Journal of Root Crops, 2018, Vol. 44 No. 2, pp. 23-31
Indian Society for Root Crops
ISSN 0378-2409, ISSN 2454-9053 (online)

Assessment of Variability in Sweet Potato (*Ipomoea batatas* (L.) Lam.) Germplasm Using Morphological and ISSR Markers

V. B. Sabarinath[1], *Shirly Raichal Anil[2], N. Krishna Radhika[2], B. S. Prakash Krishnan[2], Deo Sankar[3] and J. Sreekumar[2]

[1]College of Agriculture, Vellayani, Thiruvananthapuram 695 522, Kerala, India

[2]ICAR- Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram 695 017, Kerala, India

[3]IGKV, Raipur

*Corresponding author: Shirly Raichal Anil, email: shirlyanil@yahoo.com

Abstract

Fifty two accessions of sweet potato collected from eastern states of India and maintained in the National Active Germplasm Site at ICAR-CTCRI, along with two wild species *Ipomoea triloba* L. and *Ipomoea aquatica* Forssk. were evaluated using eighteen vegetative morphological and eleven Inter Simple Sequence Repeats (ISSR) markers. The dendrogram obtained using phenotypic characters separated the genotypes into two major clusters and an outlier at a Euclidean distance of 1.20. The first three principal components of data accounted for 67.50% of the total variance among accessions. Traits like predominant vine colour, leaf lobes type were found to be of great importance in distinguishing the accessions. The cluster diagram based on morphological data revealed that the accessions exhibited greater degree of genetic variation for the 18 different morphological traits observed. According to the morphological data, there were no duplicate accessions and S1439 and S1442 were found to be highly similar among the accessions studied. The hierarchical clustering using on ISSR profile based on Jaccard's similarity coefficient separated the accessions into three principal clusters at a similarity coefficient of 0.56. The first principal cluster consisted of 37 accessions with many sub-clusters showing high intra-clusteral variability indicating the variability in the sweet potato accessions selected for the study. Accessions collected from the same geographical area were grouped together in a single cluster. The second principal cluster comprised of 15 accessions with one set of two accessions showing 89% similarity, both collected from Bihar. This grouping was similar to that obtained with morphological data. *I. triloba* L. and *I. aquatica* Forssk. were grouped as a third cluster showing their species specificity. Mantel test indicated significant correlation between morphological and molecular marker information.

Key words: Sweet potato, germplasm, wild species, ISSR markers, morphological descriptors, duplicate identification, cluster analysis

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous plant that belongs to the family Convolvulaceae. It is one of the world's most important food crop and its large, starchy, sweet tasting tuberous roots are important root vegetable (Purseglove, 1991) produced on a large scale in tropical and subtropical countries where sufficient water is available for their growth. Sweet potatoes are rich in carbohydrates, dietary fiber, beta carotene, vitamin C and vitamin B_6 (Agbore-Egbe and Rickard, 1990). With more than 133 million tons in annual production, sweet potato currently ranks as the fifth most important food crop in developing countries after rice, wheat, maize, and cassava.

Morphological characterization has been used widely since time immemorial for different purpose such as

diversity analysis (Khalik et al., 2013, Fongod et al., 2012 and Tsegaye et al., 2007), taxonomic classification of plants (Aguoru *et al.*, 2015) and to identify and eliminate duplicates (Yada et al., 2010). Morphological characterization of plant species is important in the identification of duplicate accessions and detection of unique traits (Reed et al., 2004). Sweet potato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics.

Phenotypic characterization in sweet potato is done by assessing variations in the vine, leaf, flower and storage root characteristics (Huaman, 1999). Morphological characterization supplemented by molecular characterization provides information for comparison of individual accessions or variety thereby facilitating germplasm improvement and effectiveness of collection. But morphological markers are often providing less genetic information (Rao, 2004) and also morphological traits are highly vulnerable to the environmental conditions (Gepts, 1993; Prakash et al., 1996). So they cannot provide a scrupulous assessment regarding duplication in germplasm as it holds samples from different locations. Therefore morphological characterization is often harmonized with molecular characterization as the molecular marker are stable against any environmental vulnerability and possess greater accuracy in locating the genes in its exact place (Westman and Kresovich, 1997). The molecular characterization helps to avoid duplicates in germplasm and protect the special cultivars (Allemann et al., 2004). Since Eastern states of India are the main sweet potato growing belt in India the present study aimed to identify the diversity among the sweet potato accessions collected from the eastern states of India, which are maintained at the ICAR-CTCRI gene bank, using morphological and ISSR markers.

