Int.J.Curr.Microbiol.App.Sci (2019) *8*(6): 2310-2320

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 06 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.806.274

SSR Marker-Based Molecular Characterization of Some Upland Taro (*Colocasia esculenta* L. Schott) Cultivars of North-East India

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Keywords

Taro, cultivars, SSR, molecular and markers

Article Info

Accepted:
17 May 2019
Available Online:
10 June 2019

A B S T R A C T

Ten SSR markers were used to characterize 22 taro cultivars from North East India. The study revealed that the SSR primers like uq201- 302, Ce1 B03 and Ce1 C06 with PIC=0.82, 0.76 and 0.76 respectively were found to be the most informative markers which can be used for future molecular works on taro. The Jaccard's similarity coefficient analysis ranging from 0.103 to 0.89 presented high level of genetic distance as no true duplicates were detected. The dendrogram constructed categorized the cultivars into two main clusters I and II. Only one cultivar, Damor Dema collected from Assam clustered on its own in cluster I and the second cluster II contained the remaining 21 cultivars. It was also observed that geographical origin of the cultivars did not bear any relationship with the molecular classification. The study revealed that cross between genetically distantly related cultivars like Damor Dema and Garo Kochu; Sree Kiran and Makhuti; and Damor Dema and Bor Kochu are advisable. However, crosses between genetically closely related cultivars like Boga Ahina and Red Garo, will have to be avoided for future breeding programme. Characterization of taro cultivars using SSR markers would contribute to the knowledge of genetic relationships between different cultivars.

Introduction

Taro (*Colocasia esculenta* L. Schott.) is an ancient and important tuber crop belonging to the monocotyledonous family, Araceae. Worldwide production is on the increase with 10.13 million ton/annum (FAOSTAT, 2016). Molecular data are useful in cases where morphological characters alone are insufficient for the delineation of clear taxonomic groups. The similarity of common names and lack of obvious phenotypic variation among many taro accessions has often led scientists to suspect a high degree of genetic relatedness. This underscores the importance of the estimation of the genetic diversity within germplasm collections (Greene and Pedersen, 1996). Such

limitations have resulted in the increased use of molecular approaches in assessing genetic diversity (Karp *et al.,* 1997), and in particular the emergence of polymerase chain reaction (PCR)-based markers. DNA markers with high levels of polymorphism permit fine-scale genetic characterization of germplasm if distributed throughout the genome (Okpul *et al.,* 2005). In addition they avoid the complications of environmental effects.

Microsatellites are DNA sequences composed of a tandem repetition of a simple short sequence, occurring in the genome of many higher organisms. Multiple allelic length variants can be identified at most microsatellite loci. Microsatellite markers are highly polymorphic, co-dominant loci and are abundant in most of species genomes (Palapala, 2016). Previously, other researchers had used DNA fingerprinting techniques such as amplified fragment length polymorphism (AFLP) primers (Quero-Garcia *et al.,* 2004) and random amplified polymorphic DNA (RAPD) primers (Singh *et al.,* 2012) to characterize taro. However, SSR primers were regarded to be advantageous over AFLP and RAPD (Hamza, 2004) because PCR-based SSR markers are very powerful as they are co-dominant and multi-allelic as well as highly polymorphic.

North East India is one of the centres of origin of colocasia and a considerable level of diversity is expected among the taro cultivars. Though a good amount of molecular work has been carried out in the Pacific and South-East Asian gene pools, very less systemic studies have been carried out in colocasia in North East India. In addition to these, DNA finger printing will prevent the loss of our indigenous germplasm. It will be an immense contribution towards the food security of poor people, if we could select a high yielding colocasia variety suitable for North East India.

Materials and Methods

Site of cultivar collection

Taro cultivars were collected in the form of corms and cormels from farmers fields. The taro cultivars were collected from four states of North-East India (Assam, Meghalaya, Arunachal and Nagaland) with focus on potential production areas. Two of the cultivars were also obtained from Central Tuber Crops Research Institute at Thiruvananthapuram, Kerela- India (Table 1).

