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Morphological Characterization of above Ground Characters of Taro (*Colocasia esculenta* (L.) Schott.) Accessions from North East India

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Abstract

Taro (*Colocasia esculenta* (L.) Schott), belonging to the family Araceae, is a major tuber crop grown in the tropical and sub-tropical regions. As the North Eastern states of India are regarded to be one of the primary centres of origin of taro, a considerable level of diversity is expected among the taro accessions. Therefore, the present study attempts to morphologically characterize 25 taro accessions collected from different North Eastern states of India using 27 above ground characters based on a combination of National Bureau of Plant Genetic Resources (NBPGR)/ International Plant Genetic Resources Institute (IPGRI) descriptors. Based on the frequency of the phenotypic characters across the 25 accessions, the Shannon Weaver's diversity index (H) was found to be 0.63. PCA analysis extracted three principal components representing 67.7% of the variability in the data. These observations were consistent with the box plot and biplot representations. The biplot representation showed agreement with the PCA results with leaf margin colour, leaf sheath colour and petiole colour (top 1/3rd, middle and base) contributing more to the overall variability, whereas, the range of character distributions represented in the form of box plot revealed that leaf margin colour, leaf colour (lower), petiole colour (top 1/3rd and middle) accounted for maximum variation. The dendrogram generated from hierarchical cluster analysis grouped the accessions into two broad clusters, which were further divided into two and five sub-clusters, respectively. It was also observed that geographical origin of the accessions did not bear any relationship with the morphological classification. In addition to supporting breeding and germplasm conservation programmes, the data can serve as a baseline for correlation with other types of markers.

Key words: Taro, diversity, North East India, morphological characterization, diversity index, PCA

Introduction

Tuber crops characterized by their resilience to changing climatic conditions are gaining worldwide importance with an increased demand to expand crop diversity to sustain food production. Among the various cultivated tuber crops, taro (*Colocasia esculenta* (L.) Schott.), belonging to the family Araceae, is one of the major crops grown in India (Srinivas et al., 2012). Besides being a rich source of carbohydrates, proteins, vitamins, minerals and dietary fibre (Bradburry and Holloway, 1998), taro also possesses medicinal values against tuberculosis, ulcers and fungal infection (Singh et al., 2012). The importance of taro in India becomes even more imperative considering the fact that the primary centre of origin and diversity is believed to be in the Indo-Malayan region (Yen and Wheeler, 1968) which includes the North Eastern states of India. Given the wide distribution and importance of taro in India, assessing the genetic variability among the various germplasm accessions maintained at ICAR-CTCRI is crucial for successful taro breeding. The need for such an attempt is driven by the limited availability of previous reports on North-East Indian accessions. The knowledge would enable taro breeders to exploit hybrid vigor by selecting divergent parental accessions. According to Beeching et al. (1993), a prerequisite for any genetic improvement

programme is the knowledge of the extent of genetic variation present between genotypes and the genetic distance between all closely related species with which hybrids could be produced. However, various factors like high disease incidence, promotion of other commercial crops and intensified demands on scarce land has led to narrowing of taro diversity. Therefore, generating information on existing diversity among germplasm accessions also becomes essential for efficient *in situ* conservation and maintenance of taro germplasm as well as to eliminate duplicates.

Research to define the genetic diversity of taro has been largely based upon it's cytology (Yen and Wheeler, 1968; Kuruvilla and Singh, 1981; Coates et al., 1988; Gunman and Dongxiao, 1990) and morphology (Tanimoto and Matsumoto, 1986). Of all the available markers, morphological markers are relatively simple and cheap to exploit. These markers can also increase the resolving power of genetic diversity and the baseline data generated can be correlated to other types of molecular markers like RAPD, AFLP, SSR, etc. Much of the visible phenotypic diversity of cocoyam (taro) cultivars is attributed to vegetative mutation and selection by growers for specific attributes. Therefore, there exists great diversity in colour patterns in the corms and leaves (Mathews, 2004). The present study attempts to morphologically characterize taro accessions collected from different North Eastern states of India using 27 above ground characters based on a combination of NBPGR/ IPGRI descriptors.