Materials and Methods

Plant material

Fifty two sweet potato accessions collected from the various eastern states of India and two wild species of *Ipomoea*, *I. triloba L.* and *I. aquatica* Forssk., all maintained in the National Active Germplasm Site (NAGS) at ICAR-Central Tuber Crops Research Institute were taken for the present study. The plants were raised on ridges in the field with five plants per accession spaced at 60 cm x 20 cm. Weeding and intercultural operations were carried out as per standard package of practices. The accessions were S1404, S1405, S1408, S1409, S1437, S1438, S1439, S1440 S1441, S1442 and S1666 from Chhattisgarh, S1503 and S1509 from Nagaland, S1498, S1499,S1500 S1502, S1504, S1505, S1506, S1507, S1508, S1598 and S1600 from Meghalaya, S1510, S1511, S1512, S1514, S1515, S1527, S1516, S1517, S1518, S1519, S1522, S1523, S1524 and S1525 from Tripura, S1569 and S1572 from West Bengal, S1565 from Odisha, S1574, S1576 and S1433 from Bihar, S1662, S1665, S1660, S1661, S1656, S1658, S1663 and S1659 from Arunachal Pradesh

Morphological characterization

Observations on 18 vegetative qualitative traits were scored in all the 54 accessions based on descriptors developed by International Plant Genetic Resources Institute (IPGRI) descriptors for sweet potato (CIP et al., 1991). The observations were made after 45 days of planting. The morphological data were recorded by phenotypic observation of the selected accessions. Each character and traits were recorded by assigning certain numerical value to them based on the descriptors.

Molecular characterisation

DNA was extracted from fresh and tender young leaves of the above 54 accessions using Dellaporta et al. (1983) method of DNA extraction. The quality of the DNA was checked by agarose gel electrophoresis in 0.8% agarose gel and then quantified by measuring OD at 260nm.OD at 280nm was also recorded to check the purity of DNA. Purity of DNA sample was calculated from OD at 260/280 ratio. Concentration of DNA was calculated using the formula,Concentration of DNA (µg/ml) = OD_{260} x dilution factor x 50. All the DNA samples were uniformly diluted to 10ng/µl, irrespective of their concentrations. In the present work a total of 17 primers were tested of which 11 primers (Table 1) produced reproducible bands and these were used for screening all the accessions. PCR was carried out in Proflex™ thermalcycler programmed for an initial denaturation at 94°C for 5 minutes followed by 35 cycles with denaturation at 94°C for 30 seconds, primer annealing at 56.3°C for 1 minute and extension at 72°C for 1 minute. The final extension was performed at 72°C for 10 minutes followed by hold at 4°C. The

Table 1. List of ISSR primers used for sweet potato characterization

Primer	Sequence 5'-3'	Number of bands	Number of polymorphic bands	Polymorphism (%)
UBC-807	AGAGAGAGAGAGAGAGT	6	6	100
UBC-808	AGAGAGAGAGAGAGAGC	16	13	81.25
UBC-809	AGAGAGAGAGAGAGAGG	6	6	100
UBC-818	CACACACACACACACAG	8	8	100
UBC-825	ACACACACACACACACT	10	7	70
UBC-827	ACACACACACACACACG	5	4	80
UBC-860	TGTGTGTGTGTGTGTGRA	5	5	100
$(GA)_9AC$	GAGAGAGAGAGAGAGAGAAC	12	12	100
UBC-811	GAG AGA GAG AGA GAG AC	7	7	100
UBC-817	CAC ACA CAC ACA CAC AA	6	5	83.30
UBC-810	GAGAGAGAGAGAGAGAT.	8	7	87.50
Total		89	80	89.80

PCR products were resolved in 2% agarose gel stained with ethidium bromide along with 100 bp and 1 kb ladders to identify the molecular weight of the obtained bands and for polymorphism studies (Fig. 2). The gel images of resolved PCR products were taken. Clear and reproducible bands were scored. Scoring was carried out in the form of binary scoring format via assigning "1" for the presence of a specific band and "0" for the absence of band.