Details of the experiment

The twenty-two taro cultivars collected were then planted in Experimental Farm, Department of Horticulture, Assam Agriculture University, Jorhat-Assam in randomized block design (RBD) with three replications maintaining a spacing of 0.60 m x 0.45 m. The proper recommended cultivation practices were followed to raise a good crop.

Molecular characterization

The tender young leaves were harvested in the morning hours from each taro cultivar for extraction of genomic DNA.

Deoxyribonuclic acid (DNA) extraction

The DNA extraction was performed following the protocol given by Sharma *et al.,* (2008) with slight modification. Young leaf tissues (300 mg) were ground to a fine powder using liquid nitrogen. All the samples were transferred to 2.0 ml eppendorf tubes, 5 µL Proteinase K (10mg/ml) was added. The tube was incubated in 37ºC for 30 min and then at 65ºC for another 30 min with frequent swirling. Samples were centrifuged at 8,000 g for 10 min at RT and supernatant was transferred to fresh eppendorf tube. Equal volume of phenol: chloroform: isoamyl

alcohol (25:24:1) were added and mixed by gentle inversion for 30-40 times. The samples were centrifuged at 8,000 g for 10 min at RT and supernatant was transferred to a fresh tube. 200 µL of 2 M NaCl solutions containing 4% PEG was added. It was observed that addition of this solution and incubation of the samples for at least 15 min at 4ºC increased the recovery of DNA yield. The samples were centrifuged at 8,000 g for 10 min at RT. The supernatant was transferred to a fresh tube and precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with wash solution. The obtained nucleic acid pellet was air-dried until the ethanol was removed and dissolved in appropriate amount of TE buffer (50 - 70 µL). The nucleic acid dissolved in TE buffer were treated with ribonuclease (RNase-10 mg/ml), incubated at 37ºC for 30 min and stored at –20ºC until use. The purified DNA was visualized under UV light after electrophoresis using 0.5% (w/v) agarose gel and quantified using UV spectrophotometer. The concentration and purity of the extracted DNA were determined using a protein nucleic acid analyzer. The concentration of each DNA sample was adjusted to 20 ng/µL, and the samples were stored at -20°C until analysis.

A total of ten SSR primers were screened. PCR amplification were carried out on thermal cycler in a final volume of 10 µl containing 1µl template DNA, 1µl 10X TB buffer, 0.5 µl dNTPs, 0.5µl $MgCl_2$, 0.5µl each of forward and reverse primers, 0.2µl Taq polymerase and 5.8µl H_2O. The SSR profiles had an initial denaturation at 94ºC for five minutes, followed by 35 amplification cycles (denaturation at 94ºC for 1 minute, annealing ranging from 45 to 52ºC for 1 min and extension at 72ºC for 1 minute) and a final extension step at 72ºC for 7 minute (Table 2). Ten microlitre of amplified PCR product was separated through gel electrophoresis on 3% agarose gel stained with ethidium bromide and photographed with Digital Imaging System.

Data analysis

The bands obtained were scored '1' for presence and '0' for absence in DNA samples amplified to create a binary data matrix. Polymorphism information content (PIC) of each marker was calculated using the formula:

$$PIC = 1-\Sigma x^2 i$$

Where, xi is the frequency of i^{th} allele for each SSR locus. Similarity index were calculated employing Jaccard's coefficient to established genetic relatedness. Molecular weight of the amplified bands was determined based on their relative migration in comparison to the molecular weight standards and expressed in base pairs (bp). Genetic similarity (GS) matrix between accessions based on molecular data was computed using Jaccard's (1908) coefficient. The similarity matrix was used to produce an agglomerative hierarchical clustering by employing Unweighted Pair Group Method with Arithmetic Averages (UPGMA) with average linkage (Sneath and Sokal 1973), which was then graphically converted into a dendrogram.

