Ex situ evaluation on genetic diversity of indigenous taro landraces in North East India

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In this study, 110 taro landraces were characterized using 19 quantitative traits. Statistical tools like descriptive statistics, Shannon-Wiener diversity index, principal component analysis (PCA) and cluster analysis were used to evaluate diversity. Descriptive statistics showed significant variation among the landraces for the 19 quantitative traits studied. The highest coefficient of variation was found in the yield, number of suckers, leaf width and total oxalate. The corm length (H' - 1.06) and starch content (H' - 1.20) had the highest Shannon-Wiener diversity index. PCA resulted in seven principal components (PCs), which explain 70.65% of the total variation. PC1 was mainly associated with plant height, leaf length, leaf width, petiole length and plant spread. PC2 was associated with yield, moisture content, corm length and total oxalate. PC3 was associated with dry matter content and disease index. The cluster analysis using the weighted neighbor-joining method resulted in five major clusters based on geographical location. Cluster IV had a maximum of 54 landraces, and cluster III had a minimum of five landraces. The present study, which identified high genetic diversity and plant height, number of suckers, leaf length, leaf width, corm length, yield, total oxalate content and disease index, can be useful in taro varietal improvement programmes.

Keywords: *Colocasia esculenta*, correlation, descriptive statistics, genetic diversity, landraces.

TARO (*Colocasia esculenta* L. Schott.), the fourth most crucial tuber crop¹, an essential aroid in the family Araceae, probably originated in North East India². It is rich in starch, minerals and dietary fibre. It also has a considerable amount of potassium, magnesium and vitamin-B complex³. Taro has a low glycemic index; thereby, its consumption reduces blood glucose levels in diabetic patients. It possesses medicinal properties against fungal infections, stomach ulcers and tuberculosis⁴. Flavonoids and anthocyanins in taro tubers improve blood circulation and prevent cancer development⁵. The corms, cormels, leaves, flowers and petioles are edible⁶. These nutrient-rich, gluten-free tubers play an essential role in the food and nutritional security of indigenous tribes of NE India. Leaves, petioles and tubers in fresh, dried and fermented forms are used to prepare several traditional dishes⁷. Leaves, petioles and damaged tubers are cooked with local edible grasses and fed to pigs⁸.

NE India comprises eight states, viz. Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, which greatly vary in topography, climate, language and culture⁹. This region is rich in genetic diversity of taro which is adapted to a wide range of climatic conditions¹⁰. The farmers in NE India follow jhum or slash and burn cultivation, especially in the hilly areas. Taro occupies a premium position in home gardens and jhum cultivation, where it grows along with paddy, maize and vegetables in a mixed cropping system. Several landraces have been grown in jhum fields or home gardens, which serve as a genetic reservoir of taro¹¹. The farmers have inherited a wealth of knowledge on taro germplasm from their ancestors, which is transferred from one generation to the next. Over the ages, farmers have selected landraces suitable to their needs and agro-climatic conditions. Each ethnic group in various regions prefers different landraces according to its needs, particularly for specific cuisines.

Most of the landraces in this region are low yielders, and declining soil fertility, Phytophthora leaf blight and climate change hamper taro production. Genetic improvement is the best way to improve yield and quality in taro¹². The selection of parents is also important. Germplasm characterization is fundamental in selecting the parents for crop breeding programmes¹³. Assessing genetic diversity among indigenous taro landraces needs immediate attention to improve the yield. Several researchers have examined the diversity of taro germplasm in NE India based on morpho-logical traits^{14,15} and molecular markers^{16,17} and identified wide genetic diversity among the germplasm. However, these studies are confined to a few locations and carried out with limited germplasm and fewer markers. Despite the rich cultural importance and genetic resources, knowledge of the genetic diversity of taro in NE India is limited. Thus, the present study aimed to assess genetic diversity among 110 indigenous taro landraces collected from different states of NE India using agro-morphological and quality traits.

