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Genetic Characterization of Locally Cultivated Taro Germplasm from Eleven District of Nagaland, India

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A B S T R A C T

Colocasia or taro (*Colocasia esculanta* L.) is one of the important tuber crops, which has been grown by farmers in rain-fed ecosystem and considered as an important food crop after rice in Nagaland. The characterization of taro from 11 districts of Nagaland, Northeast India has been analyzed using microsatellite markers. Twenty eight microsatellite markers were used to analyze 50 accessions of taro collected from 11 district of Nagaland. A total of 53 alleles were amplified at an average of 1.89 alleles per locus. Both altered alleles and null alleles were observed. The number of alleles ranged from one to four. The overall size of amplified products ranged from 117bp to 685bp. Dendrogram based on UPGMA analysis separated the accessions into five clusters. Four definite clusters were identified at a level of 35% similarity among the individuals. A three dimensional plot prepared from the principal component analysis of fifty-three alleles with PIC values ranging from 0.41 - 0.93 indicating high specificity and discriminatory power of the markers. Analysis of molecular variance (AMOVA) revealed most variation among individuals within population at 100%. It appears that high within population variation is a characteristic of colocasia. The present study showed that the germplasm of Nagaland was diverse but somewhat uniformly distributed across the state. Further exploration and collection of colocasia germplasm is required.

Introduction

Northeast India is the home for large numbers of tribal communities with rich culture and traditional knowledge system. In northeast India, the state of Nagaland ($25^0$6-$27^0$4N and $93^0$20-$95^0$15E) with a geographical area of 16527 km^2 is the home for large number of Naga tribal communities with rich culture and traditional knowledge system. Nagaland is rich in biodiversity. It has a very large number of non-traditional or underutilized horticultural crops. The abundant rainfall and different agro-ecological climate condition prevailing in different altitude in various districts favor the diversity both in cultivated and the wild horticultural crops. The wide range of underutilized fruits and vegetables are available in Nagaland. From these fruits and vegetables farmers can meet their

household needs like food, nutration, medicine and general livelihood balancing. Among these crops colocasia or taro (*Colocasia esculanta* L.), a member of the Araceae family, is one of the important tuber crops which has been grown by farmers in rainfed (>2,000 mm rainfalls) ecosystem and considered as an important food crop after rice in Nagaland. Taro has basic chromosome number 14 and two cytotypes: diploid with 28 chromosomes and triploid with 42 chromosomes (Coates *et al.,* 1988). Like most other root and tuber crops, taro is vegetatively propagated, although seed production is possible. Natural breeding and production spread have been reported for wild taro (Hunt *et al.,* 2013). Cultivars are propagated with corms, cormela (also known as suckers), and while vegetative propagation occurs through stolons in the wild (Ramanatha *et al.,* 2010).

According to Lebot (2009) taro is probably one of the oldest crops. Archaeological studies indicate its usage as early as 28,000 years ago in the Solomon Islands (Loy *et al.,* 1992). The origin of center of taro is still unresolved. Research continues to elucidate the center of origin of this global crop, northeast India and New Guinea being the potential separate centers of domestication (Jianchu *et al.,* 2001; Kuruvilla and Singh 1981 and Coates *et al.,* 1988).

Estimation of the genetic diversity widespread in the germplasm needs immediate attention for the improvement of taro. Very few reports are available on the analysis of genetic diversity of this crop. Several studies have attempted to describe the use of molecular marker to study genetic diversity in taro such as restriction site variation in rDNA, mitochondrial DNA (Matthew *et al.,* 1992) and RAPD markers (Irwin *et al.,* 1998; Lakhanpal *et al.,* 2003; Pillai & Lekha 2008; Sharma *et al.,* 2008; Singh *et al.,* 2012). Globally taro genetic studies has been done with isozymes (Lebot *et al.,* 2000), AFLP (Kreike *et al.,* 2004; Callion *et al.,* 2006) and microsatellite makers (Mace and Godwin 2002; Singh *et al.,* 2008; Macharia *et al.,* 2014). Very few reports on the genetic resources of *C. esculanta* (L.) have been analyzed for diversity and evolutionary pattern, but only a few germplasm has been included from Nagaland in some of the studies. Therefore, the present study was conducted with objective of genetic diversity analysis in 50 accessions of *C. esculanta* collected from 11 district of Nagaland using microsatellite markers.