Materials and Methods

Twenty five taro accessions collected from various Northeast Indian states comprising Arunachal Pradesh, Manipur, Meghalaya and Tripura, and maintained at the ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) were selected for the present study. The present study was carried out for one season during 2013-2014. The North Eastern regions are one of the places most vulnerable to climate variability and climate change (Ravindranath et al., 2011) having quite distinct climatic conditions. Generally the daily temperature in Tripura and in the western parts of Mizo Hills is about 10 ^{0}C and may go upto 28^{0}C. Further lowest temperature below freezing point is experienced in the upper Himalayas in Arunachal Pradesh. On the other hand, Manipur, typically has a moderate climate throughout the year with the maximum temperature reaching around 32^{0}C. However, Meghalaya is the wettest place on earth harboring a rich diversity. Taro is mostly grown in the Garo Hills region during the month of May-June when the temperature is hot and dry ranging from 22^{0}C to 30^{0}C. Apart from this, one accession from Andhra Pradesh (H-6) was also included in this group for comparison, as an outlier. The plants were raised with five accessions per row in a single block, spaced 0.6 m between the plants and 0.45 m between the rows in an unreplicated augmented design. The corms/cormels were planted on the ridges during the onset of the rainy season. Mulching and weeding was carried out as and when required.

Morphological data collection

The National Bureau of Plant Genetic Resources (NBPGR) minimal descriptors (Abraham et al., 2006) for taro were followed for the characterization. Certain modifications were made to incorporate additional characters in addition to the NBPGR list using the International Plant Genetic Resources Institute (IPGRI, 1999) descriptors for taro *viz.*, lamina orientation and leaf margin. Twenty seven above ground characters comprising 5 quantitative traits were selected and the plants were scored at the maximum growth stage. An average value from three plants was used to record the quantitative traits.

Data analysis

Diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simple species richness (Magurran, 1988). They also take the relative abundances of different species into account. The Shannon diversity index (H) is a type of diversity index that is commonly used to characterize species diversity in a community. It accounts for both abundance and evenness of the species present. In the present study, the frequency distribution and Shannon Weaver's diversity indices were calculated for the various traits under each character. The Shannon Weaver's diversity index (H) was calculated based on the following formula:

$$H = -\sum [p_i \times \ln (p_i)]$$

where, p_i = proportion of a particular character i.e., number of individuals with a character/ total number of individuals.

The morphological data was recorded in the form of numerical scores assigned to each trait and tabulated in an excel worksheet. However, in the case of quantitative data, for testing the real treatment differences, analysis of variance using Duncan's multiple range test was done with the SAS/STAT software (Version 9.1.3). Principal component analysis was done to study the contribution of each character in to the overall variation. Box plot and biplot representation was constructed for analyzing the distribution of the traits under different accessions. Hierarchical cluster analysis was performed using the hclust function of the R package version. 2.8.0 (R Development Core Team, 2010), based on which a dendrogram was generated to group the accessions.

Results and Discussion

Diversity index

Figure 1 (a-g) represents the differences in few morphological traits among the various taro accessions. Based on the frequency of the phenotypic characters across the 25 accessions, the Shannon Weaver's diversity index (H) was found to be 0.63 (Table 1). This represents a higher value of diversity index when compared to a similar study conducted in Ethiopia (H = 0.27) using 100 accessions (Beyene, 2013). A higher Shannon Weaver's diversity index (H) suggests more diversity among taro germplasm as the North Eastern Region is considered as it's centre of origin is also the centre of diversity. Various lines of ethno-botanical evidence suggest that taro originated in South Central Asia, probably in India or the Malay Peninsula and wild forms occur in various parts of South Eastern Asia (Purseglove, 1972).

Analysis of variance

Analysis of variance performed on the five quantitative traits showed significant variation ($p < 0.05$) among the 25 accessions studied. The coefficient of variation (CV) was found to be higher for tillering (51.91) and plant size (54.04). Comparison of multiple means using Duncan's multiple range test gave results as shown in Table 2 with the highest mean values given by Acc.719 (tillering), B-2 (plant size), Acc. 719 (leaf L:B ratio), Acc. 741 (petiole:leaf ratio) and J-8 (petiole:sheath ratio). In both methods,