The present study was conducted at ICAR Research Complex for NEH Region, Jharnapani, Nagaland ($25^{\circ}45'N$ long. and $93^{\circ}50'E$ lat., at an altitude of 281 m above msl). The soil was classified as Inceptisol. The pH was 5.7, electrical conductivity (EC) was 0.121 d Sm^{-1} and soil organic carbon was 0.73%. It was low in nitrogen ($149.2 \text{ kg N ha}^{-1}$), moderate in phosphorus ($18.4 \text{ kg P}_2O_5 \text{ ha}^{-1}$), and low in potassium ($173.6 \text{ kg K}_2O \text{ ha}^{-1}$). The mean maximum temperature of the site was 33.0° C, minimum temperature was 10.8° C, mean maximum relative humidity was 85%, and minimum humidity was 72%, with a mean annual rainfall of 1200 mm

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Landrace no.	Landrace	State
1–14 West Garo Hills	Tamarong Bol, Tamachong Kam, Marakajatong, Tasarang, Takiltom, Tamitdim, Tadura, Tararang, Ringdubi, Tasobok, Tajekjak, Tasarang Ganching, Taring	Meghalaya Latitude: 25°29'–25°35'N Longitude: 90°12'–90°07'E Altitude: 276–495 m amel
15–21 Imphal West	Mukhi pan, Houpan, Khungupan, Pangong pan, Yarumpan, Bar, Barker	Manipur Latitude: 24°41′–24°45′N Longitude: 93°40′–93°56′E
22–29 Senapati	Ziiphat, Majangzhii, Ziishow, Abzii Katomei, Tamei, Lairouching, Lairouching	Altitude: 1426–2716 m amsi Manipur Latitude: 25°16′–25°23′N Longitude: 94°01′–94°05′E
30–36 Zunheboto	Mishmeh, Atutu, Cherimeh, Ayekhu, Kanchi, Naghi, Ayi	Nagaland Latitude: 25°59′–26°03′N Longitude: 94°29′–94°31′E
37-41 Phek	Bishe, Bibu, Tenyibe, Nyisheliibe, Bishow	Altitude: 1423–1801 m amsl Nagaland Latitude: 25°49′–25°36′N Longitude: 94°19′–94°23′E
42–52 Peren	Dzurinuo, Tsophiju, Nukruca dzunuo, Semia, Beugie, Beutei, Beusang, Mbeijukwak, Hepwapuipikam, Beureu, Kungsaibeu	Altitude: 1630–1826 m amsl Nagaland Latitude: 25°36'–28°38'N Longitude: 93°40'–93°51'E
53–65 Dimapur	Mishieli, Dzurenuno, Kotsa dzuno, Dzuse, Dzurenuno, Balkedoh, Baldosan, Balloupi, Balsan, Balkang, Saikang, Bal Ahtui, Balbom	Annude: 310–361 m ansi Nagaland Latitude: 25°40′–25°47′N Longitude: 93°51′–93°55′E Altitude: 310–317 m amsl
66–72 Wokha	Tsarhomo, Vajo, Phila, Shirochu, Yojung, Lifuro, Hanphya	Nagaland Latitude: 26°60'–26°63'N Longitude: 94°14'–94°16'E Altitude: 737–1314 m amel
73–83 Mon	Baikhi, Nalon, Tungyak, Along, Tungnyak, Tungsho, Laihi, Ganching Kohimathung, Phaksa, Local	Nagaland Latitude: 26°43′–26°46′N Longitude: 95°01′–95°09′E
84–100 West Siang	Ayang Anga, Nyata Taing, Madras Kochu, Dabat, Nyemr, Nyise, Aalo Local, Naeup, Nyita, Angatakang, Libo local, Anyno, Nymar, Nyoile, Darga, Engyo kochu, Noilie	Arunachal Pradesh Latitude: 28°10′–28°15′N Longitude: 94°14′–94°48′E
101–106 Dimapur	Obi 1, Obi 2, Obi 3, Obi 4, Obi (Red), Obi (White)	Nagaland Latitude: 25°45'N Longitude: 93°50'E Altitude: 250 m amsl
107–110 Kohima	Thupelie, Dziicha, Tefiiziinuo, Normal	Nagaland Latitude: 25°40'N Longitude: 94°72'E Altitude: 1444 m amsl

Table 1.	Geographical	location of tarc	landraces	collected	from	North	East	India
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(Source: Automatic Weather Station at ICAR Nagaland Centre Agromet Observatory, Jharnapani, Nagaland).