Results and Discussion

Molecular characterization of taro cultivars

Twenty-two cultivars of taro were subjected to molecular analysis using Simple Sequence Repeat (SSR) primer and were compared at genetic level. The data from the banding pattern of all the cultivars with ten polymorphic SSR markers were analysed and a total of sixty-one (61) scorable amplicons

were produced at an average of 6.1 alleles per locus. Band sizes amplified by the markers are presented in Table 3. The overall size of amplified product ranged from 129bp to 875bp (Table 3).

Primer uq201- 302 produced the highest number of amplified fragments whereas primer uq84-207 produced the lowest number of amplified fragments (Table 3). All the ten primers showed considerable variation and a total of fourty-nine (49) polymorphic fragments were produced. The highest polymorphism (100%) was found in two primers i.e. Ce1 F04 and uq84-207, where the most common band size was 406bp (ranging from 138-424 bp) and 257bp (ranging from 165-268 bp) respectively. On the other hand, the lowest polymorphism (60%) was detected in primer uq201- 302 where the common band size was 250bp (ranging from 129-610 bp) (Table 3).

All the markers were very informative with polymorphic information content (PIC) ranging from 0.41 to 0.82 (Table 3). The SSR primer uq201- 302 was the most informative marker (PIC=0.82) while uq84-207 was the least informative primer (PIC=0.41) because PIC value close to one (1) is considered to be more informative (Table 3; Fig. 1 and 2).

Jaccard's similarity coefficient was used to generate a dendrogram using UPGMA cluster analysis (Fig. 4). Based on the similarity coefficient, analysis of twenty-two taro cultivars presented high level of genetic distance. Jaccard's similarity coefficient showed no true duplicates and it ranged from 0.103 (between Damor Dema and Garo) and 0.89 (between Red Garo and Boga Ahina), indicating sufficient genetic variability among the cultivars under study (Fig. 4).

The dendrogram constructed based on UPGMA analysis categorized the twenty-two cultivars into two main clusters I and II (Figure 3 and Table 4). Only one cultivar, Damor Dema collected from Goalpara-Assam clustered on its own in the main cluster I (CL-I).

The second main cluster (CL-II) contained the remaining twenty-one (21) cultivars from both North-East and South India and it was further sub-divided into six sub-clusters i.e. A, B, C, D, E, F and G (Fig. 3 and Table 4). Cultivars 'Kaka' collected from Assam and 'Garo' collected from Meghalaya were grouped under cluster A.

The largest sub-cluster i.e. cluster B consisted of six (6) cultivars viz. Makhuti, Ghoti, Boga Ahina, Koni and AAU-Col-46 collected from different districts of Assam and cultivar Red Garo collected from Meghalaya (Fig. 3 and Table 4).

Sub-cluster 'C' which is the second largest sub-cluster contained four cultivars i.e. Bor, Panch Mukhi and JCC-31 (Fig. 3 and Table 4) collected from different parts of Assam and cultivar Naga collected from Nagaland.

The cultivar Arunachal-2 collected from Arunachal Pradesh was placed alone under cluster D (Fig. 3 and Table 4). Four (4) cultivars collected from different parts of Assam namely Karbi Anglong, Takali, AAU-Col-5 and Ahina were categorized under the sub-cluster E (Fig. 3 and Table 4).

The cluster II further sub-divided into clusters F and G which contained two cultivars each viz. AAU-Col-32 and AAU-Col-39 from Assam; and Sree Kiran and Muktakesh from Kerela respectively.

No two cultivars analyzed in the present study showed a similarity coefficient value of one thereby indicating their distinctness (Fig. 3 and 4).

