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Genetic Diversity Analysis of Leaf Blight Resistant and Susceptible Taro [*Colocasia esculenta* (L.) Schott] Genotypes Using ISSR Markers

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Abstract

Taro is believed to have originated in the Indo-Malayan region and hence harbors a wide genetic diversity. The major problem associated with taro cultivation is taro leaf blight (TLB) caused by *Phytophthora colocasiae*. Germplasm of taro maintained at ICAR-CTCRI was continuously screened for resistance for the last four years and as a result, few resistant genotypes were identified. An attempt was made here to assess the genetic diversity existing in this set along with susceptible genotypes present in the germplasm collection using ISSR. Thirty six genotypes of *C. esculenta* from different parts of India including five varieties were used in the study. The genotypes used were classified as resistant and susceptible based on their reactions to TLB, previous studies. Fourteen ISSR markers were used on 18 resistant and 18 susceptible taro genotypes. The primers selected were suitable for the study as reflected by the mean percentage polymorphism of 95.7%, heterozygosity ranging from 0.75-0.87, average number of alleles ranging between 1.94-6.13 and Polymorphic Information Content (PIC) ranging from 0.71-0.86. Similarity matrix based on Jaccard's coefficient ranged from 0.50 to 0.88 and the genotypes were grouped into two major clusters. The clusters were sub-divided into four and three sub-clusters, respectively along with an outlier. Cluster I comprised of 12 resistant and seven susceptible lines whereas Cluster II had eight resistant and nine susceptible genotypes grouped together. Of the seven sub-clusters, resistant genotypes were grouped together in three sub-clusters viz., 1a, 1b and 2a. The sub-cluster 2d had only susceptible genotypes pooled together. In the rest three sub-clusters viz., 1c, 1d and 2b resistant and susceptible genotypes grouped together. In sub cluster Id, of the eight genotypes grouped together, only one was resistant, IC012470 which pooled with Sree Rashmi in the dendrogram at 88% similarity. Phenotypically the genotype IC012470 caught mild infection. This work shows that ISSR is able to assess the genetic diversity present among taro genotypes and also helped to group the taro genotypes on the basis of TLB resistance/susceptibility to a large extent. Few genetically divergent genotypes could be identified, viz., IC012593 in 1a, TCR 429 in 1b, C-565 in 1c, B4 in 2a, C-679 in 2b as well as IC089583 and B2(SVP) in 2c. The divergent genotypes identified were resistant to TLB except B2(SVP), which was susceptible. The TLB resistant genetically divergent genotypes can be used as diverse parents for development of a mapping population for TLB resistance along with susceptible sources and also for introgression of resistance to popular high yielding varieties which are susceptible to TLB.

Key words: *Colocasia esculenta*, taro, molecular marker, ISSR, genetic diversity, resistance, TLB

Introduction

Taro [*Colocasia esculenta* (L.) Schott] is an edible aroid distributed in the humid tropics and subtropics. It is one of the major tuber crops belonging to the family *Araceae* and sub-family *Aroidea*. It is an important staple food crop grown throughout many Pacific Island countries, parts of Africa, Asia and the Caribbean for its

fleshy corms and nutritious leaves (Deo et al., 2009). Taro is believed to have originated in South Central Asia, probably in India or the Malay Peninsula and gradually spread worldwide by traders. Wild forms occur in various parts of South Eastern Asia (Purseglove, 1972). Hence, the probability of finding a high genetic diversity is more in India. Moreover, 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. So, in this study we have included 36 taro accessions from various parts of India, to assess its genetic diversity. The main criteria used for selection of these genotypes was done on the basis of its reaction to TLB, caused by *Phytophthora colocasiae*.