One hundred ten landraces collected from different states of NE India were planted with a spacing of 60×60 cm, and 20 plants were grown for each landrace (Table 1). The ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, India, recommended dose of fertilizers for NE India (12 t ha⁻¹ FYM, 100 : 60 : 80 kg ha⁻¹ N, P₂O₅ and K₂O respectively) were applied uniformly. The tubers were planted in May and grown rainfed. They were harvested from October to November. The experiment was conducted for two consecutive years (2014 and 2015). The growth characters, viz. plant height (cm), number of leaves, number of suckers, leaf length (cm), leaf width (cm), petiole length (cm) and plant span (cm), were recorded on five plants in each replication at 120 days after planting, and the mean was computed. The yield characters like corm length (cm), corm diameter (cm) and yield (g plant⁻¹) were recorded immediately after harvest from five plants in each replication, and the mean was computed. The quality characters like dry matter (%), moisture content (%), starch (%), total sugars (%), potassium (mg 100 g⁻¹), phosphorus (mg 100 g⁻¹), and total oxalate content (mg 100 g⁻¹) were estimated. The dry matter and moisture content¹⁸ and total

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Characters	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation (%)	Diversity index <i>H</i>
Plant height (cm)	28 40	150.90	93.24	22.09	20.43	0.80
No. of leaves (no)	2.10	7.50	4.40	0.94	20.34	0.59
No. of suckers (no)	0.00	7.90	2.38	1.38	47.53	0.44
Leaf length (cm)	11.70	60.70	37.01	9.99	20.35	0.34
Leaf width (cm)	7.60	119.80	28.57	12.46	42.87	0.10
Petiole length (cm)	13.30	112.50	67.71	17.18	20.38	0.45
East-West spread (cm)	11.20	141.60	84.51	20.70	18.45	0.81
North-South spread (cm)	24.30	133.60	83.01	20.20	19.99	0.65
Corm length (cm)	5.00	28.20	9.84	4.20	36.67	1.06
Corm diameter (cm)	3.10	19.20	5.83	1.67	26.64	0.18
Yield (g plant ⁻¹)	37.60	1319.20	376.9	225.74	47.80	0.62
Dry matter (%)	13.10	42.20	26.75	5.54	19.92	0.62
Moisture content (%)	57.80	86.90	73.25	5.53	7.28	0.85
Starch (%)	12.20	50.00	25.20	8.19	21.57	1.20
Total sugars (%)	1.60	9.00	4.81	1.66	23.73	0.82
Phosphorus (mg 100 g ⁻¹)	1.10	3.80	2.36	0.68	23.51	0.82
Potassium (mg 100 g ⁻¹)	1.00	1.50	1.15	0.10	7.39	0.33
Total oxalate (mg 100 g^{-1})	44.50	358.90	1.67	79.45	42.41	0.77
Disease index (%)	11.80	100.00	65.81	21.81	27.54	0.61

Table 2. Diversity analysis for 19 quantitative traits in 110 taro landraces

sugars and starch content¹⁹ were determined following standard methods. The potassium and phosphorus contents were determined calorimetrically²⁰. The total calcium oxalate content was determined based on the method suggested by $AOAC^{21}$. Leaf blight disease incidence was recorded with the onset of disease symptoms in the field and continued at weekly intervals until harvest. *Phytophthora* leaf blight disease incidence was assessed using the following formula²²:

Disease incidence (%) = $\frac{\text{Total infected plants}}{\text{Total number of plants observed}} \times 100.$

Descriptive statistics was performed using STAR 2.0.1 developed by the International Rice Research Institute, Philippines. The Shannon–Wiener diversity index was calculated in Microsoft Office Excel 2010 using the formula

$$H = -\sum_{i=1}^{s} (pi * \ln pi),$$

where H is the diversity index and Pi is the fraction of the entire population made up of species *i*. Principal component analysis (PCA) was carried out using 19 quantitative traits in SPSS 16.0 (ref. 23). Cluster analysis between 110 landraces was done using the continuous dissimilarity index method with Euclidean on standardized variables employing the default DICE method. The tree was constructed using the weighted neighbor-joining method in Darwin 6.0 (ref. 24).

This study characterized 110 indigenous taro landraces collected from Arunachal Pradesh (17 landraces), Manipur (15 landraces), Meghalaya (14 landraces) and Nagaland (64 landraces) for agro-morphological and quality traits. The study found significant variation for the 19 quantitative traits for all the 110 taro landraces. Table 2 gives the minimum, maximum, mean, standard deviation and coefficient of variation (CV) values recorded for all the traits. CV ranged from 7.28% to 47.80%, indicating rich genetic diversity among the taro landraces. Yield (CV = 47.8%), number of suckers (CV = 47.53%), leaf width (CV = 42.87%) and total oxalate content (CV = 42.41%) showed larger variation, while moisture content (CV = 7.28%) and potassium content (CV = 7.39%) showed least variation. Higher CV values (CV > 40%) indicate that the four traits, viz. yield number of suckers, leaf width and total oxalate content, have good breeding potential. Low CV values (CV < 10%) indicate that the two traits, viz. phosphorus content and moisture content, have good genetic stability. The larger the CV value, the higher the level of genetic dispersion. So, in any taro varietal improvement programme, the breeders should consider traits with higher CV values.