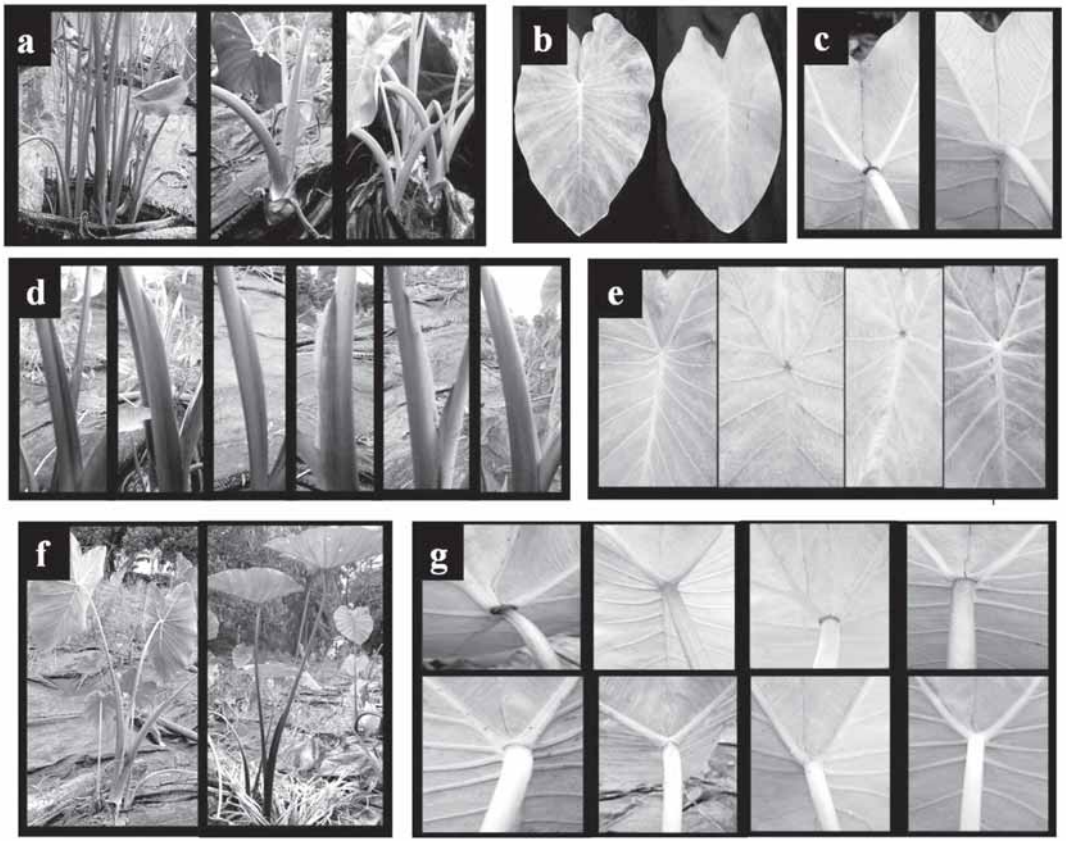

Fig. 1 (a-g). Comparison of variations in morphological traits. a - Tillering. b - Leaf variegation. c - Sinus colour. d - Sheath colour. e - Petiole junction colour and pattern (Upper). f - Lamina orientation. g - Petiole junction pattern and colour (Lower)

Table 1. Percentage distribution and Shannon-Weaver diversity indices of morphological traits in taro accessions of North East India

Sl. No.	Character	Trait	Percentage (%)	Shannon Weaver's diversity index (H)
1	Germplasm type	Cultivated	100	0
2	Growth habit	Erect	100	0
3	Type of stem	Rhizome	100	0
4	Leaf arrangement	Clockwise	76	0.55
		Anticlockwise	24	-
5	Lamina orientation	Cup-shaped	4	0.17
		Erect-apex down	96	-
6	Leaf margin	Undulate	100	0
7	Leaf margin colour	Green	24	1.6
		Light green	16	-
		Purplish green	20	-
		Purple	20	-
		Dark purple	20	-
8	Leaf colour (U)	Green	24	0.55
		Dark green	76	-
9	Leaf colour (L)	Green	60	1.05
		Light green	8	-
		Glaucous green	24	-
		Purplish green	8	-
10	Sinus colour	Green	56	1.29
		Light green	12	-
		Dark green	12	-
		Purplish green	12	-
		Dark purple	8	-
11	Leaf variegation	Present	92	0.28
		Absent	8	-
12	Petiole junction pattern and colour (U)	Spotted (yellow and purple)	20	0.99
		Asterisk		
		Solid	56	-
			24	-
13	Petiole junction pattern and colour (L)	Semilunar	4	1.22
		Colour spread to veins	28	-
		Colour not spread to veins	36	-
		No pattern		-
			32	-
14	Vein colour (U)	Green	28	0.59
		Dark green	72	-
15	Vein colour (L)	Green	4	0.82
		Light green	20	-
		Whitish green	72	-
		Purplish green	4	-
16	Petiole colour (top 1/3rd)	Light green	24	1.46
		Whitish green	24	-
		Purplish green	8	-