Materials and Methods

Fifty accessions of *C. esculenta* var. *esculenta* collected from all the 11 districts of Nagaland were used to investigate the genetic diversity by studying the level of polymorphism with SSR markers. For Plant DNA extraction, young unrolled leaves were harvested and 150-200 mg leaf tissue was used. Total genomic DNA was extracted using the DN easy Plant Mini (QIAGEN). The isolated DNA was quantified and purity checked by running 0.8% agarose gel followed by spectrophotometer.

Twenty eight Colocasia microsatellite markers were used for amplification (Table 2). PCR was carried out in a 25-µl reaction mixture consisting of 1 µl of template DNA, 5 *p* mol of each reverse and forward primer, 7.1 µl PCR Master-mix (Qiagen) and nuclease-free water.

Amplification was carried out in a thermal cycler (Thermal Scientific, Veriti PCR) with a 5 min initial denaturation at 94ºC followed by 30 cycles of 94ºC for 40 sec, annealing at primer-specific temperature for 1 min and extension at 72 for 32 sec. A final extension at 72ºC for 5 min was given after the last cycle. After the completion of the PCR, the

products were stored at 4ºC refrigerator until the gel electrophoresis was done. 10 µl of the PCR product was electrophoresed in 3% agarose gel (with ethidium bromide) at 120V cm^{-1} for 4 hours. The gels were then visualized and photographed using a gel documentation system (Alpha Innotech, USA). Bands in gel images were corrected for smiling effect using the Alpha Imager FC software (Alpha Innotech, USA) and were scored for the presence or absence in the genotypes. The presence of an amplified band in each position was scored as '1'; absence was scored as '0'.

Data analysis

The data were entered into an Excel sheet as a rectangular matrix. Polymorphism information content (PIC) of each marker was calculated using the formula;

PIC = *1*-Σx2i

Where, xi is the frequency of i^{th} allele for each SSR locus (Sajeev *et al.,* 2011). 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.

Null alleles were treated as missing data. The similarity matrix was used to produce an agglomerative hierarchical clustering by employing UPGMA with average linkage (Sneath and Sokal 1973), which was then graphically converted into a dendrogram. To test the goodness of fit of clustering to the band scoring data, 'cophenetic correlation coefficient' was estimated. All the above calculations were made using NTSYS-pc software (Rohlf 2001). To get an idea about community / ecology-based preference of accessions / sharing of seeds/corms among communities, test accessions were grouped into six hypothetical populations (Pop. I–VI) based on their location of collection. Principal Coordinates (PCA) in different populations was calculated to get a first-hand idea about variations that exist within and among the hypothetical populations. A hierarchical analysis of molecular variance (AMOVA) with populations nested within types was performed (Excoffierer *et al.,* 2005). For average gene diversity, PCA (populations) and AMOVA, the computer program GenAlEx 6.5 Beta (Peakall and Smouse 2012) was used.

Results and Discussion

Out of the fifty *Colocasia esculanata* genotypes collected, forty-eight genotypes germinated and were selected for Simple Sequence Repeat (SSRs) analysis. CV5 and CV38 could not be analysed in molecular studies due to non -germination of corm as the corms were rotten. Altogether twenty eight colocasia SSRs markers were used to study the genetic diversity. All the primers used produce scorable amplicons. Band sizes amplified by the markers are presented in Table 2. Out of 28 markers, 16 showed considerable variation from the expected band sizes. A total of 53 alleles were amplified at an average of 1.89 alleles per locus. Both altered alleles and null alleles were seen. The number of alleles ranged from one to four. The overall size of amplified products ranged from 117bp to 685bp.