Data analysis

Multivariate analysis was performed by numeric taxonomic techniques using the procedure of principal component analysis (Sneath and Sokal, 1973). To bring out the patterns of similarity and dissimilarity, data were subjected to UPGMA method of clustering which grouped the 54 accessions into sound clusters based on phenetic resemblances as determined in terms of Euclidean distance coefficient. Morphometric analyses were performed using Multivariate Statistical Package MVSP Version 3.1 (Kovach computing Services, Wales, UK).The binary data obtained from ISSR profile was used to generate a dendrogram which grouped the 54 accessions, on the basis of Jaccard's similarity coefficient and unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) using NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U. S. A., software version 2.02 package) (Rohlf, 1998). To determine the similarity and diversity between every two accessions, pair-wise distance (similarity) matrices was computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC. Morphological and molecular data were compared using Mantel's test (Mantel, 1967) by adopting random permutations.

Results and Discussion

Morphological characterisation

The morphological data analysis using Multivariate Statistical Package (MVSP 3.22) generated a dendrogram (Fig. 1) which separated all the 54 accessions into two major clusters and one outlier at a Euclidean distance of 1.2.

The pattern of clustering of accessions is described in Table 2. Among the two principal clusters, the second principal cluster was having the maximum number of accessions. No two accessions were 100% similar. The maximum similarity was obtained between S 1439 and S 1432 at a Euclidean distance of 0.2 (Log 10 transformed). Thus, the study identified no sets of duplicates. This is very low compared to Koussao et al. (2014) where, eight duplicates were obtained among 112 sweet potato germplasm collections taken from Burkina Faso in a diversity study. The duplicated accessions may occur due to different accessions given similar names or same accessions having different names or due to a wide spread exchange of propagules all over the world (Ahikpa et al., 2013). Often, it leads to unnecessary expense in