		Cream	8	-
		Purple shaded cream	36	-
17	Petiole colour (middle)	Green	4	1.2
		Light green	56	-
		Whitish green	16	-
		Purplish green	20	-
		Cream	4	-
18	Petiole colour (base)	Green	52	1.15
		Light green	28	-
		Purplish green	12	-
		Purple	8	-
19	Leaf sheath pattern	Closed	100	0
20	Leaf sheath colour	Green	44	1.31
		Light green	32	-
		Purplish green	8	-
		Purple	12	-
		Dark purple	4	-
21	Flower formation	Non-flowering	100	0
22	Seed formation	Absent	100	0
23	Tillering*	Low (1-3)	72	0.78
		Medium (4-6)	12	-
		Other	16	-
24	Plant size*	Dwarf (< 50 cm)	16	0.44
		Medium (50-100)	84	-
25	Leaf L:B ratio* (Leaf type)	Narrow	100	0
26	Petiole: leaf length ratio* (Petiole type)	Short	28	0.84
		Medium	64	-
		Long	8	-
27	Petiole: sheath ratio* (Sheath type)	Short	52	0.69
		Medium	48	-

* Quantitative traits Mean – 0.63

tillering and plant size showed high CV values indicating that there was more variation for these phenotypic characters. Taro is highly pan-tropical in its distribution and cultivation and it is probably the variability in these important phenotypic characters that has helped the crop survive and adapt to different agro-ecological conditions.

Principal component analysis

The first principal component explained 35.25% of the variability with leaf margin color, leaf color (L), sinus color, petiole color (top 1/3rd, middle and base) and leaf sheath color as traits with the highest loadings. The second component accounted for 17.74% of the variability with petiole colour top 1/3rd and leaf margin colour as the prominent traits. Leaf margin color, sinus color and petiole colour middle gave high loadings in PC3. These three components together explained 67.77% of the variability among the accessions. Hence, it is evident that leaf margin color and petiole color are important in distinguishing the various accessions. These observations are consistent with the box plot (Fig. 2) and biplot (Fig. 3) representations that depicts the distribution range of various traits in different accessions. The box plot indicate that the traits, leaf margin color and petiole color middle explain maximum variability followed by petiole color top and leaf color lower. The traits dispersed from the origin explain greater variation than those clustered around the origin of the biplot, which include leaf margin color, leaf sheath color, petiole color (top 1/3rd, middle and base), sinus color and leaf color - lower. The scattered traits are found to be in positive correlation as the angle between the trait vectors are $< 90^{o}$.