Highest number of heterozygotes was detected by the primer COLGCC73-164 (Plate-26). Highest variation was seen in COL-GCC 211-202, where the most common band size was 414bp (rang 139-414 bp

compared to 202-211 bp expected). Similarly high variations were seen in COL-GCC 249-155 and COL-GCC-220-211. On the other hand, COL-GCC 233-167, COL-GCC 118-221, COL-GCC 77-174, COL-GCC 95-219 *etc*., showed similarity/closeness with the expected band size. PIC values of the markers ranged from 0.41 (COL-GCC228-110) to 0.93 COL-GCC 211-202). Two markers amplified 4 alleles each (COL-GCC 208-253 and COL-GCC 206-122) while three markers amplified 3 alleles each (COL-GCC 56-191, COL-GCC 98-294 and COL-GCC 211-202). PIC values of the markers ranged from 0.41 (COL-GCC228-110) to 0.93 COL-GCC 211-202). Two markers amplified 4 alleles each (COL-GCC 208-253 and COL-GCC 206-122) while three markers amplified 3 alleles each (COL-GCC 56-191, COL-GCC 98-294 and COL-GCC 211-202).

Dendrogram (Figure 2) based on UPGMA analysis separated the accessions into five clusters with a Jaccard's similarity coefficient indicating high genetic variability among the accessions. Four definite clusters were identified at a level of 35% similarity among the individuals. A three dimensional plot prepared from the principal component analysis of the genotypic data also showed similar results. Cluster V was the biggest containing 38 genotypes followed by cluster-III with 5 genotypes. Cluster II contained 3 genotypes while both cluster I and cluster IV contained 1 genotype each (Figure 2). Clusters were determined based on discriminate analysis with the genotypic data. Clusters V showed 5 subclusters of which subcluster A was the biggest and subcluster E contained a unique genotype- 14. Cluster-III also showed two small subclusters. Among the 48 genotypes, genotype Tong II was unique as it formed a single genotype cluster at levels of cluster discrimination. At the discrimination level CUT-3, genotype Ati also produced a single genotype cluster. The discriminate analysis showed a clear distinction of the accession of the accessions of different clusters with the group (cluster) centroids placed distinctly apart from one another. The groups were placed distinctly apart both horizontally and vertically although vertical distinction was less prominent. There was no overlapping of group centroid. (Figure 2)

All genotypes were grouped into six hypothetical populations based on their location of collection (Table 1, Figure 1). These were again grouped into two regions based on altitude. Analysis of molecular variation (AMOVA) indicated that there were no variations among population or between the regions (Table 3). Within population the variation accounted for 100% of the observed molecular variation. A principal co-ordinate analysis was carried out to visualize the distribution of different hypothetical population across the regions. As seen from Figure 4, the populations were uniformly distributed which supported the observation from AMOVA that within population variation accounts for the observed molecular variation.

The present report on genetic characterization of colocasia germplasm of Nagaland using molecular markers is the first of its kind. Amplification pattern of the markers indicated that although some of the markers behaved as expected, others showed prominent variation. This indicated that in the population of Nagaland there is a large amount of inherent variation which might have accumulated due to vegetative propagation. There are four previous studies on the genetic diversity of Indian colocasia using either or a combination of random markers like RAPD, ISSR or isozyme. The current study is the first of its kind using the robust SSR marker with Indian germplasm. In the first study with Indian germplasm, Lakhanpal *et al.*, (2003) analyzed

32 colocasia genotypes and showed 100% polymorphism among the RAPD primers. Pillai & Lekha (2008) and Sharma *et al.*, (2008) studied 14 and 45 genotypes respectively and the RAPD primers showed 97% polymorphism. In a more recent study, Singh *et al.*, (2012) reported diversity analysis using RAPD and ISSR markers in 24 colocasia genotypes collected from Andaman Islands. They also reported 70.60% and 77.30% polymorphism in the RAPD and ISSR markers, respectively. The present study is the first of its kind with a collection from North East India. Also, this is so far the only study with largest number of germplasm from India using the robust SSR marker. The markers showed 100% polymorphism indicating the extent of diversity in the genotypes studied.