Table.1 Place of taro cultivar collection

Sl. No.	Cultivars	State	District
1.	Kaka	Assam	Jorhat
2.	Garo	Meghalaya	Resubelpara- North Garo Hills
3.	Makhuti	Assam	Kokrajhar
4.	Ghoti	Assam	Jorhat
5.	Boga Ahina	Assam	Jorhat
6.	Koni	Assam	Jorhat
7.	Red Garo	Meghalaya	Garobadha- West Garo Hills
8.	Karbi Anglong	Assam	Karbi Anglong
9.	Bor Kochu	Assam	Dibrughar
10.	AAU-Col-46	Assam	Karbi Anglong
11.	Arunachal 2	Arunachal	Pasighat
12.	Panch Mukhi	Assam	Jorhat
13.	Naga	Nagaland	Mokokchung
14.	JCC-31	Assam	Karbi Anglong
15.	Damor Dema	Assam	Goalpara
16.	AAU-Col-5	Assam	Karbi Anglong
17.	Ahina	Assam	Jorhat
18.	AAU-Col-32	Assam	Karbi Anglong
19.	Takali	Assam	Jorhat
20.	AAU-Col-39	Assam	Karbi Anglong
21.	Muktakesh	Kerela	CTCRI- Thiruvananthapuram
22.	Sree Kiran	Kerela	CTCRI- Thiruvananthapuram

Table.2 Taro SSR-marker and their sequences used to study

Primer name	Forward primers	Reverse primers	Temperature (^{o}C)
Ce1 A06	5’-GCT TGT CGG ATC TAT TGT- 3’	5’-GGA ATC AGT AGC CAC ATC-3’	51
Ce1 B03	5’- TTG CTT GGT GTG AAT G-3’	5’- CTA GCT GTG TAT GCA GTG T-3’	51
Ce1 C03	5’- TGT TGG GAA AGA GGG-3’	5’- GGG GAA TAA CCA GAG AA-3’	51
Ce1 C06	5’- CCA GAA GAG ACG TTA CAG A-3’	5’- ACG ACT TTG GAC GGA-3’	47
Ce1 F04	5’- AGG GAA TAC AAT GGC TC-3’	5’- ACG AGG GAA GAG TGT AAA-3’	47
Ce1 H12	5’- TAG TTA GCG TGC CTT TC-3’	5’- CAA CAA CTT AAT GCT TCA C- 3’	51
uq73-164	5’- ATG CCA ATG GAG GAT GGC AG-3’	5’- CGT CTA GCT TAG GAC AAC ATG-3’	47
uq84-207	5’- AGG ACA AAA TAG CAT CAG CAC- 3’	5’- CCC ATT GGA GAG ATA GAG AGA- 3’	51
uq97- 256	5’- GTA ATC TAT TCA ACC CCC CTT-3’	5’- TCA ACC TTC TCC ATC AGT CC-3’	49
uq201-302	5’- CTA AGG AGA GGA GAT CCG AAC- 3’	5’- CAA GAC GAT GCT GAA CCA- 3’	49

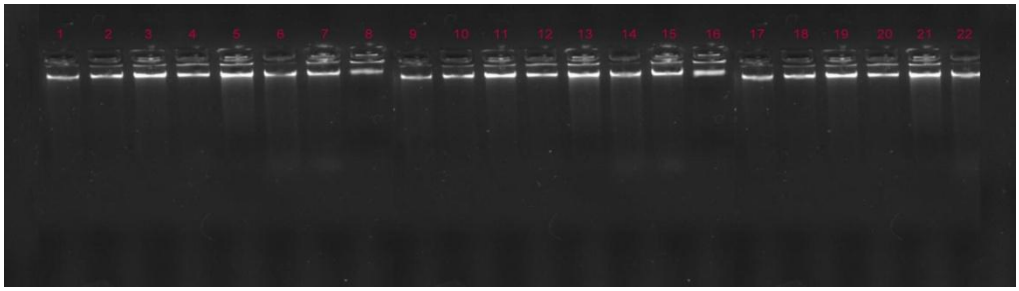
Table.3 Polymorphism detected in twenty-two taro cultivars by ten SSR primers

Primer	No. of alleles amplified	Polymorphic allele	Size of bands (bp)			Polymorphism (%)	PIC value
			High	Low	Most frequent		
Ce1 A06	8	6	277	205	205	75	0.74
Ce1 B03	8	5	250	157	157	62.5	0.76
Ce1 C03	5	4	875	435	523	80	0.70
Ce1 C06	8	7	857	168	440	87.5	0.76
Ce1 F04	6	6	424	138	406	100	0.73
Ce1 H12	4	4	237	129	191	75	0.59
uq73-164	4	4	531	168	168	75	0.67
uq84-207	3	3	268	165	257	100	0.41
uq97- 256	5	4	510	160	305	80	0.63
uq201- 302	10	6	610	129	250	60	0.82