Table 2. Duncan's multiple range test for the various quantitative traits

Accessions	Tillering	Plant size	Leaf L:B ratio	Petiole:leaf ratio	Petiole:sheath ratio
719	5.00^{a}	57.17^{b}	1.71^{a}	2.04^{abcd}	1.62^{ef}
C-14	4.00^{ab}	61.67^{b}	1.42^{efg}	1.83^{bcdef}	1.99^{abcd}
L-14	3.67^{ab}	66.83^{ab}	1.38^{gh}	2.09^{abc}	2.06^{abc}
I-14	3.33^{bc}	57.67^{b}	1.43^{efg}	1.85^{bcde}	2.11^{abc}
G-14	3.00^{bcd}	62.00^{b}	1.36^{gh}	1.89^{bcde}	2.16^{ab}
E-8	2.67^{bcde}	67.87^{ab}	1.40^{fgh}	1.88^{bcde}	1.92^{abcd}
K-15	2.67^{bcde}	59.00^{b}	1.52^{cde}	1.74^{cdef}	1.90^{abcde}
C-9	2.00^{cdef}	60.33^{b}	1.41^{fgh}	1.66^{def}	1.58^{f}
H-9	1.67^{cdefg}	59.67^{b}	1.39^{gh}	1.67^{def}	1.82^{cdef}
A-12	1.67^{cdefg}	61.00^{b}	1.40^{fgh}	1.83^{bcdef}	2.01^{abcd}
L-8	1.67^{cdefg}	71.00^{ab}	1.31^{h}	2.01^{abcd}	1.99^{abcd}
741	1.67^{cdefg}	55.83^{b}	1.60^{bc}	2.38^{a}	1.87^{bcdef}
724	(1.33)^{defg}	82.50^{ab}	1.36^{gh}	1.44^{fg}	1.61^{f}
H-2	1.00^{efg}	41.67^{b}	1.33^{gh}	1.88^{bcde}	2.13^{abc}
J-8	1.00^{efg}	78.00^{ab}	1.52^{cde}	2.1^{abc}	2.21^{a}
717	1.00^{efg}	44.83^{b}	1.33^{gh}	1.74^{bcdef}	1.87^{bcdef}
A-6	1.00^{efg}	58.50^{b}	1.40^{fgh}	1.70^{cdef}	2.02^{abc}
I-15	0.67^{fg}	54.00^{b}	1.41^{fgh}	1.87^{bcde}	2.19^{a}
F-9	0.67^{fg}	79.00^{ab}	1.57^{bcd}	2.09^{abc}	2.10^{abc}
J-13	0.67^{fg}	71.83^{ab}	1.50^{efd}	2.14^{ab}	2.14^{ab}
B-4	0.67^{fg}	72.83^{ab}	1.40^{fgh}	1.71^{cdef}	2.01^{abcd}
H-6	0.33^{fg}	49.5^{b}	1.39^{gh}	1.27^{g}	1.71^{def}
L-12	0.33^{fg}	73.83^{ab}	1.37^{gh}	1.86^{bcde}	1.93^{abcd}
B-3	0.33^{fg}	64.50^{b}	1.37^{gh}	1.58^{efg}	2.05^{abc}
B-2	0.00^{g}	135.50^{a}	1.63^{ab}	1.59^{efg}	2.02^{abc}
F value	6.16**	0.73	10.55**	3.08	3.88**

*Mean values with the same letter are not significantly different ** Significant at $P < 0.01$ level

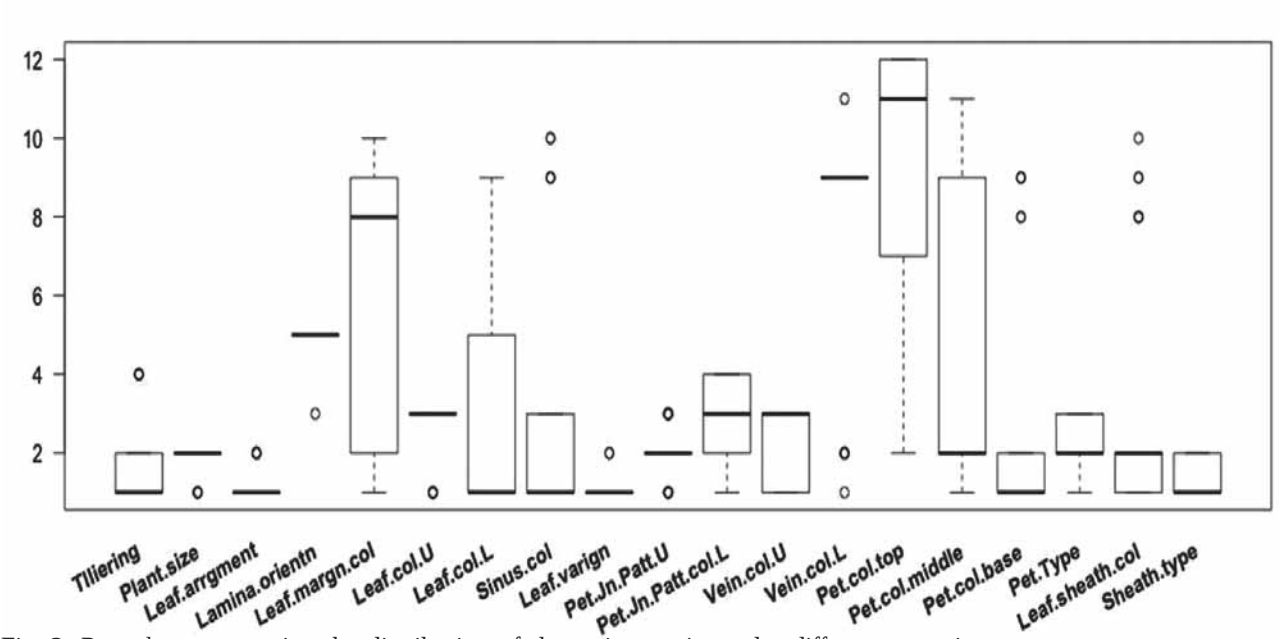

Fig. 2. Box plot representing the distribution of the various traits under different accessions