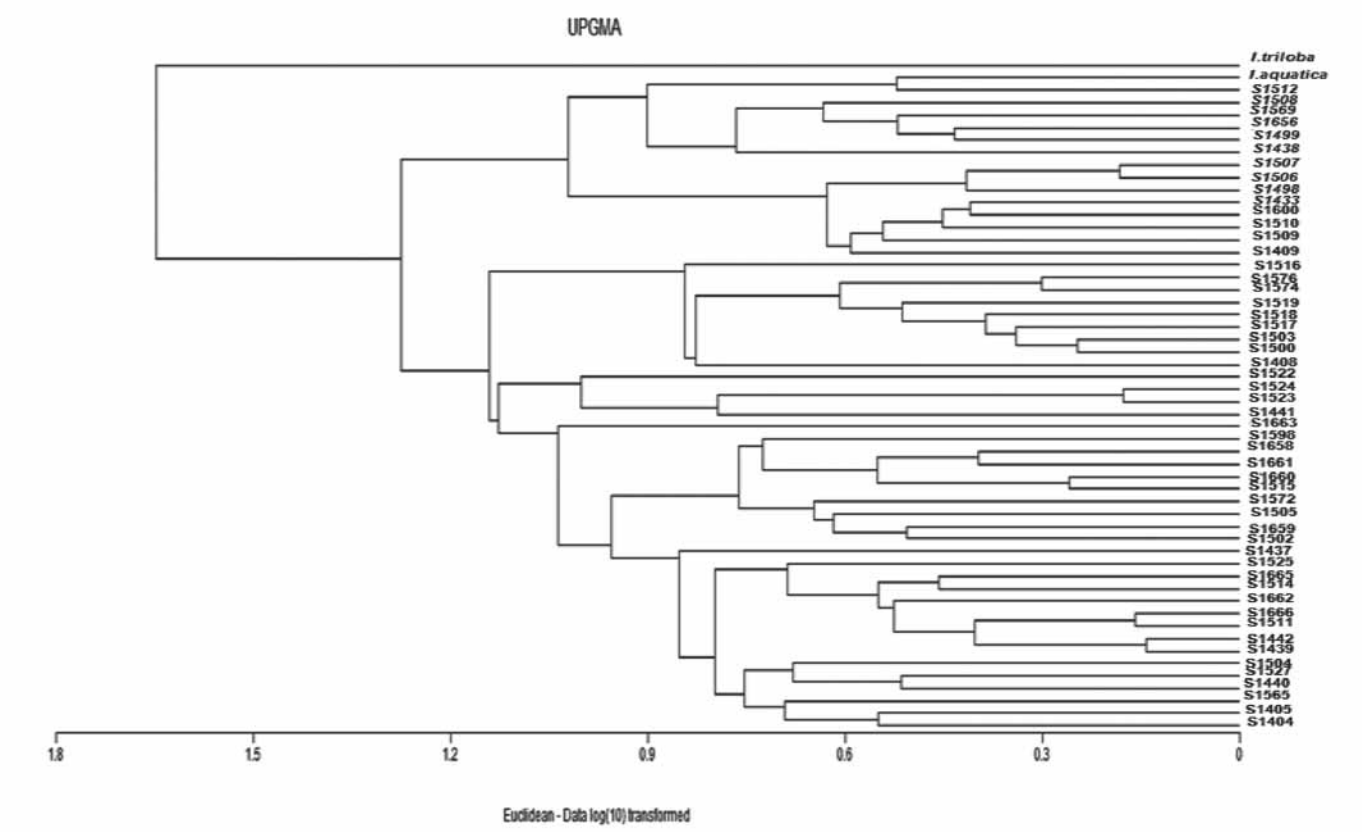

Fig.1. Dendrogram based on morphological data

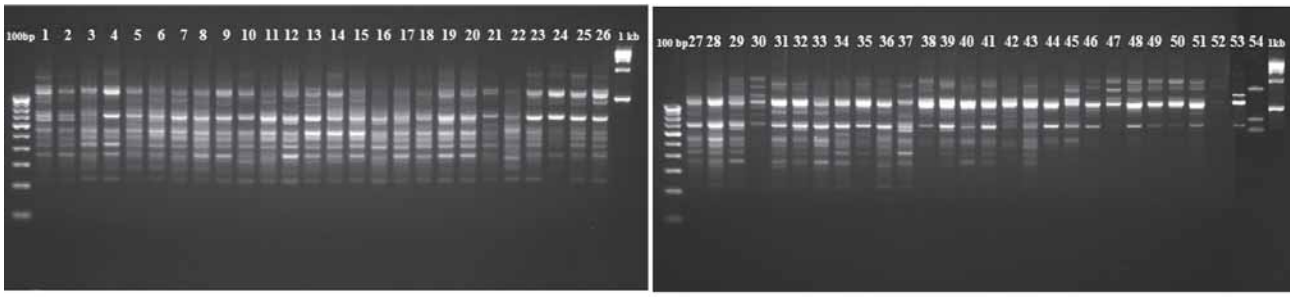

Fig. 2. Electrophoretic pattern of 54 accessions of *Ipomoea in* 2% agarose gel using UBC 808

terms of labour, area and maintenance of germplasm. So duplicates must be brought into a reference status by the elimination of their copies (Naik et al., 2006).

Principal component analysis (PCA)

Principal component analysis indicated high Eigen values for the first three principal components (PC) which accounted for the 67.5% of the variability among the characters studied (Table 3). PC1 accounted for 36.3% of the variation with predominant vine colour, leaf lobes type, abaxial vein pigmentation and petiole pigmentation as highly loaded characters. The second principal component accounted for 19% of the variation which included vine tip pubescence, leaf lobes type and immature leaf colour. The third principal component accounted for 12.30% of the 262variation with predominant vine colour, secondary vine colour and petiole pigmentation with high values. The trait included in both PC1 and PC2 was leaf lobes type. Predominant vine colour was the trait included in both PC1 and PC3. Hence predominant vine colour and leaf lobe type can be considered as important in distinguishing the accessions.

Among eighteen morphological characters recorded using IPGRI descriptors, predominant vine colour, leaf

Table 2. Thematic map of the pattern of clustering of 54 accessions based on morphological descriptors giving distribution of accessions (based on Fig.1.)