Table.4 List of clustered taro cultivars using UPGMA based on molecular relationship (Jaccard's similarity coefficient)

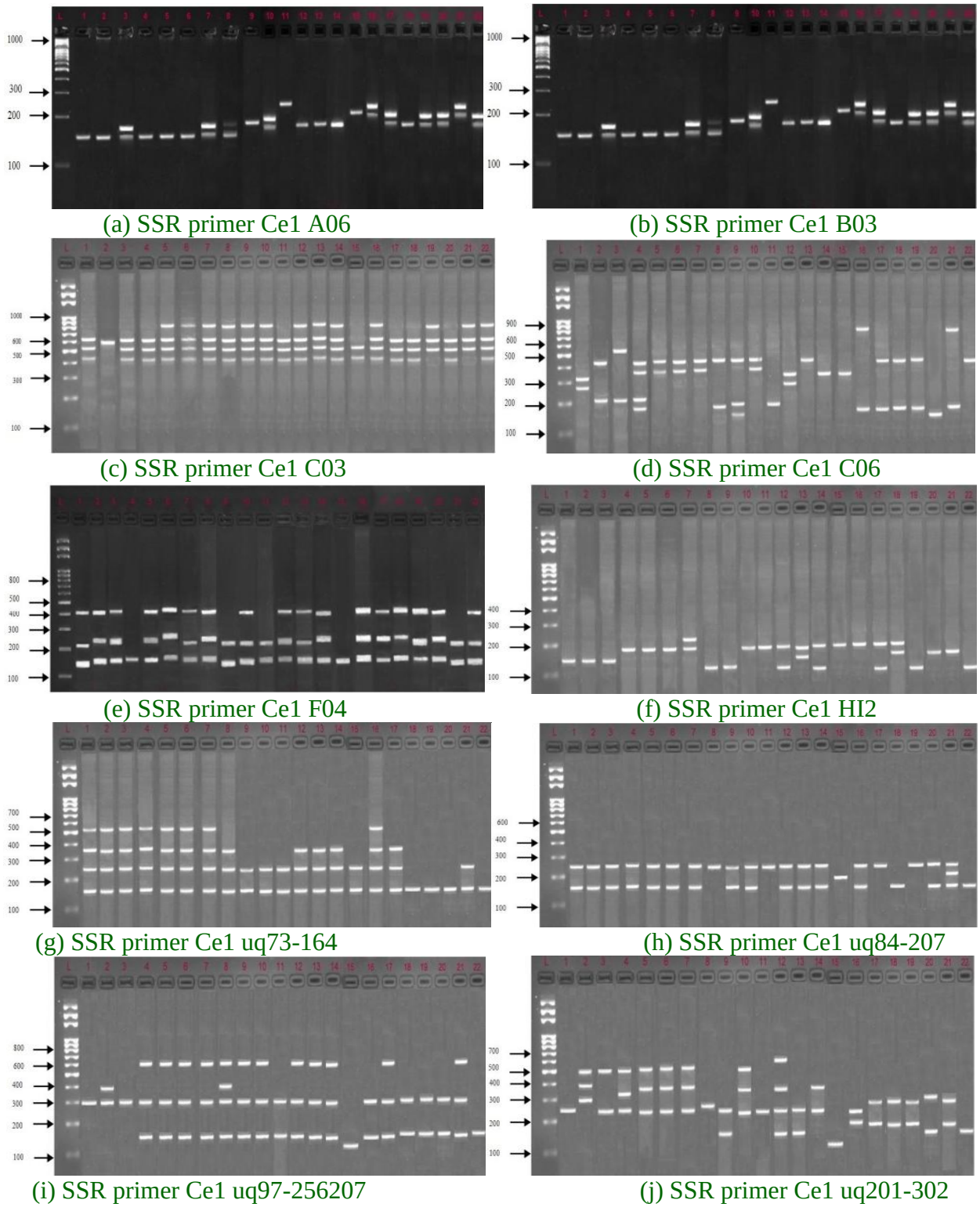
Cluster		No. of cultivar	Cultivars
I		1	Damor Dema
II	A	2	Kaka and Garo
	B	6	Makhuti, Ghoti, Boga Ahina, Red Garo, Koni and AAU-Col-46
	B1	2	Boga Ahina and Red Garo
	C	5	Bor, Panch Mukhi, JCC-31 and Naga
	D	1	Arunachal-2
	E	4	Karbi Anglong, Takali, AAU-Col-5 and Ahina
	F	2	AAU-Col-32 and AAU-Col-39
	G	2	Sree Kiran and Muktakesh