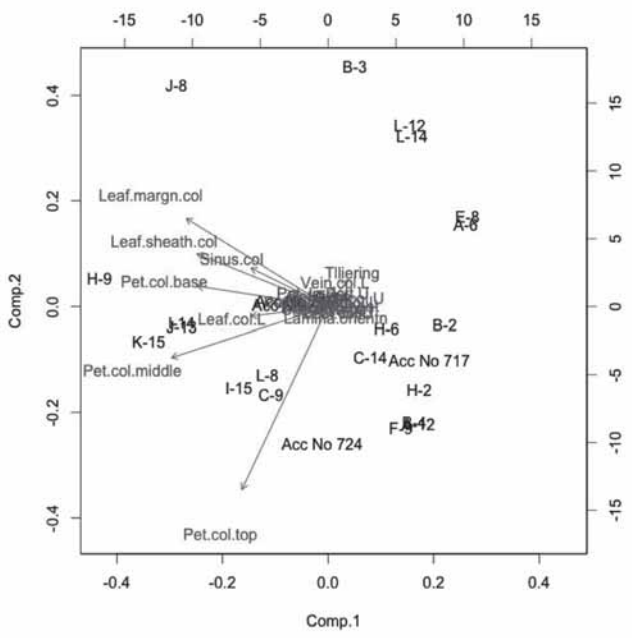

Fig. 3. Biplot representing the distribution of the various traits under different accessions

Dendrogram analysis

Dendrogram generated (Fig. 4) showed that the 25 accessions were broadly grouped under two clusters (cluster 1 and cluster 2), which were further sub divided into two and five sub-clusters each, respectively (clusters 1a and 1b; clusters 2a, 2b, 2c, 2d and 2e). Of these, cluster 2a included the maximum number of accessions (n = 7). It was also observed that geographically closer accessions were grouped under different clusters. Accession No. 724 from Meghalaya was grouped with H-6 from Andhra Pradesh. Except for some leaf characters (leaf colour and margin colour), petiole characters and plant size, they were similar for the other traits screened. Similarly, accession No. 719 from Meghalaya was grouped in 2b along with accession No. 741 from Tripura. These lines showed more similarity, with variations observed only in tillering, vein colour and leaf sheath colour. These results suggest that the geographical origin of the accessions does not bear any relationship with the morphological classification. This finding is consistent with other reports based on morphological analysis (Zubair et al., 2007; Ahmad et al., 2008; Ali et al., 2008).

The cluster data can aid taro breeders to make informed decisions in selecting parental accessions for hybridization from diverse clusters. In addition, knowledge about certain morphological peculiarities like presence of long stolons which is a characteristic of wild taro is often associated with small elongated corms, continuous growth and high concentration of calcium oxalate that causes acridity (Lebot et al., 2004) and can be avoided while selecting parents. The present study was instrumental in highlighting the incongruence between geographical location and accession similarity. The variation for biochemical compounds as well as morpho-physiological traits in *C. esculenta* germplasm has been created by evolutionary forces (Stebbins, 1957) including

