideal marker system which is genetically co-dominant and highly polymorphic allowing precise discrimination even in closely related individuals is important due to high levels of heterozygosity in individual genotypes (Elizabeth, *et al*., 2003).

## 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 potato and cassava largely due to constrains of pests and diseases becoming a major limiting factor for taro production (Ivancic, 1992). TLB constitutes a significant threat to food security and economy in those countries which do not have resistant varieties and where taro is a major staple and an export commodity (Sharma *et al*., 2008). Studies on the development of resistant varieties to leaf blight are still going on around the world. However, none of the workers in taro succeeded to identify any molecular markers associated with TLB with RAPD, ISSR and/or SSR markers. Till date no molecular markers exists for TLB worldwide. Though scattered reports are there where few accessions gave specific bands for the resistant lines (Anand, 2016). Another study done was by Sharma *et al*. (2008) who employing AFLP markers. Some of the major constraints for taro breeding programs including TLB resistance breeding are the lack of knowledge on genetic diversity in cultivars, limitations in access to and knowledge of additional sources of

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disease resistance as well as the absence of information on the potential agronomic and processing value of genotypes (Ivancic, *et al.*, 2000).

In this connection, for the present work, 36 genotypes (18 each of resistant and susceptible) were selected for the study. These lines were screened previously at ICAR-CTCRI for the last few years. Varieties like Sree Rashmi and Sree Pallavi are highly susceptible, whereas, Bhu Sree (Sonajuli) and Bhu Kripa (Jhankri) are moderately susceptible. Muktakeshi on the other hand is tolerant and all these were included in this study. Among the 18 resistant genotypes, seven (E-10, B-4, IC310104, 370, 203, 679 and 84) were found to be resistant for the last four years under artificial screening. Amongst these the first three are from the NEH region, which is now designated as the Centre of origin for taro (Chair *et al.*, 2016). Hence, there is every chance that we can find some good resistant source for TLB from this area.

5.2.1 RAPD MARKER ANALYSIS

RAPD primers that were employed to find out molecular markers associated with leaf blight resistance in taro were of the OPW series (OPW1, OPW2, OPW5, OPW6, OPW8, OPW12, OPW16). Hussain and Tyagi (2005) had used these sets of primers in their study for studying the uniformity of tissue culture regenerated plants with corms of taro and the same set were repeated here. The results obtained for the primer screening viz., percent polymorphism, PIC, number of alleles per locus, He, etc. also supported the usefulness of these primers in distinguishing the variability existing in this set of genotypes.

In a study with RAPD based DNA markers linked to anthracnose disease resistance in *Sorghum*, the locus for disease resistance in *Sorghum*

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accession G73 was found to segregate as a simple recessive trait in a cross with susceptible cultivar HC136. In order to identify molecular markers linked to disease resistance locus, Random Amplified Polymorphic DNA (RAPD) analysis was coupled with bulk segregant analysis. Six disease linked markers were screened with individual resistant and susceptible genotypes to observe degree of linkage of identified RAPD markers with the gene for resistance. Two primer sequences (OPI 16 and OPD 12) were found to be closely linked to the locus for disease resistance (Panday *et al.*, 2002). Another study by Mukesh *et al.* (2006) showed that a set of 104 wheat recombinant inbred lines (RILs) obtained from a cross between resistant parent (HD 29) and susceptible (WH 542) to Karnal bunt (KB) were screened and used to identify (RAPD) markers linked to Karnal bunt resistance. The two parents were analysed with 92 RAPD primers. A total of 65 primers proved to be functional by giving scorable polymerase chain reaction (PCR) products, of which, 21 primers detected polymorphism (32%) between the two parental genotypes. Using these primers, bulked segregant analysis was carried out on two bulked DNA, one obtained by pooling DNA from 10kb resistant RILs and the other by pooling 10kb susceptible RILs. One marker, OPM-20 showed apparent association with resistance to KB which was confirmed following selective genotyping of individual RILs included in the bulks. In another study by Gygax, *et al.* (2004), RAPD markers were used for apple scab resistance gene, *Vbj* derived from *Malus baccata* Jackii. It was confirmed that breeding for scab-resistant apple cultivars by pyramiding several resistance genes is a promising way to control apple scab caused by the fungus, *Venturia inaequalis* and found that three RAPD markers were linked to *Vbj*. In a similar way, a marker system could be developed and employed to breed new varieties resistance to TLB by pyramiding several resistance genes. In the present study, a resistant genotype

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R16 revealed a unique band at the 1000bp region with the primer OPW1 which is completely absent in all other genotypes and hence cannot be confirmed that the band is specific for resistance. This could be related to some other character/trait. With the primer OPW16 some resistant genotypes (IC012601, TCR 429, 723, 679, Muktakeshi) had a specific band at 300bp and is present in only one susceptible genotype 276 and hence this was not considered a marker specific for resistance. With yet another primer, OPW5 a resistant genotype, TCR 429 expressed a unique banding pattern. This analysis is important as this marker if validated, can be utilized for further breeding programs for screening resistant progenies at very early stages and thus speeding up the process of developing TLB resistant varieties.