Table.1 The list of the Genotypes and their groups into six hypothetical population based on their location of collection

Population	Sample ID	Genotype name	Place of collection	Population	Sample ID	Genotype name	Place of collection
pop 1	CV2	Chugoma	Zunheboto	pop2	CV14	Manie I	Wokha
	CV34	Aiie	Zunheboto		CV27	Lijalanii	Mokokchung
	CV46	Chuyali	Zunheboto		CV28	Pajo	Wokha
	CV4	Beithola	Phek		CV31	Manie II	Wokha
	CV22	Beyo	Phek		CV37	Kotaknii	Mokokchung
	CV24	Bei II	Phek		CV43	Wasii nii	Mokokchung
	CV49	Beizo	Phek		CV44	Manyii	Mokokchung
	CV32	Banu sam sam	Kiphire		CV45	Tejongnii	Mokokchung
	CV33	Bao	Kiphire	pop4	CV1	Waipong	Peren
pop3	CV16	Tong I	Mon		CV3	Bei I	Peren
	CV36	Tinopang	Longleng		CV11	Beidimai I	Peren
	CV39	Tino II	Tuensang		CV12	Loudoubei	Peren
	CV40	Tino III	Tuensang		CV29	Beidimai I	Peren
	CV47	Tong II	Mon				
	CV48	Tong III	Mon				
pop5	CV6	Tephfii dziinuo	Kohima	pop6	CV9	Dziinuo I	Dimapur
	CV7	Ati	Kohima		CV13	Dzii dziinuo	Dimapur
	CV8	Obei	Kohima		CV15	Dziirinuo I	Dimapur
	CV10	Thegabeizii	Kohima		CV17	Dziinuo II	Dimapur
	CV18	Thupela	Kohima		CV19	Dziinuo III	Dimapur
	CV21	Sama	Kohima		CV20	Keriila	Dimapur
	CV23	Dziinuo IV	Kohima		CV26	Chiicha	Dimapur
	CV25	Tefiidzii	Kohima		CV41	Dziinuo V	Dimapur
	CV30	Dziirinuo II	Kohima		CV50	Dziitii	Dimapur
	CV35	Wolikhuo	Kohima				
	CV42	Atsantu	Kohima				

Table.2 List of Colocasia SSR markers, their amplification pattern and PIC value in the accessions