Fig.1 DNA extracted from twenty-two taro cultivars visualized under UV-rays (Lane 1-22)



Lane 1- Kaka, 2- Garo, 3- Makhuti, 4- Ghoti, 5- Boga Ahina, 6- Koni, 7- Red Garo, 8- Karbi Anglong, 9- Bor Kochu, 10- AAU-Col-46, 11- Arunachal 2, 12- Panch Mukhi, 13- Naga, 14- JCC-31, 15- Damor Dema, 16- AAU-Col-5, 17- Ahina, 18- AAU-Col-32, 19- Takali, 20- AAU-Col-39, 21- Muktakesh and 22- Sree Kiran

Fig.2 DNA fingerprints of twenty-two taro cultivars amplified with ten SSR primers



Lane 1- Kaka, 2- Garo, 3- Makhuti, 4- Ghoti, 5- Boga Ahina, 6- Koni, 7- Red Garo, 8- Karbi Anglong, 9- Bor Kochu, 10- AAU-Col-46, 11- Arunachal 2, 12- Panch Mukhi, 13- Naga, 14- JCC-31, 15- Damor Dema, 16- AAU-Col-5, 17- Ahina, 18- AAU-Col-32, 19- Takali, 20- AAU-Col-39, 21- Muktakesh and 22- Sree Kiran

Fig.3 Upgma dendrogram showing the genetic relationship between twenty-two taro cultivars based on SSR markers