UPGMA analysis utilizing RAPD markers proved to be a meaningful with respect to detecting the genetic diversity amongst the genotypes tested. The set was divided into two major clusters. Cluster-I and Cluster-II contained 9 each resistant and susceptible genotypes. The three released varieties Bhu Sree, Bhu Kripa and Sree Rashmi grouped together, where, Bhu Sree and Bhu Kripa pooled together revealing 88% similarity. Another set of 2 susceptible genotypes, 557 and 628 pooled together revealing a similarity of 81%. Though the studies performed were of preliminary type, specific clustering pattern within the sub-clusters. Nevertheless, this data can help the breeders in identifying genotypes which can be used as divergent parents for hybridization programmes so as to exploit heterosis.

The polymorphism of the RAPD primers studied ranged from 50% to 100%. OPW12 recorded the lowest (50%) polymorphism followed by OPW6 (81.8%). The observed heterozygosity values (He) ranged between 0.66 (OPW16) to 0.89 (OPW1, OPW2 and OPW8). For most of the RAPD primers

studied He were found to be >0.8 indicating that the primers used could detect variability present and hence are useful.

The polymorphism Information Content (PIC) were highest for OPW8 (0.888) primer followed by OPW2 (0.886) and OPW1 (0.885) and least with OPW16 (0.615). Number of alleles per locus ranged from 4 - 8.19 with the maximum alleles shown by OPW8 and the minimum by OPW5. Polymorphism observed in present study was able to differentiate taro genotypes which revealed high polymorphism. The results of the study regarding the genetic polymorphism in rye cultivars using RAPD by Petrovicova *et al.* (2014) where PIC values of all RAPD markers were higher than 0.8 indicating high level of polymorphism between rye genotypes proved RAPD to be a rapid, reliable and practicable method which agrees with the present study too.

## 5.2.2. ISSR MARKER STUDY

ISSR primers (UBC 841, UBC 811, UBC 810, UBC 817, UBC 847, UBC 871, UBC 827, UBC 836, UBC 825, UBC 809, UBC 818, $(GA)_9AC$, $(GA)_9AT$, $(ACC)_9$ Y) were used to find out markers associated with leaf blight resistance in taro. Ratnaparkhe *et al.* (1998) were the first to demonstrate that ISSR markers are useful in gene tagging and can be employed for identifying markers linked to a gene of interest. They found out that Simple Sequence Repeat $(AC)_8YT$ when used directly in a PCR reaction, amplifies a marker, UBC-855, which is linked to gene for resistance to fusarium wilt race 4. The repeat $(AC)_8T$ amplified a marker UBC-825 which was located at 5.0cM from the gene for resistance, which was closer than the UBC-855 and CS-27 markers (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) using ISSR. In lettuce, ISSR markers were used to

identify the 13 resistance genes for downy mildew and mapped into four clusters (Hulbert *et al.*, 1985). In the present study, for primer UBC 836, a resistant genotype IC089624 (R5) expressed a unique band at 1000 bp. The primer $(GA)_9AC$ also produced an unique band for resistant genotype, IC089624 (R5) at 800 bp, which are found to be promising if validated and can be utilized in further breeding programs for screening resistant progenies at a very early stages and thus speeding up the process of developing TLB resistant varieties. The clustering of resistance genes at a specific chromosomal region is advantageous, 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 *et al.*, 1998). ISSR was included in this study as a previous study by Anand (2016) showed that when six resistant and six susceptible lines of taro was screened using 13 ISSR markers, UBC 825 and $(AG)_9AC$ gave an extra band in all the resistant varieties in 685 bp and 808 bp regions, respectively in all the resistant lines screened. However, in the present study, though $(AG)_9AC$ gave a unique band at 800 bp for IC089624 (R5), the result could not be obtained for the other genotypes tested. This indicates that the unique band is not tightly linked to TLB resistance and more primers needs to be screened.