Marker	Primer sequences		No. of alleles amplified	Expected band size	Size of bands (bp)			PIC value
	Forward	Reverse			High	Low	Most frequent	
COL-GCC56-191	TGTCCCTTTTGATCTGTACAAG	CTCAACGGCTCATACACAC	3	56-191	249	115	134	0.78
COL-GCC82-117	TCAAGCGTAGGGGAAAAAC	CACAACACAAAACTGTAAACC	1	82-117	193	153	188	0.52
COL-GCC111-300	AGTGTATCCTACGTCCACGG	CAACCTTCTCCATCAGTCCAG	2	111-300	367	221	289	0.89
COL-GCC192-245	GGACTAACCGTTATGCTGC	CTATGACTCGCCGTCATTG	2	192-245	427	202	236	0.73
COL-GCC132-147	ACCCCGAAAAAGCCAATG	CTATCACTTGTTCCTCCTTCTC	1	132-147	156	113	139	0.65
COL-GCC233-167	TGCACAGTCAACAATGTCG	ATCTCCAAGCCCAATCTCC	2	233-167	182	140	164	0.73
COL-GCC88B-94	CACACATACCCACATACACG	CCAGGCTCTAATGATGATGATG	2	88-94	117	66	108	0.59
COL-GCC75-100	TTGGTCAGATCAAGGCTAGAG	GACTAACATCACACACACACG	2	75-100	117	73	88	0.54
COL-GCC118-221	GACTAACCGTTATGCTGCC	TAGATTGGAGCCCTTGGAC	1	118-221	217	156	213	0.71
COL-GCC103-220	GGATCTCTGGATTGGCTTCC	ATGATGCACTCACACCCAC	1	103-220	351	169	176	0.48
COL-GCC77-174	GATCTCAAGCACAAGAGACG	TCAACCTTCTCCATCAGTCC	2	77-174	178	158	167	0.66
COL-GCC95-219	ACAACTCGTGTATCCTACATCC	TCAACTCTCAAACCCTTCCC	1	95-219	219	191	206	0.43
COL-GCC98-294	AGTCCAGAGCACTCAAGTCG	CACAACAGTGTATCCTACGTCC	3	98-294	339	125	278	0.83
COL-GCC206-122	CGTTCAACACAGACCACTAC	TCCTTTGGAAAGGAGGTCC	4	206-122	272	105	126	0.91
COL-GCC223-157	GAGATGGTGTGAGTAAAGGAAG	TGGACTACTACTGAAGCAGAG	1	223-157	190	163	166	0.46
COL-GCC220-211	CTAAGGAGAGGAGATCCGAAC	CTGATACCACTTGTTGCCC	2	220-211	361	153	351	0.72
COL-GCC119-367	GGTCAAGCGTAGGGGAAAAAC	AGCTAGGGAGCACCAAACAC	1	119-367	355	302	312	0.58
COL-GCC91-262	GTCCAGTGTAGAGAAAAACCAG	CACAACCAAACATACGGAAAC	2	91-262	311	219	239	0.73
COL-GCC249-155	GACGGTCCAAATGTTAG	CCAAGGAAGATATTACCAAG	3	249-155	454	224	234	0.76
COL-GCC110-283	AGCCACGACACTCAACTATC	GCCCAGTATATCTTGCATCTCC	2	110-283	257	222	243	0.65
COL-GCC211-202	CTAACCACACACACATGAGCAC	TACTGTCCTGCTTCATCCCTCC	3	211-202	414	139	414	0.93
COL-GCC228-110	CCAGACTTCTCTCTACACCAAG	GATCTGTTGAAGAGATCCGTTG	1	228-110	126	104	116	0.41
COL-GCC240-223	ACTAACACGAGCACTCTCC	ACCATTTCCTACCACCTCC	1	240-223	250	199	203	0.52
COL-GCC105-267	CACCAAGGCATGGGAAAC	CCTGAAAATGGCAAAATACTTTAC	2	105-267	341	231	231	0.66
COL-GCC208-253	TAGAGGGTGGACAGGAG	CTAGAGGCACTGATGTAAC	4	208-253	685	229	244	0.89
COL-GCC209-120	CTACTCTACTGCCATCTAC	GTGAGTGAGAAAGTGAATG	1	209-120	130	67	70	0.51
COL-GCC90-102	TGGTGCGTTGGTCAGATCAAGG	ACAACACACACACGAGCACAC	1	90-102	185	92	94	0.57
COL-GCC73-164	ATGCCAATGGAGGATGGCAG	CGTCTAGCTTAGGACAACATGC	2	73-164	184	137	140	0.68

Table.3 Analysis of Molecular variation

Source	df	SS	MS	Est. Var.	%
Among Regions	1	12.218	12.218	0.013	0%
Among Pops	4	48.119	12.030	0.000	0%
Within Pops	42	564.413	13.438	13.438	100%
Total	47	624.750		13.451	100%

Fig.1 Map of Nagaland showing the genotypes grouped into six hypothetical population based on their location of collection

Fig.2 Dendrogram of colocasia varieties obtained by UPGMA cluster analysis based on microsatellite data