Taro is an important vegetable crop in India. All parts of the plant including the corm, cormels, rhizome, stalk, leaves and flowers are edible and prized in various food cultures. Taro corm is an excellent source of carbohydrate, vitamins, proteins, elements like potassium, calcium, phosphorus, iron and dietary fiber. Its corm and leaves have some medicinal properties against tuberculosis, ulcers, pulmonary congestion, fungal infection and is consumed by people allergic to cereals and children who are sensitive to milk. Taro flour is a good baby food (Kaushal et al., 2015). Taro leaf blight (TLB) caused by *Phytophthora colocasiae* Raciborski, is the most destructive disease of taro. The occurrence of TLB has been reported from many countries. Butler and Kulkarni (1913) reported this for the first time in India. This disease has destroyed taro plantings in Papua New Guinea (Packard, 1975) and in American Samoa (Gurr, 1996). In India, TLB is 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). Occasional sunlight with intermittent rain is most favorable for disease severity compared to prolonged cloudy weather with rainfall (Misra and Chowdhury, 1997). In the case of taro, TLB scoring is done on a 1-6 scale, where 1 - no infection, 2 - < 10% infection, 3 - 11 to 25% infection, 4 - 26 to 50% infection, 5 - 51 to 75% infection and 6 - > 75% infection. If the infected area is less than 10% and the spots are restricted, the genotype is considered as a tolerant ones. In a resistant variety the size of the infected area increases more slowly than a susceptible variety (Misra et al., 2008) Different taro genotypes respond differentially against *P. colocasiae*. This is mainly due to the genetic make-up of taro that may favor the growth and spread of the pathogen or may resist and eliminate its spread. With the high diversity existing in the crop, it is highly probable that we may identify few genotypes which are resistant/tolerant to the disease.

Assessment of genetic variability in a species is a prerequisite for initiating any efficient breeding programme, as it provides the basis for tailoring desirable genotypes. The molecular characterization of germplasm could be a major factor in rationalizing the national collections and in establishing a core collection for the region, which would comprise a restricted range of genotypes that are more accessible to regional breeding programs chosen to represent the genetic diversity of the region (Mace and Godwin, 2002). In recent years, several molecular techniques have been used for germplasm characterization, identification of varieties, molecular diagnostics, phylogenetic studies and diversity analysis (Nusaifa Beevi, 2009). Among the different molecular markers, inter simple sequence repeats (ISSR) approach offers the advantage of being technically undemanding, without the use of radioactivity or poly acrylamide gel and are relatively cost effective as compared to other procedures. More recently, characterization using simple sequence repeats (SSR) and inter simple sequence repeat (ISSR) markers were used for both *C. esculenta* and *Xanthosoma* (Godwin et al., 2001; Mace and Godwin, 2002). PCR-based SSR markers are very powerful as they are co-dominant and multi-allelic as well as highly polymorphic. However, the major drawback of SSRs is the cost and time necessary for their development. In contrast, the ISSR approach requires no prior sequence knowledge and can be immediately applied to any plant species. Although there is no single dominant marker system that meets all the needs, ISSR markers are considered to be rapid, robust and provide more informative data sets with less effort and cost than other dominant molecular marker techniques. So they

are mainly used in genetic diversity studies (Godwin et al., 1997; Salimath et al., 1995; Yang et al., 1996). The method provides highly reproducible results and generates abundant polymorphisms in many systems. This technique can rapidly differentiate between closely related individuals (Zietkiewicz et al., 1994).

The present study was conducted with an objective to analyze the genetic diversity existing amongst 36 genotypes of taro based on their reaction to *Phytophthora colocasiae*. These genotypes were screened for TLB for the past four years using artificial screening techniques and resistant ones identified. Genetically distinct genotypes identified could be used as diverse parents for development of a mapping population for TLB resistance and also for introgression of resistance to popular high yielding varieties which are susceptible to the disease. Eighteen each of TLB resistant and susceptible genotypes of taro collected from different parts of India was tested using 14 ISSR markers.

Materials and methods

The material for molecular characterization comprised of young leaves from 36 accessions (18 resistant and 18 susceptible ones, previously screened) of *Colocasia esculenta* (L.) Schott collected from the field genebank maintained at ICAR – CTCRI, Sreekariyam. The details of the accessions used are given in Table 1.