UPGMA analysis utilizing ISSR markers proved to be a successful one with the formation of 2 clusters, Cluster-I comprised 12 resistant and seven susceptible lines and Cluster-II with eight resistant and nine susceptible lines. Though the studies were of preliminary type, specific clustering pattern within the sub-clusters were noticed where, the two traits pooled along with each other, except a few exceptions. In the current study bands were obtained with ISSR primer, UBC 811 producing a band of 1270 bp, specific to seven resistant genotypes (IC012601, IC089624, TCR 429, 679, 370, 84 and 565) and

completely absent in susceptible genotypes which could be related to resistant gene. It is reported that a 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). Marker assisted selection (MAS) using DNA markers tightly linked to wilt resistance genes was used to screen a large number of germplasm lines without subjecting them to the pathogen and to pyramid them into agronomically superior varieties (Padaliya *et al.*, 2013). Present results could also pay way for utilization of ISSR markers after their proper validation.

The polymorphism of the ISSR primers employed ranged from 60 to 100% with UBC 827 recorded the lowest (60%) while UBC 818 recorded the next highest value of 80% while remaining primers showed 100% polymorphism. Average percentage of polymorphism is 95.7 The observed heterozygosity value (He) ranged between 0.75 (UBC 827) to 0.87 (UBC 809, UBC 818 and UBC 811). He values for most of the primers were found to be >0.8. Polymorphism Information Content (PIC) were highest for UBC 818 (0.862) followed by UBC 811 (0.861) and UBC 809(0.857). The primer, UBC 827 (0.709) recorded the lowest PIC content. Number of alleles per locus ranged from 2.38-6.13 with the maximum shown by UBC 811 and the minimum by UBC 817. However, Ramirez *et al.* (2014) could only get moderate PIC estimates for the ISSR markers in *Dioscorea* germplasm. The polymorphic information content measures the informativeness related to the expected heterozygousity (He) which can also be estimated from allele frequencies. The higher PIC and He values obtained in the present study indicated higher variability of the population and also indicated the usefulness of ISSR markers identified in elucidating genetic diversity among taro.

## 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 which prevented the broad use of microsatellites in plants (Ratnaparkhe *et al.*, 1998). In the present study, previously identified SSR makers were used, among which, primer Ce1 A08 didn't produced any specific band in the expected product size and hence was not considered for the study. The primers chosen for the study were Ce1 F04, Ce1 B03, Ce1 F12, Uq 97-256, Uq 201-302, Uq 73-164, Uq 84-207, Uq 110-283 and Uq 132-147 since they produced polymorphic bands. 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 were done worldwide to find out markers linked to disease resistance gene and to incorporate those genes into new varieties by breeding. Though in the present study, no specific bands were obtained associated with TLB, further studies needs to be performed to identify if any such marker(s) are available by employing more number of markers and genotypes.

In the cluster analysis, three clusters were obtained while generating a dendrogram using NTSYS software. Cluster-II contained five susceptible

genotypes including Bhu Sree, Bhu Kripa and Sree Pallavi along with a resistant genotype 557 which showed that 557 could have a genetic makeup similar to the susceptible varieties. The second cluster showed a mixed pattern which comprised both susceptible and resistant genotypes. In the fourth cluster five susceptible genotypes grouped with a lone resistant genotype, 679. Further studies are needed to confirm if the resistant genotypes are really resistant due to the fact that they also grouped together with susceptible ones. Similar results were also obtained in chickpea genotypes for wilt resistance by Padaliya *et al.*, (2013) where 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 tolerant genotypes such as, ICCV-2 and GG-1 were grouped in the same sub-cluster of cluster-II, the susceptible genotypes GG-4 and JG-62 were present in separate cluster.

It is difficult to pyramid two or more disease resistance genes conventionally 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 into a single line using marker assisted selection (MAS) and tagging of genes is a pre-requisite for MAS (Dhillon *et al.*, 2011). The future strategy should be to oriented towards development of new elite taro varieties along with enrichment of the germplasm from different regions to further diversify the existing diversity and conserve them. Use of these genotypes for crop improvement needs will strengthen the capacities of the national taro breeding programmes and to develop disease resistant varieties.