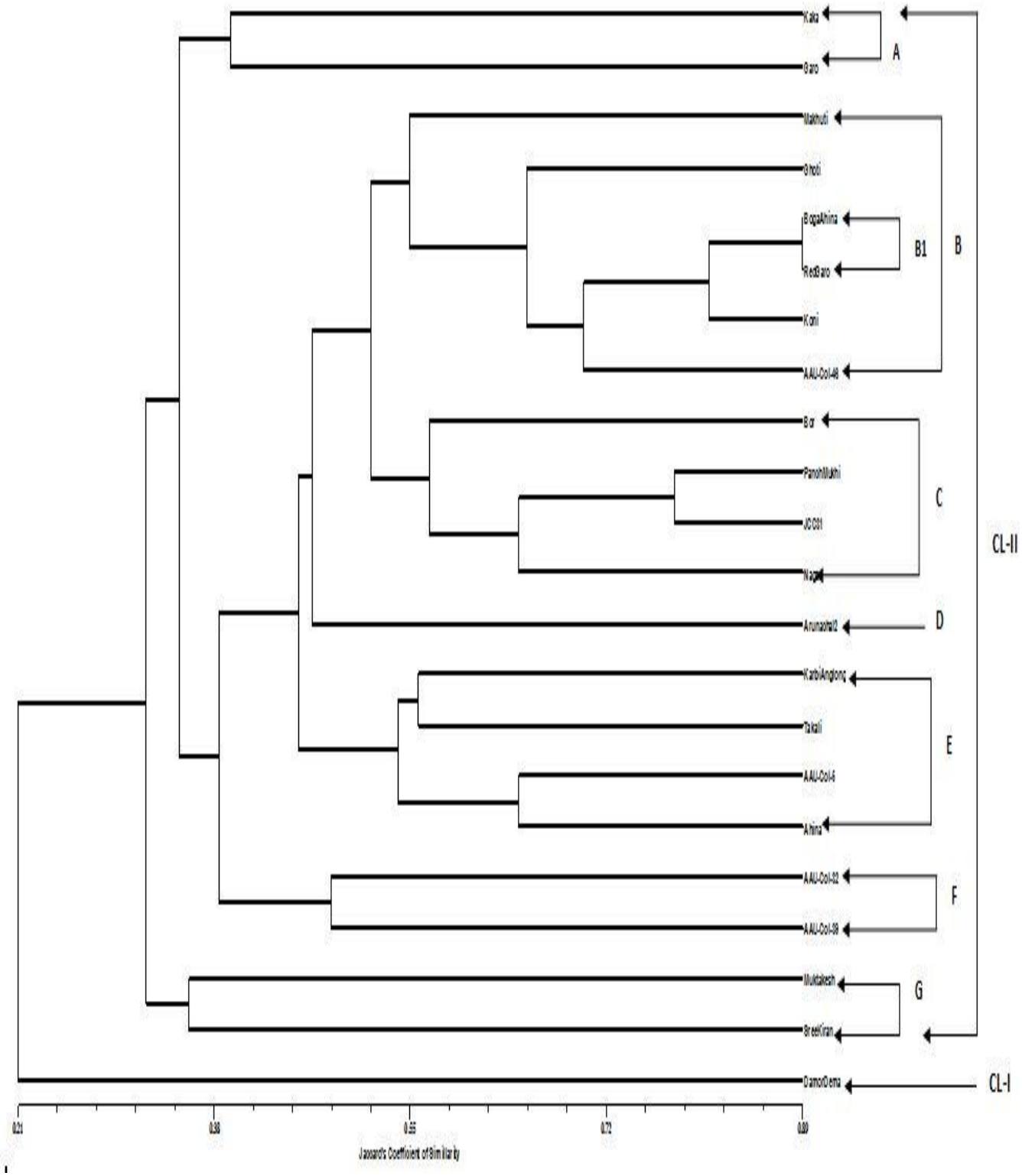


Fig.4 Similarity matrix based on Jaccard's Coefficient of Similarity

	Kaka	Garo	Makhuti	Ghoti	Boga Ahina	Koni	Red Garo	Karbi Anglong	Bor	AAU-Col-46	Arunachal 2	Panch Mukhi	Naga	JCC31	Damor Dema	AAU-Col-5	Ahina	AAU-Col-32	Takali	AAU-Col-39	Muktakesh
Garo	0.393																				
Makhuti	0.538	0.519																			
Ghoti	0.387	0.375	0.552																		
BogaAhina	0.419	0.452	0.586	0.750																	
Koni	0.406	0.394	0.516	0.667	0.821																
RedGaro	0.406	0.438	0.621	0.667	0.889	0.793															
KarbiAnglong	0.313	0.344	0.419	0.469	0.500	0.581	0.485														
Bor	0.333	0.242	0.355	0.500	0.484	0.382	0.424	0.467													
AAU-Col-46	0.313	0.344	0.467	0.516	0.714	0.633	0.750	0.394	0.467												
Arunachal2	0.308	0.250	0.500	0.500	0.481	0.414	0.464	0.407	0.440	0.462											
PanchMukhi	0.419	0.286	0.394	0.441	0.613	0.500	0.545	0.455	0.586	0.548	0.429										
Naga	0.414	0.355	0.536	0.533	0.679	0.600	0.655	0.552	0.593	0.607	0.542	0.679									
JCC31	0.400	0.265	0.375	0.469	0.600	0.581	0.531	0.533	0.517	0.533	0.407	0.778	0.607								
DamorDema	0.192	0.103	0.222	0.241	0.233	0.226	0.226	0.207	0.138	0.207	0.350	0.233	0.259	0.250							
AAU-Col-5	0.419	0.324	0.484	0.485	0.515	0.545	0.500	0.455	0.353	0.455	0.481	0.429	0.516	0.500	0.233						
Ahina	0.290	0.281	0.400	0.500	0.484	0.516	0.469	0.630	0.400	0.467	0.500	0.438	0.536	0.517	0.269	0.643					
AAU-Col-32	0.310	0.300	0.333	0.387	0.375	0.364	0.324	0.448	0.379	0.355	0.360	0.375	0.464	0.448	0.192	0.467	0.481				
Takali	0.188	0.258	0.333	0.303	0.375	0.324	0.364	0.556	0.429	0.400	0.417	0.375	0.464	0.355	0.148	0.467	0.600	0.462			
AAU-Col-39	0.321	0.226	0.345	0.313	0.303	0.333	0.294	0.367	0.393	0.323	0.320	0.387	0.481	0.414	0.154	0.433	0.393	0.480	0.370		
Muktakesh	0.281	0.200	0.265	0.278	0.270	0.263	0.263	0.324	0.344	0.286	0.276	0.237	0.333	0.250	0.133	0.424	0.387	0.323	0.464	0.333	
SreeKiran	0.296	0.125	0.233	0.250	0.323	0.313	0.313	0.393	0.480	0.300	0.240	0.367	0.462	0.345	0.167	0.323	0.370	0.346	0.458	0.417	0.357