Table 1. List of the 36 taro accessions used for the study

Code	Acc. No./ Name	Area of collection	Code	Name Acc. No./	Area of collection
R1	C-450	Kerala	S1	Sree Rashmi	Kerala
R2	IC012601	NEH	S2	C-276	Madhya Pradesh
R3	TCR 429	KeralaA	S3	C-557	NEH
R4	C-723	Meghalaya	S4	C-628	NEH
R5	IC089624	NEH	S5	TCR 514	Kerala
R6	IC122159	NEH	S6	VRS	Kerala
R7	C-66	New Delhi	S7	Bhu Sree	Odisha
R8	C-565	Odisha	S8	Bhu Kripa	Odisha
R9	IC012470	NEH	S9	Sree Pallavi	Meghalaya
R10	B4	NEH	S10	C-22	Kerala
R11	IC310104	NEH	S11	C-485	Odisha
R12	IC012294	NEH	S12	IC420620	Mizoram
R13	C-370	Kerala	S13	IC089583	NEH
R14	IC012593	NEH	S14	C-553	Unknown
R15	C-203	Kerala	S15	C-85	Kerala
R16	C-679	Bihar	S16	C-621	Meghalaya
R17	C-84	Kerala	S17	TCR 961	Andhra Pradesh
R18	Muktakeshi	Odisha	S18	B2 (SVP)	Kerala

R - Resistant genotypes; S - Susceptible genotypes

For isolation of genomic DNA, 160 mg fresh leaf was used following the CTAB extraction method developed by Sharma et al. (2008). The purified DNA was visualized under a gel documentation system (G:BOX), after electrophoresis using 0.8% (w/v) agarose gel and was quantified using Nano spectrophotometer (NANODROP®ND-1000). The DNA was diluted in autoclaved ddH_2O and stored at -20°C. A total of 18 ISSR (UBC primers) were screened initially. PCR amplification were carried out in a thermal cycler (Biorad) with a final volume of 20 µl containing 4 µl template DNA, 0.16 µl of dNTP mix containing 25mM each of the four dNTPs, 0.5µl of primer, 0.4 µl $MgCl_2$, 2µl Taq buffer (with $MgCl_2$ 15mM) and 0.2 U Taq DNA polymerase (Genei). 10 µl of the amplified PCR product was resolved in 1.5% agarose gel electrophoresis stained with ethidium bromide and the gel image was captured using gel documentation system (G:BOX).

Among 18 ISSR primers used in this study, 14 were from UBC and the rest were ISSR primers reported earlier for taro (Okpul et al., 2005). Polymerase chain reaction was done using a thermal cycler (BIORAD). PCR programming for ISSR primers was standardized with the following steps - initial denaturation (94°C - 5 min), denaturation (94°C - 30 sec), annealing (56.3°C - 1 min), extension 72°C - 1 min), for 30 cycles followed by a final extension (72°C - 10 min) and cooling (4°C - 00).

The PCR products were then resolved in 1.8% agarose gel along with 100bp and 1kb ladder and the images were documented in a gel documentation system for further analysis.

The size of the DNA fragments were estimated by comparison with 100 bp

and 1 kb DNA size markers were run on the same gel using GenSys software. The bands were scored as '1' for presence and '0' for absence to create a binary data matrix. The scored molecular data was subjected to various measures of degrees of polymorphism viz., heterozygosity (He), which is a method to measure the degree of polymorphism. It is defined as the probability that a random population is heterozygous at a locus and is given in a randomly mating population by $H = 1 - \sum_i Pi^2$, where, p is the frequency of the i^{th} allele in the population. Polymorphism information content (PIC) is another measure of polymorphism commonly used as a measure of polymorphism for a co-dominant marker locus used in linkage analysis. It was calculated using the formula $PIC = 1 - \sum_i Pi^2 - \sum_{i,j} Pi^2 Pj^2$, where, p is the frequency of the allele in a population. The average number of alleles per locus was calculated as $n = (1/K) \sum ni$ Where, n_i is the no. alleles per locus and k is the no. of loci. The binary data obtained was used for the construction of similarity matrix using Jaccard's coefficient followed by cluster analysis. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering was done using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. All these analyses was done using NTSYS-PC, version 2.02 (Rohlf, 1998).