States / Cluster	Sub-clusters	Chhattis-garh	Nagaland	Meghalaya	Tripura	West Bengal	Odisha	Bihar	Arunachal Pradesh	Wild species
Cluster 1	A	S1438	S1508 S1499	S1512	S1569				S1656	*I.aquatica*
	B	S1409	S1509	S1600 S1498 S1506 S1507	S1510			S1433		
Cluster 2	A	S1408	S1503	S1500	S1517 S1518 S1519			S1574 S1576		
	B	S1441			S1522				S1663	
	C				S1523 S1524					
	D				S1515 S1516					
	E			S1502 S1598 S1505		S1572			S1658 S1659 S1661 S1660 S1515	
	F	S1404 S1405 S1437 S1439 S1440 S1442 S1666		S1504	S1511 S1514 S1525 S1527		S1565		S1662 S1665	
Outlier										*I.triloba*

Table 3. Principal component analysis in 54 accessions of sweet potato

Variables	PC1	PC2	PC3	PC4
Twining	-0.02	-0.145	0.013	0.16
Plant type	0.01	-0.152	-0.027	0.255
Ground cover	-0.045	-0.013	0.141	0.507
Vine internode length	0.003	-0.045	0.015	0.095
Vine internode diameter	0.007	0.036	0.027	0.052
Predominant vine colour	0.457	0.173	0.345	0.053
Secondary vine colour	0.175	0.275	-0.819	-0.09
Vine tip pubescence	0.152	0.585	0.082	0.165
General outline of leaf	-0.161	0.155	0.143	-0.137
Leaf lobes type	-0.443	0.433	0.143	-0.139
Leaf lob number	-0.286	0.266	0.077	-0.139
Shape of central leaf lobe	-0.265	0.254	0.099	-0.102
Mature leaf size	-0.006	0.044	0.007	-0.022
Abaxial leaf vein pigmentation	0.377	0.133	0.034	-0.376
Mature leaf colour	0.121	0.042	-0.098	-0.08
Immature leaf colour	0.035	0.319	-0.198	0.556
Petiole length	-0.005	0.081	0.063	0.271
Petiole Pigmentation	0.45	0.169	0.267	-0.061
Eigenvalues	19.829	10.352	6.688	3.471
Percentage	36.331	18.968	12.254	6.360
Cum. Percentage	36.331	55.299	67.553	73.913

lobes type, abaxial leaf vein pigmentation, petiole pigmentation were the main variable characters observed within the accessions and these could be used in distinguishing the accessions. The accessions showed similarity in characters such as twining, plant type, mature leaf colour, vine internode diameter and vine internode length. Karuri et al. (2010) in his study with 89 genotypes of sweet potato observed that the general outline of leaf and the shape of central leaf lobe were the characters which allowed separation of accessions. The PCA revealed a very high value for leaf lobes type in this study, which indicates that this character has played a major role in clustering of the accessions studied.

Molecular characterisation

The present study revealed moderate level of polymorphism of ISSR primers as the selected ISSR primers provided 89.8% polymorphic gel profile and obtained an average number of 7.3 polymorphic bands per primer. A total of 80 polymorphic bands were obtained with 11 primers (Table 1). The polymorphism obtained can be considered similar with the study of Moulin et al. (2012) done on sweet potato landraces. Using 19 primers they obtained 146 bands, 92.4% of which were polymorphic and each primer generated a mean of 7.1 polymorphic fragments. A higher value of mean band number per primer was obtained by Qiang *et al*. (2009). They used 10 ISSR primers and obtained a mean band number of 13.8 per primer and a total of 124 polymorphic bands were generated.

The highest band number of 16 was obtained from UBC 808 in the present investigation. The lowest number of bands (5) was obtained using the primers UBC 827 and UBC 860. So the number of polymorphic bands in the study ranged from 4 to 13 which are in contrast to that obtained in the study conducted by Qiang et al. (2009) among sweet potato accessions where the number of polymorphic bands ranged from 7 to 17 with 10 ISSR primers and generated a total of 138 bands which is also high when compared to the present study.