Clustering pattern did not show any strict relationship between geographical and genotypic diversity as the cultivars from different geographic regions were grouped together indicating that the cultivars under investigation were genetically related. Similar results were also reported by previous researchers like Irwin *et al.,* (1998) and Lakhanpaul *et al.,* (2003).

In practical terms, the present result obtained from the SSR characterization revealed that cross between genetically distantly related cultivars like Damor Dema and Garo, Sree Kiran and Makhuti, Damor Dema and Bor, Muktakesh and Damor Dema are advisable. However, crosses between genetically closely related cultivars such as those identified by the SSR analysis like Boga Ahina and Red Garo, will have to be avoided for future breeding programme.

From the study, SSR microsatellite proved to be a valuable tool for cultivar identification especially in such a collection with a narrow genetic base. Results agreed with that of earlier studies by Singh *et al.,* (2008), Mwenye (2009), Faveretto *et al.,* (2011), Macharia *et al.,* (2014) and Palapala *et al.,* (2016).

It is concluded that, among the molecular markers available, SSRs are fast emerging as markers of choice, mostly due to their co-dominant nature, transferability, reproducibility and amenability to high

throughput. The current study showed SSR markers are suitable for genetic diversity analysis of *Colocasia esculenta* (L.) Schott because they showed high level of polymorphism and phylogenetic differentiation with corresponding results. SSR markers like Ce1 F04 and uq84-207 which gave 100 percent polymorphism can be selected for molecular characterization of taro. These primers will be useful for future molecular analysis and will provide taro breeders with a genetic basis for selection of parents for crop improvement.

The taro cultivars were diverse even within their location as they were not grouped according to their geographic origin. The present investigation revealed that crosses between genetically distantly related cultivars like Damor Dema and Garo Kochu; Sree Kiran and Makhuti; Damor Dema and Bor Kochu; and Muktakesh and Damor Dema are advisable. However, crosses between genetically closely related cultivars such as those identified by the SSR analysis like Boga Ahina and Red Garo, will have to be avoided for future hybridization programme. Therefore, characterization of taro cultivars using molecular markers is of prime necessity for plant breeding programmes and *ex-situ* conservation of plant genetic resources.

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How to cite this article:

Khatemenla, S. Alam, Madhumita Barooah, Deepa B. Phookan, Prakash Kalita, Madhumita C. Talukdar and Star Luikham. 2019. SSR Marker-Based Molecular Characterization of Some Upland Taro (*Colocasia esculenta* L. Schott) Cultivars of North-East India. *Int.J.Curr.Microbiol.App.Sci.* 8(06): 2310-2320. doi: https://doi.org/10.20546/ijcmas.2019.806.274