The polymorphism of the SSR primers studied ranged from 33.33% to 100% with Uq 73-164 recorded the lowest polymorphism (33.33%) followed

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by Uq 201-302 (50%). Primers Ce1 F12 and Uq 97-256 revealed 100% polymorphism. Average percent polymorphism of SSR primers was 71.29%. The observed heterozygosity value (He) ranged between 0.33 (Uq 84-207) to 0.74 (Uq 132-147 and Uq 201-302). For most of the SSR primers studied He was found to be less than 0.8. The polymorphism Information content (PIC) of the primers were highest (0.69) for Uq 132-147 and Uq 201-302 followed by Uq 97-256 (0.61) and Uq 73-164 (0.59). The primer, Uq 84-207 (0.30), recorded the lowest PIC content. Number of alleles per locus ranged from 1.08 - 6.22 with the maximum with Uq 97-256 and the minimum with Ce1 B03.

Nunes *et al.*, 2012 who investigated the genetic diversity of seven regional core collections of Brazilian taro using seven microsatellite loci, found polymorphism information content (PIC) ranging from 0.75 to 0.91, and the polymorphism was able to differentiate the taro cultivars. The results of the present study are in accordance with that of Nunes *et al.* (2012).

5.2.4. MANTLE'S TEST

Mantel's test was performed to access correlation between the different markers used. No correlation was observed between the three molecular markers employed, which clearly indicated that as there is no trait specific marker among all the three marker system employed and only genetic diversity is revealed. Comparison with different type of markers (RAPD, ISSR, SSR,) showed that even though numbers of polymorphic bands are higher for ISSR and RAPD, the average polymorphism information content was not lesser for SSR as compared to RAPD and ISSR primers evaluated. The results clearly indicate that all the types of markers employed were efficient in discriminating the genotypes evaluated. Similar works was done by other researchers too where they used more than one marker system in identifying the diversity

existing in taro (Singh *et al.,* 2011). Here they used both RAPD and ISSR to study the diversity in tarp along with morphological characters. In this study, both RAPD and ISSR was able to give a more or less similar pattern. But, it was much varied from the morphological data. The special distribution of the genetic diversity can be related to evolution of the species (Pissard *et al.*, 2007).

## 5.2.5 MARKER LINKED TO TLB

Though much work has not been done to identify molecular markers associated with TLB, few preliminary works are reported. One of the main works was reported by Sharma *et al.* (2009) using AFLP. They employed suppressive subtractive hybridization cDNA libraries, Northern blot analysis, high throughput DNA sequencing and bioinformatics to identify the defence related gene in taro induced by *P. colocasiae*. However, in the present study, an attempt was made to identify the presence of any marker through screening of resistant and susceptible genotypes using various marker systems. Though a tightly bound marker was not identified, we could identify similarity with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), mRNA from R13 (370) with the sequence TTTGAAGAAGATAGCCT (17 bp). Studies by Ivanicic *et al.* (1995) showed that resistance genotypes appeared in the population resulting from crosses between two partially susceptible genotypes, which indicates that minor genes associated with partial resistance are involved in leaf blight resistance. Hence, for further studies for identification of markers linked to TLB, more genotypes and markers needs to be screened and so also a mapping population can be developed.

# Summary

# 6. SUMMARY

The study entitled "**Identification of molecular markers for resistance to taro leaf blight in (*Colocasia esculenta* L.) Schott**" was carried out at the Division of Crop Improvement, ICAR-CTCRI, Sreekariyam, during the year 2017-18 with the objective to identify molecular markers associated with TLB by RAPD, ISSR and SSR markers; to characterize the identified markers by sequencing of trait specific genes followed by BLAST and assessing the genetic diversity existing in the resistant and susceptible genotypes studied. A total of 36 taro genotypes were selected from the taro field gene bank maintained at ICAR - CTCRI. Eighteen genotypes each of susceptible and resistant ones were screened to identify molecular markers associated with leaf blight resistance in taro.

DNA was isolated from the selected 36 genotypes by employing the method standardized by Doyle and Doyle (1987) and modified by Sharma *et al.* (2008) (CTAB method) to obtain good quantity DNA (278 ng/μl to 4200 ng/μl) with purity range from 1.9-2.3.

PCR conditions already standardized at the Molecular Biology Laboratory of Crop Improvement Division, ICAR-CTCRI was used for PCR amplification (RAPD & ISSR). Out of 15 RAPD primers from preliminary analysis of screening data, ten primers were screened to analyse its amplification profile, of which 9 primers were selected for the study. The annealing temperature for RAPD-PCR condition already standardized was optimized at $32^0$C. The primers selected (OPW series) for TLB screening were OPW1, OPW2, OPW5, OPW6, OPW8, OPW12, OPW14, OPW16. The presence of amplicons was confirmed by resolving them in 2% agarose gel.