The Shannon–Wiener diversity indexes (H') exhibited 19 quantitative traits ranging between 0.10 and 1.20 (Table 2). Among the quantitative traits, the highest diversity index was corm length (H' - 1.06) and starch content (H' - 1.20), which displayed a significantly higher level of genetic diversity than the other traits. The lowest diversity index was for leaf width (H' - 0.10) and corm diameter (H' - 0.18), which displayed low genetic diversity. The other traits like plant height, number of leaves, plant spread, yield, dry matter content, moisture content, total sugars, total oxalate and disease index exhibited a high diversity index (H' > 0.5), indicating these traits are highly variable.

PCA identifies the relationship between variables. PCA of the 110 indigenous taro landraces based on 19 quantitative traits resulted in seven principal components (PCs; Table 3).

Table 3. Eigenvectors from principal component analysis of agro-morphological and yield traits in taro landraces								
Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	
Plant height	0.836	0.047	0.060	-0.160	-0.130	-0.029	-0.073	
No. of leaves	0.386	-0.140	-0.120	-0.080	0.548	0.232	0.282	
No. of suckers	0.290	0.170	-0.052	0.133	0.549	-0.236	0.513	
Leaf length	0.855	-0.006	0.024	0.040	-0.231	-0.025	-0.080	
Leaf width	0.624	-0.017	-0.003	0.069	-0.253	0.150	-0.029	
Petiole length	0.887	-0.032	0.075	-0.010	-0.034	0.013	-0.003	
East-West spread	0.895	0.060	0.023	0.018	0.070	-0.039	-0.012	
North-South spread	0.868	0.041	-0.057	-0.119	0.037	-0.099	-0.077	
Corm length	0.000	0.430	0.416	0.140	-0.472	0.241	0.315	
Corm diameter	0.010	0.328	0.206	0.332	0.325	0.005	-0.513	
Yield	0.021	0.644	0.509	0.134	-0.021	-0.130	0.111	
Dry matter	0.035	-0.789	0.591	0.079	0.064	-0.046	0.053	
Moisture content	-0.038	0.789	-0.591	-0.080	-0.063	0.043	-0.054	
Starch	0.013	0.308	0.198	-0.665	0.265	-0.233	-0.201	
Total sugars	0.086	0.292	0.077	0.023	0.119	0.583	0.244	
Phosphorus	0.239	-0.110	-0.203	0.526	0.112	-0.012	-0.280	
Potassium	-0.034	0.251	0.141	0.097	-0.152	-0.668	0.219	
Total oxalate	-0.047	0.348	0.324	0.501	0.261	0.043	-0.141	
Disease index	-0.086	0.200	0.488	-0.471	0.150	0.223	-0.211	
Eigenvalues	4.473	2.425	1.654	1.433	1.291	1.116	1.032	
% Total variance	23.544	12.761	8.707	7.543	6.793	5.873	5.432	
% Cumulative variance	23.544	36.304	45.011	52.554	59.347	65.220	70.652	





Figure 1. Cluster analysis of 110 taro landraces based on 19 quantitative traits.

These seven PCs exhibited 70.65% of the total variation. The leaf length, petiole

leaf length, petiole length, plant spread (E–W, N–S) and leaf width contributed more to genetic variation among the landraces. PC2 explained 12.76% of the total variation and

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first component (PC1) explained 23.54% of the total varia-

tion, and plant growth characteristics such as plant height,

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was mainly associated with yield, moisture content, corm length and total oxalate. Yield, dry matter content and disease index contributed more to the variation among taro in PC3, which explained 8.7% of the total variation. Earlier studies on taro germplasm in Ghana, India and China reported that the first three PCs contributed more to the variation and were mainly associated with plant growth and yield characters^{25–29}. In PCA, the first three PCs are important, and their associated traits contribute more to the genetic variation³⁰. These traits could be helpful in crop improvement programmes.