Using the molecular scoring data, hierarchical clustering was done based on UPGMA using Jaccard's similarity coefficient and the 54 accessions were divided into two principal clusters and one outlier at a similarity coefficient of 0.56 (Fig. 3). Both principal clusters were further divided into many sub clusters. The first principal cluster

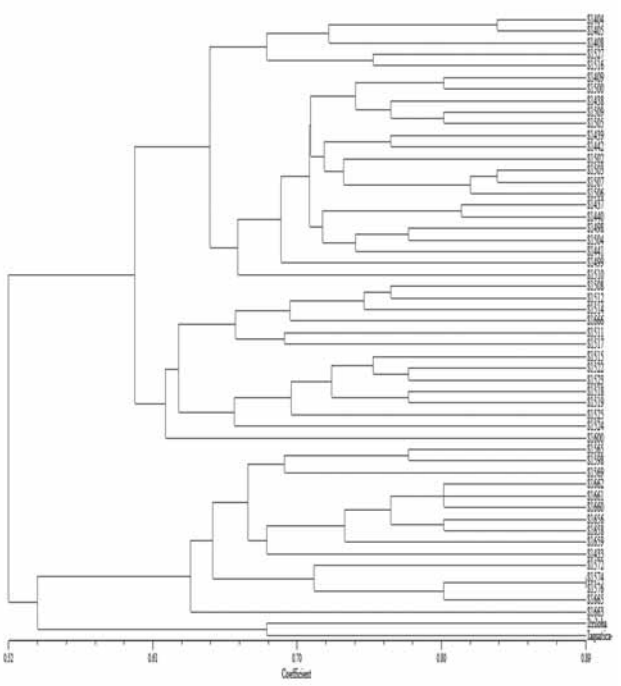

Fig. 3. Dendrogram based on ISSR data

consisted of 37 accessions in many sub clusters which indicates high intra clusteral variability indicating the variability in the sweet potato accessions selected for the study (Fig. 3). Accessions collected from the same geographical area were seen grouped together in sub clusters within this principal cluster. However, a few accessions collected from the same locality were also separated apart and found intermixed between the different clusters. The accession from the West Garo Hills,Meghalaya (S1600) was seen as an outlier within the first principal cluster. Fajardo et al. (2002) observed a high level of genetic similarity between accessions collected from the same locality. The dendrogram also indicated that some genotypes were genetically quite dissimilar from other genotypes collected from the same locale. The occurrence of these dissimilar accessions may be attributable to the outcrossing of the cultivated genotypes with sweet potato growing in the nearest area or the occurrence of an occasional off-type (introduction) within the locale (Veasey et al., 2008; Katayama et al., 2017).

The second principal cluster consisted of 15 accessions in 3 sub clusters. In this principal cluster S1574 and S1576 were grouped together (Fig. 4) with 89% similarity both from the state of Bihar. This set can be considered as the most similar accessions in the study.