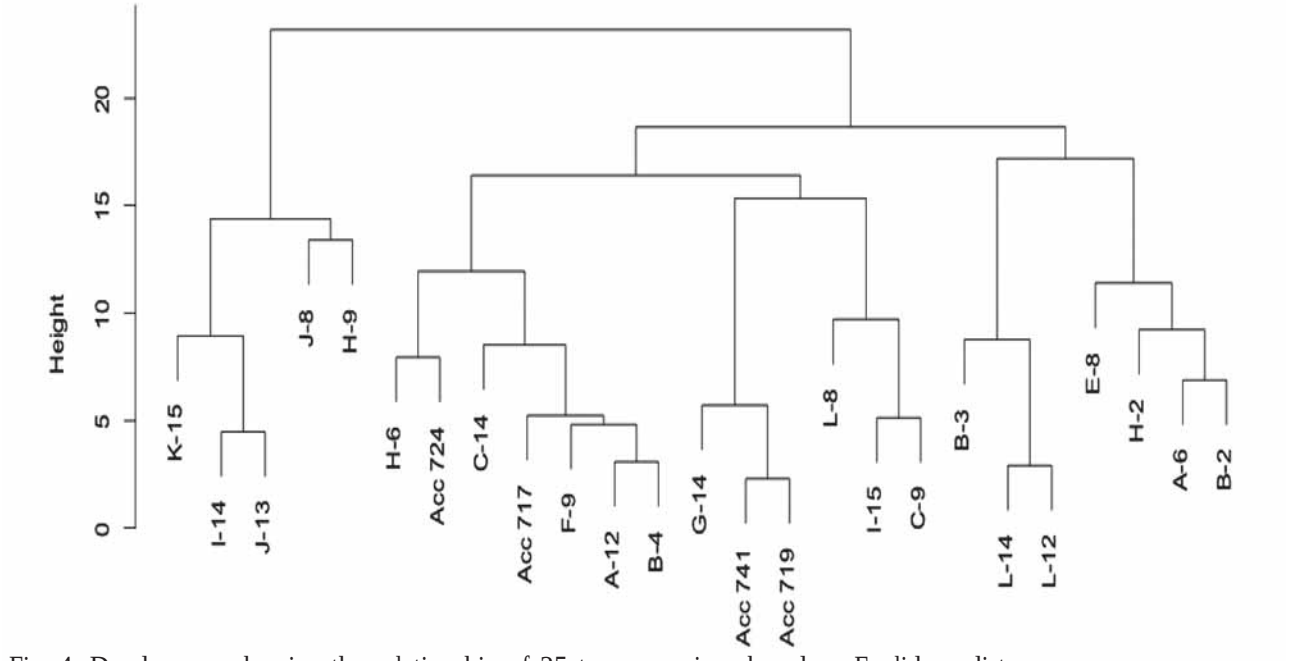

Fig. 4. Dendrogram showing the relationship of 25 taro accessions based on Euclidean distance

geographical speciation (Singh et al., 2008). Moreover, India being nearer to the equator, there is abundance of intense sunlight including ultra violet rays which can probably influence the evolution through mutations (Singh et al., 2012). As per the Climate Change Adaptation in the North Eastern Region of India report (2012), climate change will affect particularly terrestrial forest ecosystems like those in India's North Eastern Region,which will be among those affected most by climate change. Traditional approaches for the measurement of diversity have relied upon the ability to resolve differences in morphological traits (Karp et al., 1996). These approaches have been successful in taro germplasm characterization (Prana, 2000; Jianchu et al., 2001). As in the present study, genetic resources have been characterized in taro in the past in various countries (Lebot et al., 2004; Quero-Garcia et al., 2004; Trimanto et al., 2010; Singh et al., 2012). However, phenotypic variations might not reflect genotypic variation at molecular level, as previously reported by Okpul et al. (2005). As such, information derived from agro-morphological characterization should be treated with caution; however the use of predominant and stable agro-morphological traits can provide basic information and stratification prior to thorough molecular characterization (Singh et al., 2008).

Conclusion

Taro holds great potential in commercial agriculture as it produces fine granule starch desired in specialized industries (Onwueme, 1978). Its future depends on the selection of high yielding, good quality genotypes. However, in order to exploit this crop better, an understanding of its genetic diversity and distribution is essential for its conservation and use (Ramana and Hodgkin, 2002). Hence, this study was undertaken with 25 taro accessions collected from the main centre of diversity, the North East India and analyzed to assess the diversity existing amongst them. The study revealed that, a high level of diversity existed as evidenced by a high Shannon Weaver's diversity index (H) of 0.63. The coefficient of variation was found to be higher for tillering (51.91) and plant size (54.04), which are useful for the adaptability of the plant. PCA analysis revealed 3 principal components revealing 67.7% of the variability. In the fast changing agricultural scenario induced by climate change, where a high degree of vulnerability to the water and climate induced disasters is likely to make the North Eastern region environmentally insecure in the future, it is highly essential to conserve this vast genetic diversity for posterity. The study will be extended further to include molecular characterization using SSR markers for identifying the extent of genetic diversity present in this set of taro accessions. The markers thus identified will be used along with important morphological traits for the identification of duplicates within the vast germplasm collection as well as to identify diverse lines for any future breeding programs at the Centre.

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