Results and Discussion

Taro is believed to have had its origin in northeast India and hence, the probability of finding a high genetic diversity is more in India. So, in this study we have taken up 36 taro genotypes from various parts of India, to assess its diversity. If a high degree of diversity exists within a population, it could be utilized for further breeding experiments in order to widen its genetic base and to get more elite hybrid varieties. In addition, taro germplasm characterization using molecular markers will contribute to the knowledge of genetic relationships between genotypes of wild and cultivated gene pool, and hence facilitate the breeding of taro cultivars to satisfy the market needs and respond to diverse biotic (e.g., taro leaf blight) and abiotic (e.g., drought and salinity) challenges (Sharma et al., 2008). A total of eighteen ISSR primers were used to study the 36 genotypes of *C. esculenta* (L.) Schott. Among these, fourteen primers (Table 2) produced reproducible and clear bands which were taken for further studies. In $(ACC)_9$ Y, Y = Pyrimidine. The

Table 2. Percent polymorphism with ISSR primers

Primers	Total no. of bands	Total no. of polymorphic bands	Percent polymorphism (%)
$(GA)_9$AC	7	7	100
$(ACC)_9$Y	8	8	100
$(GA)_9$AT	7	7	100
UBC 809	9	9	100
UBC 810	9	9	100
UBC 811	9	9	100
UBC 817	7	7	100
UBC 818	10	8	80
UBC 824	8	8	100
UBC 825	8	8	100
UBC 827	6	4	60
UBC 836	8	8	100
UBC 841	7	7	100
UBC 847	5	5	100
Total	108	105	
Mean			95.7

total number of bands per ISSR primer ranged from 5 (UBC 847) to 10 (UBC 818). The lowest number of bands was recorded for UBC 847 (5) followed by $(GA)_9$AT, UBC 817, $(GA)_9$AC and UBC 841 with seven bands each. Primer UBC 818 which produced the highest number of bands also recorded the highest number of polymorphic bands (10) followed by UBC 809, UBC 810 and UBC 811 (Fig. 1) with nine bands, each. The percent polymorphism of the ISSR primers studied ranged from 60% to 100% with UBC 827 recording the lowest (60%) while, UBC 818 was the second (80%). The rest of the primers showed 100% polymorphism. A total of 108 bands were formed from these 14 primers of which, 105 were polymorphic. Average percent polymorphism for the 14 ISSR primers was 95.7% (Table 2). The RAPD and ISSR markers were also used by Hussain and Tyagi (2005) to assess the genetic variability in *C. esculenta* (L.) Schott. The work of Singh et al. (2012) showed that both RAPD and ISSR markers were suitable for genetic diversity analysis of *C. esculenta* (L.) Schott as they showed high level of polymorphism and phylogenetic differentiation. Around 77.30% polymorphism was observed in their study whereas, in the present study the percent polymorphism recorded was much higher at 95.7%.

The number of alleles per locus ranged from 2.38 to 6.13 with the maximum alleles shown by UBC 811 and the minimum shown by UBC 817. The PIC, Number