All the primers produced polymorphic bands and showed high allelic frequency. The polymorphism Information content (PIC) were highest for OPW8 (0.888) primer followed by OPW2 (0.886) and OPW1 (0.885) and least with OPW16 (0.615). Number of alleles per locus ranged from 4-8.19 with the maximum alleles shown by OPW8 and the minimum with OPW5. Observed heterozygosity values (He) ranged between 0.66 (OPW16) to 0.89 (OPW1, OPW2, OPW8). For most of the RAPD primers employed, OPW12 recorded the least polymorphism (50%) followed by OPW6 (81.8%). Average percentage polymorphism varied from 86.22%. OPW1 and OPW16 produced 100% polymorphism. In short, all the primers selected were able to detect the variability present in the crop and hence can be used for further studies to identify diversity in taro.

Among the 36 taro accessions, the similarity index values ranged from 0.47 to 0.88 with the lowest similarity index (0.47) was observed between 557 (S3) and 370 (R13) and also between Sree Pallavi (S9) and 370 (R13) whereas, the highest similarity index (0.91) was observed between IC310104 (R11) and L-12 (R14). Amongst the susceptible lines, the highest similarity index (0.88) was shown between 22 (S10) and 621 (S16) as well as Bhu Sree (S7) and Bhu Kripa (S8). A dendrogram was generated which grouped the 36 genotypes into two major clusters where, no separate cluster was observed between susceptible and resistant genotypes. However, within the sub-clusters they grouped separately. Sub-cluster 1a had grouped Bhu Sree and Bhu Kripa pooling together showing 88% similarity. This could be due to the fact that both are landraces from Odhisha and hence could have some common features. In the sub-cluster 1b, Sree Pallavi a susceptible variety was pooled with two other susceptible ones, S5 and S16, but Pallavi was found to be different from them. On the other hand, the sub-cluster 1c pooled six resistant lines, where IC089624

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showed divergence. The susceptible line, 276 formed an outlier. In the second Cluster also, mostly the resistant and susceptible genotypes grouped together. However, some exceptions were there, in sub-cluster II b and c, which showed mixed grouping. Here, one of the resistant line, 679 (R16) formed an outlier and was found to be divergent from the rest. Two resistant lines, IC310104 (R11) and L-12 (R14) revealed 91% similarity. Both these have their origin in the NEH region and hence probably due to some common ancestry, they are showing high degree of similarity. In sub-cluster IIa only two genotypes were present - the resistant variety Muktakeshi (R18) and a susceptible genotype B2-(SVP) (S18) which revealed 80% similarity. Except Muktakeshi, all other released varieties of taro *viz.*, Sree Rashmi, Sree Pallavi, Bhu Sree and Bhu Kripa were included in the Cluster I. Of the 7 resistant lines which showed consistency with respect to resistance in the pot studies, identified earlier, only E-10 grouped in the Cluster-I and remaining six lines were grouped in the Cluster II. Bhu Sree, Bhu Kripa and Sree Rashmi existed in the same sub-cluster-I and showed a similarity of around 83%.

The annealing temperature for ISSR-PCR condition already standardized was done at $56.3^0$C. Nineteen ISSR primers were screened to analyse their amplification profile, out of which 14 primers were selected for the study. The primers selected (UBC series) for TLB screening were UBC 841, UBC 811, UBC 810, UBC 817, UBC 847, UBC 871, UBC 827, UBC 836, UBC 825, UBC 809, UBC 818, $(GA)_9AC$, $(GA)_9AT$ and $(ACC)_9$ Y. Presence of amplicons at expected size was confirmed by resolving in 1.8% agarose gel.

With respect to ISSR markers, the polymorphism information content (PIC) of the primers were highest for UBC 818 (0.862) followed by UBC 811(0.861) and UBC 809 (0.857) whereas UBC 827 (0.709) recorded the

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lowest PIC. Number of alleles per locus ranged from 2.38 - 6.13 with maximum alleles revealed by UBC 811 and the minimum with UBC 817. The observed heterozygosity value (He) varied between 0.75 (UBC 827) to 0.87 (UBC 809, UBC 818 and UBC 811). The He values were found to be >0.8 for most of the ISSR primers. Polymorphism varied from 60% to 100% with UBC 827 recorded the lowest (60%) while UBC 818 recorded the 80% polymorphism. All the other primers evaluated showed 100% polymorphism with average percentage of polymorphism of 95.7%. All the primers tested were hence found to be useful in detecting variability.