The cluster analysis between the 110 taro landraces based on weighed neighbor-joining method using 19 quantitative traits formed 5 major clusters (Figure 1). Cluster IV had the maximum landraces (54), followed by cluster I (28) and cluster II (16). Cluster III (five) and cluster V (seven) had the minimum number of landraces. The analysis formed clusters based on geographical location. In general, landraces that shared close state boundaries and similar agro-ecological conditions were grouped in one cluster. However, few landraces from close geographical locations representing different agro-eco-regions were grouped in different clusters. This indicates the movement of taro landraces to other adjoining states, and a similar clustering pattern has been reported for taro germplasm in NE India¹⁵. PCA and cluster analysis showed high genetic diversity. The results indicate that PCA is a more precise indicator than cluster analysis for genetic diversity. Further, this study highlights the incongruence between landraces and geographic location. The agro-morphological variation in taro landraces has resulted due to mutations and geographical speciation³¹. It may not reflect genotypic variation at the molecular level, so breeders must be cautious. However, agro-morphological variation provides basic information about the indigenous taro landraces. The breeders should pay attention to growth and yield traits, total oxalate content and *Phytophthora* leaf blight disease index for selecting the parents in taro improvement. It is also suggested that selecting parents from diverse clusters for specific traits aid in taro improvement.

Taro is one of the important components in the cuisine of the ethnic people of NE India. The future of taro production depends on acrid-free, high-yielding genotypes. Understanding the genetic diversity is essential to exploit taro in this region. Hence, the present study analysed the diversity among 110 germplasms collected from NE India. A high level of genetic diversity was found among the taro collections, as evidenced by the high Shannon-Wiener diversity index (up to H' - 1.20). PCA analysis resulted in seven components that exhibited 70.65% variability. Cluster analysis resulted in five major clusters based on geographical location. This study reveals that traits like growth, yield, corm length, starch, dry matter, total oxalate content and the Phytophthora leaf blight disease index influence the diversity, and these breeders should consider these traits in taro improvement programmes to select superior genotypes. Further, molecular characterization is necessary to assess the extent of genetic diversity present in the taro germplasms of NE India.

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REE-mineral phases in Indian red mud from the east coast bauxite deposit

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Red mud is often considered a prospective secondary resource of rare earth elements (REE). The Indian red mud samples were characterized using XRD, WD-XRF, ICP-OES and SEM-EDS to study their REE mineralogy and REE content. A major fraction (77%) of the sample was below 45 μ m, with total REE content of 433 ppm. There was an enrichment of LREE over HREE, and La, Ce, Nd and Sc were the main contributors to the total REE value. REE-bearing minerals like monazite and zircon occurred as discrete mineral phases in the bauxite residue.

Keywords: Bauxite deposit, monazite, rare earth elements, red mud, zircon.

RED mud or bauxite residue is a solid waste that is produced by aluminium industries during the extraction of alumina from bauxite. It mainly comprises iron oxide, aluminium oxide, silicon oxide and titanium oxide. Generally, it is reddish-brown in colour, and the characteristic red colour is due to the presence of large amounts of iron oxide. The average composition of red mud is Fe₂O₃ (48–54%), Al₂O₃ (17-20%), TiO₂ (3-4%), SiO₂ (4-6%), CaO (1-2%), Na₂O $(3-5\%)^1$. It also contains many trace elements such as Ga, V, Mn, Cr, Ni, Cu, Pb, Zn, Cd, P, Mg, Hf, Zr, Th, U, Nb, Ba, Sr, K and rare earth elements (REEs) in varied proportion^{2,3}. However, the mineralogical and chemical composition of red mud varies widely depending on the source of bauxite and the technological processing conditions applied to recover alumina^{4,5}. It is reported that 0.8–1.5 tonnes of red mud is generated per tonne of alumina produced from bauxite⁶. World inventory of red mud shows that about 60-120 million tonnes are produced annually from the bauxite industry. India is one of the largest producers of red mud, producing about 2 million tonnes⁷. The production of red mud has been rising rapidly⁸⁻¹⁰ and its disposal is one of the major concerns as red mud is highly alkaline and is a potential threat to air, water and land. Hence, red mud is a source of environmental pollution on the one hand^{11,12} and a promising source for obtaining valuable elements on the other¹³. It can be utilized to recover Fe, Al, Ti, Na and REE^{3,14}. It is reported that bauxite residue contains a high amount of scandium (70-260 ppm) that is close to its primary resources^{15,16}. The approximate range of total REE content in red mud is about 500-1700 ppm (ref. 17). Several studies have reported that red mud is a secondary source

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