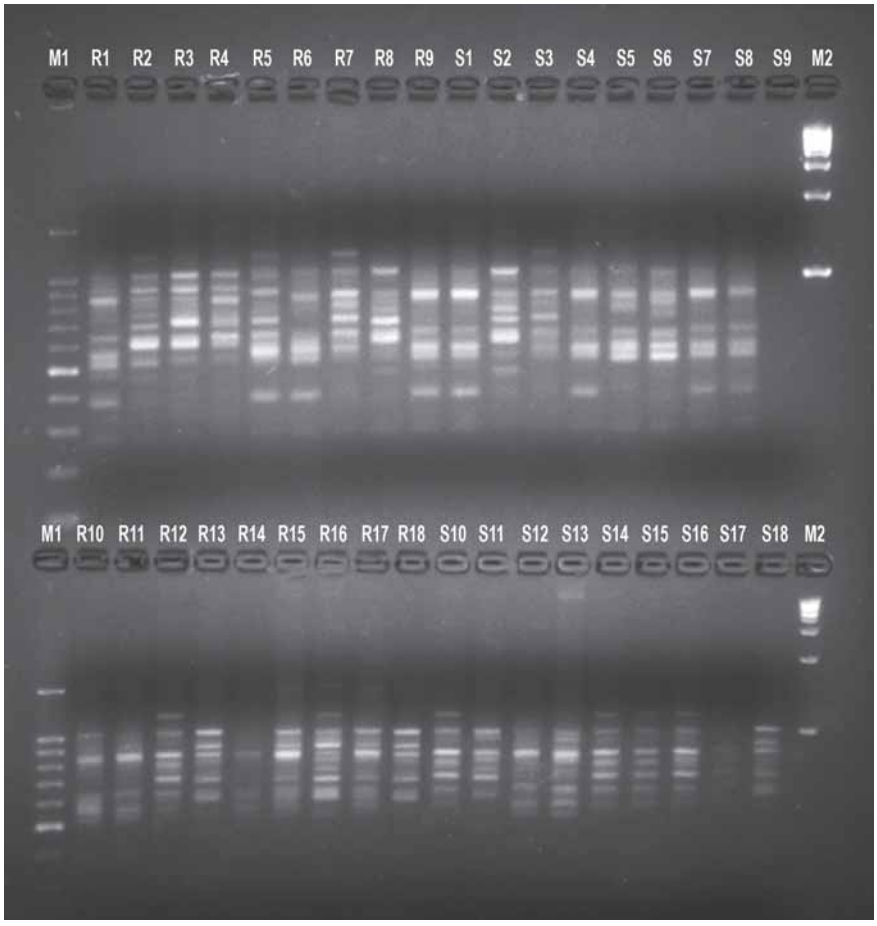

Fig. 1. Agarose gel profile of primer UBC 810. R1: 450, R2: IC012601, R3: TCR 429, R4: C-723, R5: IC089624, R6: IC122159, R7: C-66, R8: C-565, R9: IC012470, S1: Sree Rashmi, S2: C-276, S3: C-557, S4: C-628, S5: TCR 514, S6: VRS, S7: Bhu Sree, S8: Bhu Kripa, S9: Sree Pallavi, R10: B-4, R11: IC310104, R12: IC012294, R13: C-370, R14: IC012593, R15: C-203, R16: C-679, R17: C-84, R18: Muktakeshi, S10: C-22, S11: C-485, S12: IC420620, S13: IC089583, S14: C-553, S15: C-85, S16: C-621, S17: TCR 961, S18: B-2 (SVP), M1: 100bp, M2: 1Kbp

Table 3. Diversity estimates for ISSR primers

Primer	Avg. no. of alleles per locus	Polymorphism Information Content (PIC)	Heterozygosity
$(ACC)_9Y$	5.05	0.837	0.85
$(GA)_9AC$	3.83	0.808	0.83
$(GA)_9AT$	3.88	0.788	0.81
UBC809	5.55	0.857	0.87
UBC810	4.61	0.830	0.84
UBC811	6.13	0.861	0.87
UBC817	2.38	0.809	0.83
UBC818	6.00	0.862	0.87
UBC824	1.94	0.767	0.79
UBC825	4.61	0.826	0.84
UBC827	4.33	0.709	0.75
UBC826	5.25	0.800	0.82
UBC841	2.72	0.791	0.81
UBC847	3.63	0.756	0.78

of alleles and He values of the ISSR primers studied are given in Table 3.

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 was the highest for UBC 818 (0.8623) followed by UBC 811 (0.8614) and UBC 809 (0.857). The primers, UBC 827 (0.7094) recorded the lowest PIC content of < 0.8. Number of alleles per locus ranged from 2.38 to 6.13 with the maximum alleles shown by UBC 811 and the minimum by UBC 817. However, Velasco-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.

The similarity indices obtained for each pair wise comparison among the 36 taro genotypes based on fourteen ISSR markers ranged from 0.50 to 0.88 (Fig. 2.). Most of the similarity coefficient values ranged between 0.61 and 0.79. Among the 36 taro genotypes, the lowest similarity index (0.50) was

	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	0.69	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																		
S1	0.69	0.69	0.63	0.71	0.69	0.72	0.71	0.67	0.68	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	0.68	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	0.79	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	0.69	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	0.76	0.72	0.69	0.81	0.81	0.76	0.82	1.00										
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	0.69	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	0.69	0.73	0.79	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	0.69	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	0.69	0.72	0.66	0.68	0.72	0.80	0.79	1.00						
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	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	0.61	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	0.69	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	0.59	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. 2. Similarity coefficient of 36 taro genotypes with ISSR markers

observed between Sree Rashmi (S1) and B2 (SVP) (S18), showing that Sree Rashmi was different from B2 (SVP) by almost 50%. Interestingly, both are collections from Kerala and both are susceptible to TLB. On the other hand, the highest value of similarity coefficient (0.88) was obtained between Sree Rashmi (S1), a collection from Kerala and IC012470 (R9), an NEH collection. Here, the NEH collection is a resistant one, but showed 88% similarity with the susceptible variety, Sree Rashmi. Phenotypically resistance broke down in this genotype in the fourth year and it had shown infection by *P. colocasiae*.