A similarity matrix generated using NTSys showed a similarity index ranging from 0.50 to 0.88 with lowest value (0.50) for Sree Rashmi (S1) and B-2 (SVP) (S18) and highest (0.88) was between Sree Rashmi (S1) and E-10 (R9). Cluster analysis resulted in a dendrogram where again the susceptible genotypes clustered along with the resistant ones, however they pooled together in sub-clusters with few exceptions. Like in the case of RAPD, the susceptible variety, Sree Rashmi (S1) pooled together with the resistant genotype, E-10 (R9) and revealed the same 88% similarity, which indicates that they share a similar genetic makeup. In the sub-cluster 1d, only one resistant genotype E-10 (R9) was included while, the rest were susceptible ones including the variety Sree Rashmi (S1) which grouped together with E-10 (R9) and revealed 88% similarity. All the four susceptible varieties were also included in this sub-cluster and expressed 74% similarity. In the sub-cluster IIb, genotypes 203 (R15) and J-14 (R12) which are resistant showed 81% similarity. ISSR too showed mostly the genetic diversity that exists in taro.

Standardization of SSR-PCR conditions was attempted to obtain consistently good amplification at the expected product size. Twelve SSR

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primers, were used for initial screening of which nine primers, which gave good amplification at the expected product size and showed polymorphic bands were selected based on its agarose gel profile. Annealing temperature of the SSR-PCR condition was optimized at $56^0C$. The primers selected from Uq series (Mace and Godwin, 2002) and Ce1 series (Noyer *et al.*, 2004) were Ce1 A08, Ce1 F04, Ce1 B03, Ce1 F12, Uq 97- 256, Uq 201- 302, Uq 73-164, Uq 84-207, Uq 110-283 and Uq 132-147. These were selected on the basis of their quality of polymorphic bands and the electrophoretic patterns at the expected product size. The annealing temperatures of these primers varied from the literature and were standardized using gradient PCR. The presence of amplicons at the expected size was confirmed by resolving it in 2.5% agarose gel.

In case of SSR, the polymorphism Information content (PIC) of the primers were highest (0.69) for Uq 132-147 and Uq 201-302 followed by Uq 97-256 (0.61) and Uq 73-164 (0.59). The primer, Uq 84-207 (0.30), recorded the lowest PIC content. Number of alleles per locus varied from 1.08 - 6.22 with the maximum alleles shown with Uq 94-256 and the minimum with Ce1 B03. The Observed heterozygosity value (He) ranged between 0.33 (Uq 84-207) to 0.74 (Uq 132-147 and Uq 201-302). Percent polymorphism ranged from 33.33% to 100% with Uq 73-164 recording the lowest (33.33%) followed by Uq 201-302 (50%). Ce1 F12, Uq 97-256 revealed 100% polymorphism with average polymorphism of 71.29%. In SSR markers, all the primers used could not detect polymorphism that well as indicated by the low values of PIC by few of them. So screening of more SSR markers will have to be resorted to get a better result with this marker system.

The binary data obtained from the gels were subjected to analysis for similarity coefficient values, which ranged from 0.49 to 0.89 concentrating between 0.56 to 0.86 where the lowest similarity index (0.49) was observed

between 485 (S11) and 450 (R1), whereas, the highest (0.89) was between 679 (R16) and TCR 961 (S17). Here, the dendrogram grouped the 36 genotypes into five major cluster and one outlier. Here too, the susceptible ones clustered along with with the resistant genotypes as in RAPD and ISSR. Cluster-I comprised three resistant genotypes. Second cluster consisted of six genotypes of which, five were susceptible (including three varieties viz., Bhu Sree, Bhu Kripa and Sree Pallavi) and only one resistant genotype, TCR 429 (R3). The third cluster consisted the maximum of 20 genotypes of which nine were resistant including Muktakeshi and eleven susceptible genotypes including the variety, Sree Rashmi. 370 (R13) and 84 (R17) revealed 95% similarity and both were resistant ones. The fourth cluster comprised of only two genotypes, both susceptible. Fifth cluster contained of four genotypes and all were resistant. J-14 (R12), a resistant genotype was found to be highly divergent than the remaining ones and formed as an outlier showing a similarity of only 62% with the rest. As per this result the divergent parents can be used for the development of a mapping population for further studies.

Mantel's test also established that no correlation existed between the three marker systems studied. The test result between RAPD and ISSR was $r = 0.43105$ and $p = 1.0000$; between RAPD and SSR was $r = 0.17271$ and $p = 0.9982$ and that between ISSR and SSR was $r = 0.09405$ and $p = 0.9360$. This could be due to the fact that the markers were not able to reveal any trait specificity and only the genetic diversity was exposed.