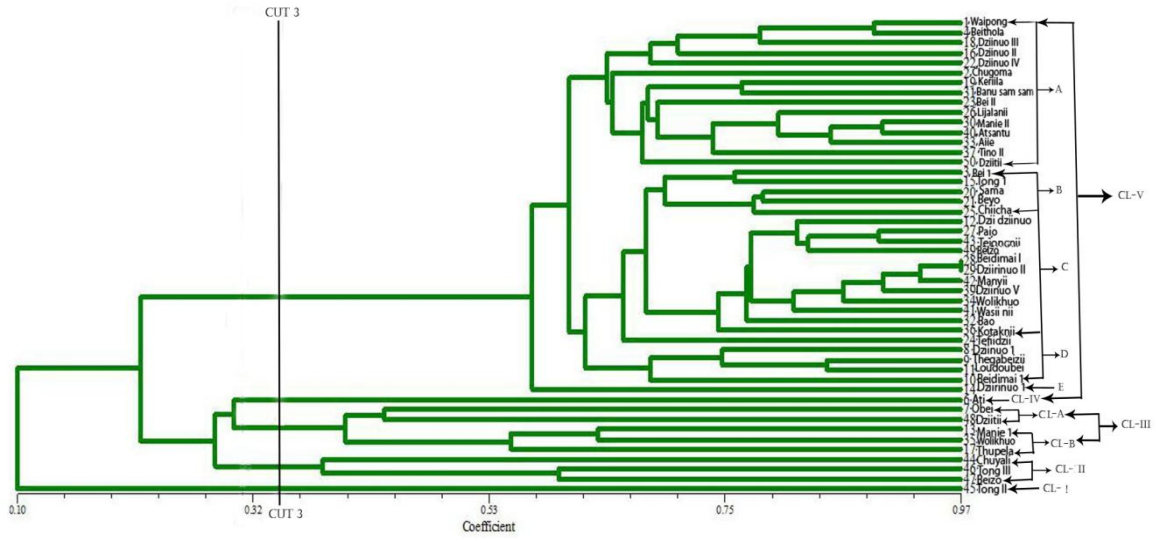


Fig.3 The discriminate analysis showed a clear distinction of the accessions of different clusters with the group (cluster) centroids placed distinctly apart from one another

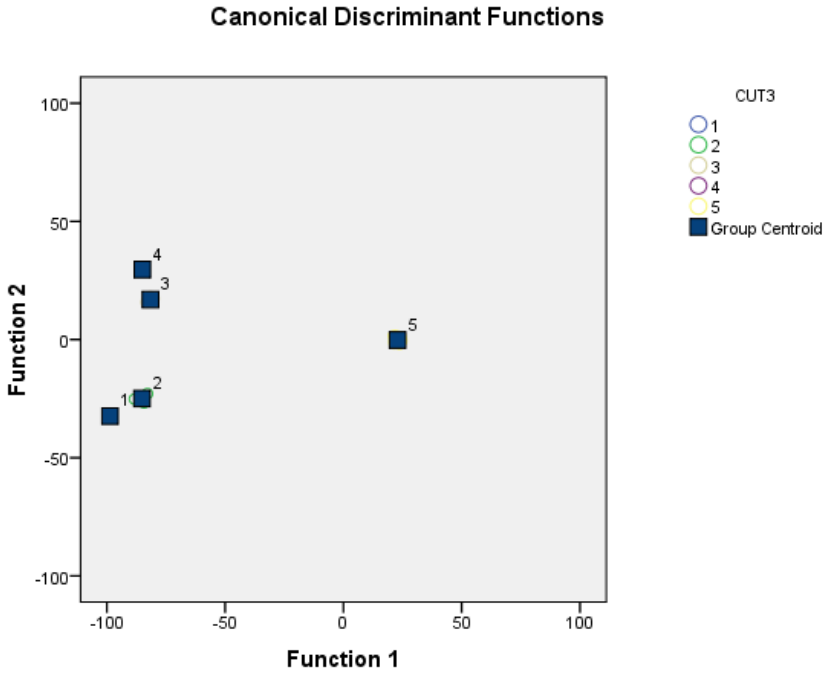
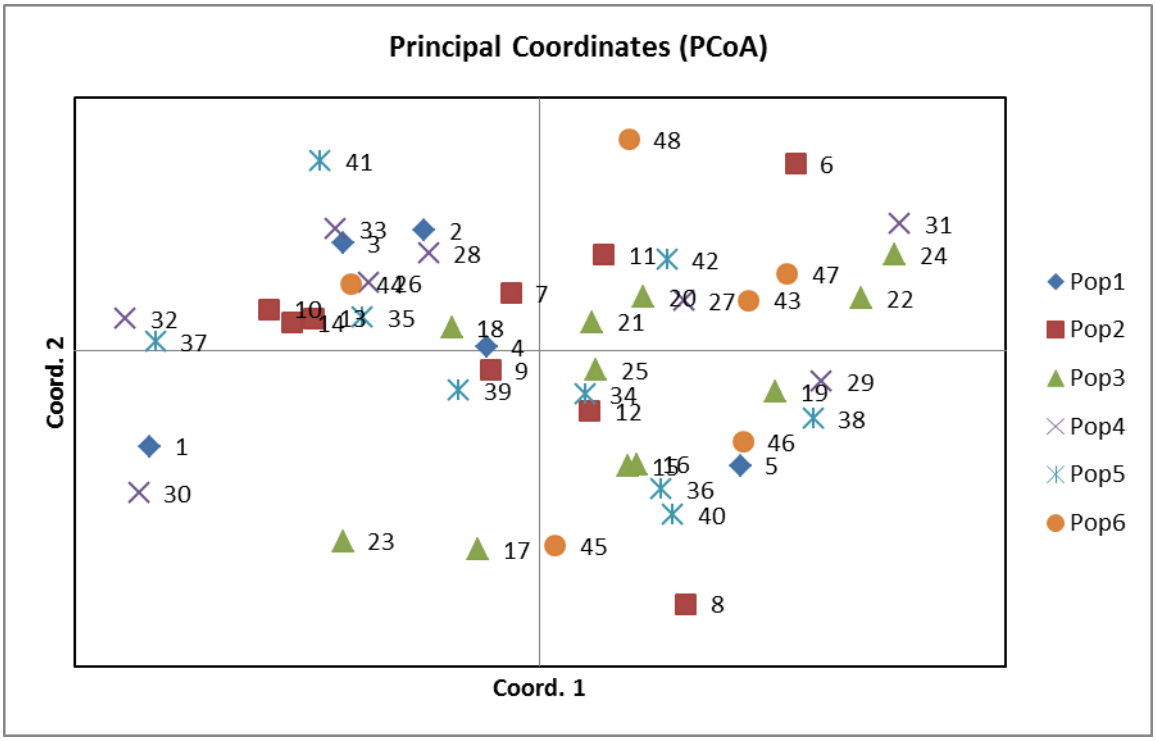