A dendrogram generated using UPGMA cluster analysis separated the 36 taro genotypes into two major clusters where, the genotypes from the different states clustered in a mixed way. When the reaction to TLB was taken into account, the susceptible genotypes clustered together with the resistant genotypes in sub-cluster 1c, 1d and 2b (Fig. 3). No strict relationship was found with geographical distribution as genotypes from different States pooled in the same cluster. Similar results were obtained by Lakhanpaul et al. (2003) using RAPD, where the clustering pattern did not show any strict relationship with geographical distribution, morphotype classification and genotypic diversity. Further, genotypes classified as belonging to the same morphotypic group did not always cluster together. On the contrary, Kreike *et al*. (2004) clearly observed that in the dendrogram, the accessions from clusters that reflect their geographic origin, with the taro from different countries pooled together in the

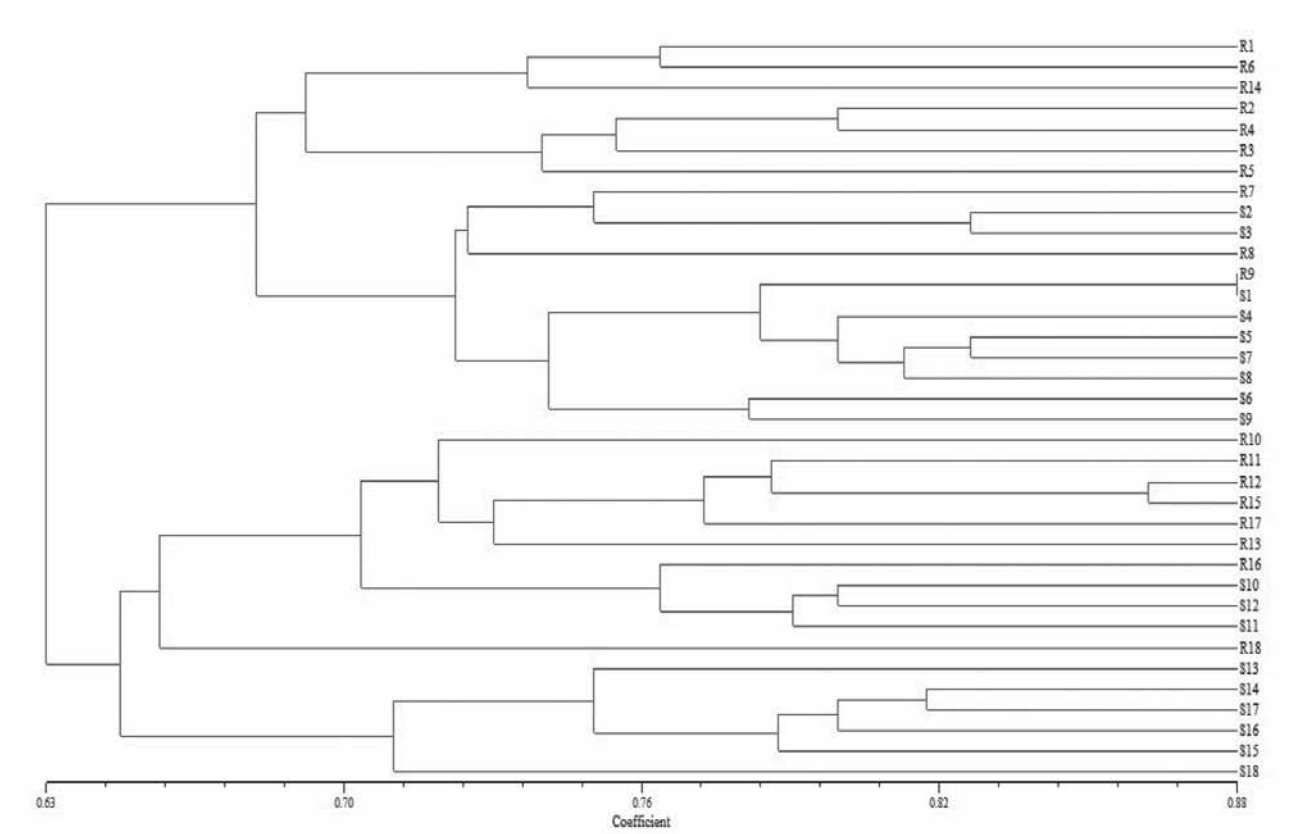

Fig. 3. Dendrogram showing the clustering with ISSR markers

same cluster. Here, the dendrogram was divided into two major clusters - one cluster showed the Pacific germplasm from countries like Papua New Guinea, the Philippines and Vanuatu and another cluster comprising the Asian germplasm including Indonesia and Malaysia. In the present study too, as all the genotypes belong to the Asian pool, they showed incongruity in pooling.

Cluster-1 and two had four sub-clusters each. Cluster-1 comprised 12 resistant lines and seven susceptible genotypes and was divided into four sub-clusters comprising genotypes belonging to all the States from where they were collected. The first sub-cluster included three resistant genotypes wherein IC012593 (R14), an NEH collection, was divergent. The second sub-cluster included 4 resistant genotypes wherein, TCR 429 (R3) (Kerala) and IC089624 (R5) (NEH) were divergent. The third sub-cluster had two resistant and two susceptible genotypes each, where, the resistant genotype C-565 (R8) from Odisha was divergent. In the last sub-cluster, only one resistant genotype from NEH, IC012470 (R9) was included while, remaining all were susceptible

Table 4. Distribution of genotypes into different clusters with ISSR primers

Cluster	No. of genotypes	Genotypes