In the present study with three marker systems viz., RAPD, ISSR and SSR. RAPD and SSR primers could not produce any specific band which was present in all the resistant genotypes and absent in susceptible ones. However, in the case of ISSR markers, primer UBC 811, produced a unique band at 1270

bp in seven of the 18 resistant genotypes and this was completely absent in the susceptible ones. Hence, this was eluted using QIAquick Gel Extraction kit. Eluted DNA was checked on 1.0% agarose gel for its presence. As the size of the band was very high and concentration was less, it was re-amplified with the same primer. For re-amplification, only four genotypes (IC012601-R2; 370 - R13; 679 - R16 and 84 - R17) were selected based on the band intensity. This product was then checked in agarose gel, which gave two bands of which one was very prominent at approximately 280 bp. This band was isolated and sequenced. Sequence data showed that the size ranged from 242 bp, 252 bp, 247 bp and 252 bp, respectively. Upon blast analysis of the DNA sequence from R13 (370), it was found to share 100% identity and 8% query cover with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), mRNA and the following is the sequence which showed similarity with the above mRNA - TTTGAAGAAGATAGCCT (17 bp). The result shows that the marker identified from this resistant genotype, 370, has some utility in identification of disease resistance. Further conversion of this sequence into a SCAR marker and further validation with more resistant genotypes / population is needed to confirm this result and its application as a marker for identification of TLB resistant lines.

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# Appendix

# APPENDIX

## APPENDIX I

CTAB EXTRACTION BUFFER (CTAB METHOD)

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

## APPENDIX II

TE BUFFER (10 X)

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

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# 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 IV

AGE LOADING DYE (1X)

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

The loading dye is made up to 600 ml.

APPENDIX V

100 BP LADDER FOR AGE GEL

| Composition | Concentration |
| --- | --- |
| 100bp Ladder | 5μl |
| Dye | 40μl |
| Distilled water | 55μl |

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# IDENTIFICATION OF MOLECULAR MARKERS FOR RESISTANCE TO TARO LEAF BLIGHT IN *Colocasia esculenta* L. Schott

By

## ANJITHA NAIR U.M.

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Abstract Of The Thesis

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Kerala Agricultural University, Thrissur



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## COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

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# ABSTRACT

The study entitled "**Identification of molecular markers for resistance to taro leaf blight in** *Colocasia esculenta* **(L.) Schott**" was carried out at the Division of Crop Improvement, ICAR-CTCRI, Sreekariyam, during 2017-18 with the objective to identify molecular markers associated with leaf blight resistance in taro, sequencing and analysis using BLAST. RAPD, ISSR and SSR markers were used for the study. A total of 36 taro genotypes were selected consisting of 18 each susceptible and 18 resistant genotypes. DNA was isolated by employing the CTAB method of Sharma *et al.* (2008).

Out of 10 RAPD primers screened, 7 were selected whose annealing temperature were optimized at $32^\circ C$ and the presence of amplicons were confirmed in 2% agarose gel. For the selected primers, percent polymorphism ranged from 50 to 100% where, OPW12 recorded the least polymorphism (50%) followed by OPW6 (81.8%). The highest was shown by OPW1 and OPW16. The average percent polymorphism was 86.22%. The PIC values were highest for OPW8 (0.888) followed by OPW2 (0.886) and OPW1 (0.885) while least with OPW16 (0.615). Number of alleles per locus varied from 4-8.19 with the maximum by OPW8 and minimum by OPW5. The He values ranged between 0.66 (OPW16) to 0.89 (OPW1, OPW2, OPW8) and mostly found to be >0.8. Dendrogram generated using UPGMA analysis grouped the 36 genotypes into two major clusters. Cluster I with four susceptible varieties includes Bhu Sree, Bhu Kripa and Sree Rashmi where, Bhu Sree and Bhu Kripa pooled together showing 88% similarity. The resistant line, IC310104 and a susceptible line, L-12 expressed 91% similarity. Similarity index values varied from 0.47 to 0.88 with lowest (0.47) between 557 (S3) and 370 (R13) and between Sree

Pallavi (S9) and 370 (R13) while, the highest similarity index (0.91) was observed between IC310104 (R11) and L12 (R14).