Fig.4 Distribution of population on the scatter plot



At the global level, genetic studies with isozymes (Lebot *et al.*, 2000) and AFLP (Kreike *et al.*, 2004) showed a greater diversity in the South East Asian germplasm compared to germplasm from Oceania. Another study on a set of 96 germplasm form Vanulatu Lava, A Pacific Ocean Island, using AFLP markers identified eight clusters with varying degrees of similarity (Callion *et al.*, 2006). Macharia *et al.*, (2014) studied 98 germplasm (5 populations) from East Africa using 6 microsatellite markers and amplified 31 alleles of which 85% were polymorphic.

The first set of 7 colocasia specific SSR markers was developed by Mace and Godwin (2002). Singh *et al.*, (2008) used these seven markers to assess diversity in 859 Papua New Guinea colocasia collections. They obtained 30 polymorphic alleles with a PIC value ranging from 0.0 - 0.59 and twenty three clusters were identified in their study. Macharia *et al.*, (2014) identified 31 alleles in 98 East African germplasm accessions using six micro satellite markers developed by Mace and Godwin (2002). In the present study, 28 SSR markers were used of which 4 were from the study of Mace and Godwin (2002). However, the new markers used in this study showed higher allelic variation. Fifty three alleles were identified with PIC values ranging from 0.41 - 0.93 indicating high specificity and discriminatory power of the markers. Four definite clusters were identified based on a discriminatory analysis. In none of the previous study, discriminatory analysis was done to delineate clusters. Two very distinct genotypes; genotype 9 from Dimapur and genotype 45 from Mokokchung were identified.

Six populations divided into two regions were hypothetically constituted to assess regional or altitudinal variation, if any, in the collection. However, AMOVA did not show inter-population or inter-regional variation. With-in population variation accounted for 100% of the molecular variation. This

supports the results obtained in all the previous studies (Singh *et al.,* 2008, Kreike *et al.,* 2004, Mace *et al.,* 2006, Macharia *et al.,* 2014) carried out in two different parts of the world where more than 80% of the variation was represented by with-in population variation. Thus, it appears that high with-in population variation is a characteristic of colocasia and movement/establishment of outside germplasm into a country or region specific population is low. However, it would be interesting to compare the two distinctly isolated population of India, one from the North East and the other from the Andaman Island.

The present study showed that the newly developed SSR markers were robust and informative, and also the germplasm of Nagaland was diverse but somewhat uniformly distributed across the state. Further exploration and collection of colocasia germplasm is required.

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