1 a	3	C-450, IC122159, IC012593
b	4	IC012601, C-723, TCR 429, IC089624
c	4	C-276, C-66, C-557, C-565
d	8	IC012470, Sree Rashmi, C-628, TCR 514, Bhu Sree, Bhu Kripa, VRS, Sree Pallavi
2 a	6	B4, IC310104, IC012294, C-203, C-84, C-370
b	4	C-679, C-22, C-485, IC420620
Outlier	1	Muktakeshi
d	6	C-553, IC089583, TCR 961, B2 (SVP), C-621, C-85

including the taro variety Sree Rashmi. Sree Rashmi (Kerala) pooled with IC012470 (R9) (NEH) showing 88% similarity. This clearly shows that the clustering pattern did not show any definite relationship with geographical distribution as well as TLB resistance.

The Cluster-2 comprised eight resistant and nine susceptible genotypes, was also subdivided into three sub-clusters and a divergent line, Muktakeshi from Odisha. The first sub-cluster with six resistant genotypes had one resistant genotype, B4 (R10), collected from NEH, which was divergent. Genotypes C-203 (R15) (Kerala) and IC012294 (R12) (NEH) which were resistant, showed 81% similarity. The second sub-cluster had pooled three susceptible and one resistant genotype, C-679 (R16) from Bihar. Muktakeshi (R18) formed an outlier and was found divergent. In the third sub-cluster, only susceptible genotypes were pooled showing a similarity of 71%, where genotype B2 (SVP) (S18) from Kerala was found divergent. In short, the ISSR markers have proved to be more informative due to higher multiplex ratio of ISSRs as compared to other marker systems like SSRs (Godwin et al., 2001).

The results of the present study have an important implications for the future breeding programme of taro. Taro leaf bight is one of the major problems facing taro cultivation. Screening has resulted in the identification of many resistant/tolerant lines of taro. For any successful breeding programme, a high genetic diversity between the parents is desirable which can lead to new combinations of desired characters. From the present study, we can select divergent parents identified having resistance to TLB viz., IC012593, TCR 429, C-565, B4, C-679 and IC089583 as the male parents and the popular varieties having all other desirable characters such as good cooking quality, non-acridity, high yield, etc. as the female parent for capturing as much heterosis as possible and developing a TLB tolerant/resistant variety.

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