Fourteen ISSR primers were selected whose annealing temperature was optimized at 56.3°C and amplicons were confirmed in 1.8% agarose gel. Percent polymorphism of primers varied from 60 to 100% where UBC 827 recorded lowest (60%) while UBC 818 recorded the 80% polymorphism. Rest of the primers showed 100% polymorphism with an average polymorphism of 95.7%. The PIC values of the primers were highest for UBC 818 (0.862) followed by UBC 811 (0.861) and UBC 809 (0.857) while UBC 827 (0.709) recorded lowest PIC content of <0.8. Number of alleles per locus varied from 2.38 - 6.13, where the maximum was shown by UBC 811 and minimum by UBC 817. He values varied between 0.75 (UBC 827) to 0.87 (UBC 809, UBC 818 and UBC 811). Similarity matrix index values varied from 0.50 to 0.88 with lowest (0.50) shown between Sree Rashmi (S1) and B-2 (SVP) (S18) and highest (0.88) between Sree Rashmi (S1) and E-10 (R9). A dendrogram was constructed using UPGMA, which grouped the genotypes into two major clusters. In the first cluster susceptible variety, Sree Rashmi (S1) pooled together with resistant, E-10 (R9) and it revealed 88% similarity.

Out of 14 primers tested, the primer (UBC 811) gave an extra band for 7 resistant genotypes (IC012601, IC089624, TCR 429, 679, 370, 84 and 565) out of the total 18 selected in 1270 bp region and it included the three resistant lines, 679, 370 and 84 which showed consistency in resistance reaction for the last four years under artificial screening. For primer UBC836, a resistant genotype IC089624 expressed a unique band at 1000 bp. The primer (GA)$_9$AC also produced a unique band for the resistant genotype, IC089624 at 800 bp. For primer, UBC824, a resistant line, 565 showed a different banding pattern. From these primers, the one showing unique bands for the maximum genotypes

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*viz.,* UBC811 was selected. The band of size 1270 bp was cut and eluted. However, as the size of the band was very high and concentration was less, it was reamplified with the same primer. For reamplification, only four resistant genotypes (IC012601- R2; 370 - R13; 679 - R16 and 84 - R17) were selected based on the band intensity. This product was then checked in agarose gel, which gave two bands of which, one was very prominent at approximately 280 bp. This band was isolated and sequenced. Sequence data showed that the product size ranged from 242 bp, 252 bp, 247 bp and 252 bp, respectively. The sequences obtained were used for similarity search in BLASTn and 100% identity and 8% query cover with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), mRNA was obtained for the DNA sequence from R13 (370). The following sequence (TTTGAAGAAGATAGCCT - 17 bp) showed similarity with the above mRNA.

Nine out of ten SSR primers screened were selected based on their agarose gel profile whose annealing temperatures were optimized at 56$^0$C and presence of amplicons was confirmed in 2.5% agarose gel. The percent polymorphism of the nine primers ranged from 33.33% to 100%, Uq 73-164 with the lowest (33.33%) followed by Uq 201-302 (50%). Primers, Ce1 F12 and Uq 97-256 revealed 100% polymorphism with average polymorphism shown was 71.29%. The PIC values of the primers were highest for Uq 132-147 (0.69) and Uq 201-302 (0.69) followed by Uq 97-256 (0.61) and Uq 73-164

(0.59). Primer, Uq 84-207, recorded the lowest PIC (0.30). Number of alleles per locus varied from 1.08 - 6.22 with maximum for Uq 97-256 and minimum with Ce1 B03.

The He values varied between 0.33 (Uq 84-207) to 0.74 (Uq 132-147 and Uq 201-302) whereas similarity coefficient values ranged from 0.49 to 0.89 concentrating between 0.56 to 0.86 with lowest (0.49) between 485 (S11) and 450 (R1) while highest (0.89) between 679 (R16) and TCR 961 (S17).

In present study with three marker systems, the 30 odd primers did not produce any trait specific band(s) in all the resistant genotypes tested. However, with ISSR markers, primer UBC 811, expressed unique band in seven resistant genotypes which was completely absent in the susceptible ones. The specific band obtained were eluted and sequenced. The sequence showed 100% identity and 8% query cover with *Arabidopsis lyrata* subsp. *lyrata* disease resistance protein RML1B (LOC9323997), mRNA. This was obtained for the DNA sequence from R13 (370). The following is the sequence which showed similarity with the above mRNA - TTTGAAGAAGATAGCCT (17 bp). Mantel's test established no correlation between the marker systems employed since they did not reveal any trait specific marker and only the genetic diversity was revealed. Therefore, further studies must be performed by employing more genotypes with increased primers to arrive at a definite consensus. The sequence obtained can be converted to a SCAR marker and validated with more resistant genotypes.