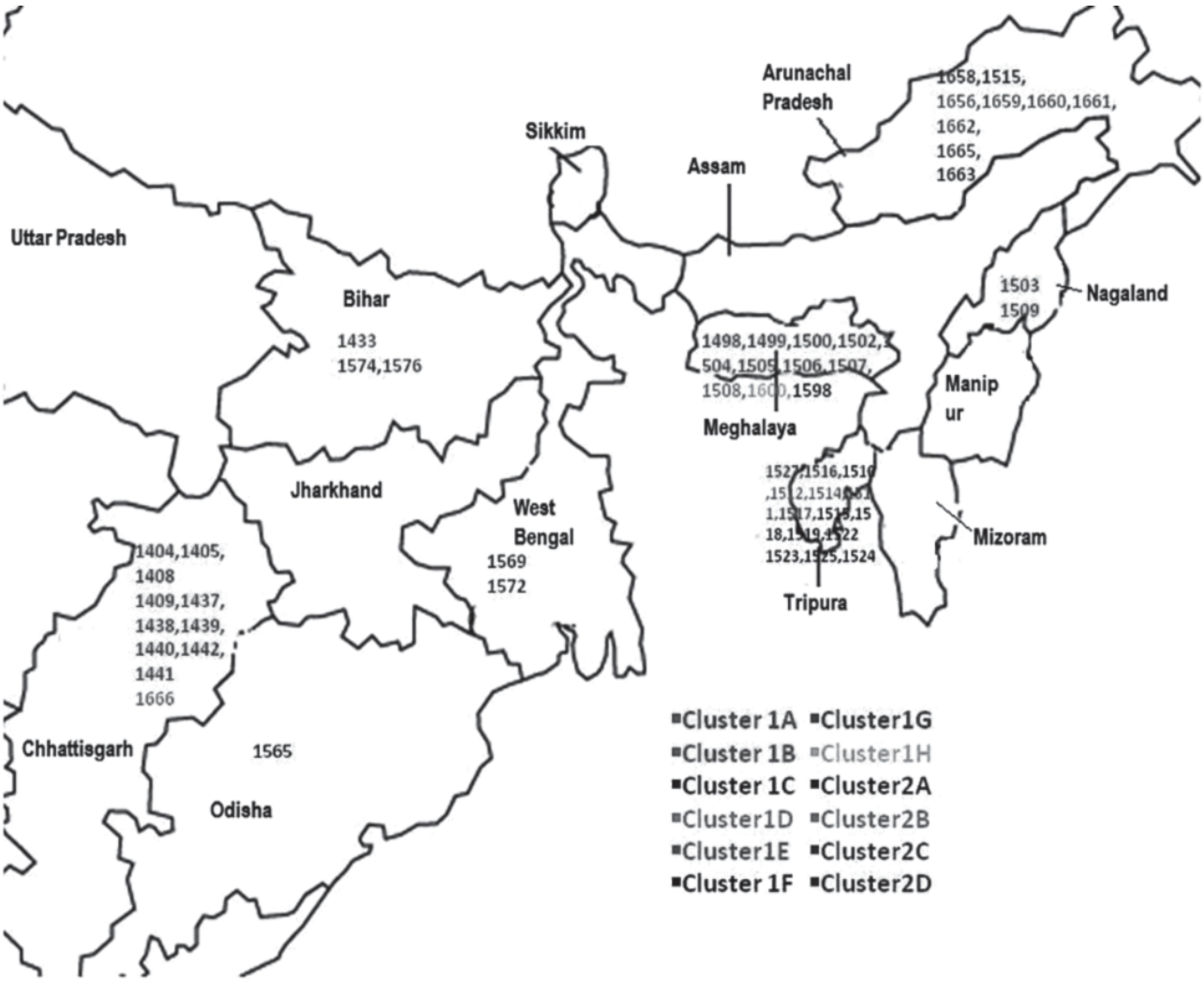

Fig. 4. Thematic map of the pattern of clustering of 54 accessions based on ISSR markers giving distribution of accessions (based on Fig. 3.)

This grouping was similar to that obtained with morphological data. Accessions collected from Arunachal Pradesh were grouped together in a single cluster (sub cluster 2B). The third principal cluster comprised of the two wild species, *I. triloba and I. aquatica*. There was lot of intra-clusteral variability within each principal cluster. Within first cluster the intra-clusteral variation ranged from 0.58-0.84. Within principal cluster 2, variability ranged from 0.63-0.89. The third cluster comprised of the two wild species. *I. triloba* and *I. aquatica* were grouped as outliers because of their difference in banding patterns compared to all other sweet potato accessions and they showed their species specificity.

One of the factors leading to variation between the accessions within landraces may be related to the mating system of sweet potato, a cross pollinating and hexaploid species (Ozias-Akins and Jarret, 1994). The intraclusteral variability between accessions can be attributed to the high incidence of random somatic mutations already reported in this crop (Hernandez et al., 1964) and anthropogenic factors such as the selection of different types and the maintenance of these within the households, eventually contributing to evolution of landraces (Veasey et al., 2008) and variability. The exchange of plants among the farmers can be considered as probably the most important factor for genetic diversity (Veasey et al., 2008) which give an explanation to the variability among the landraces in the eastern states. This intensive exchange of sweetpotato landraces between farmers, especially between neighbours and relatives, may explain the absence of defined groups in the dendrogram irrespective of the place of collection.

Genetic relationships based on similarity matrix

An estimate of genetic relationships was derived from the marker data using Jaccard's similarity coefficient. Pair-wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 89% to a minimum of 37% with an average similarity of 60% . This means the dissimilarity ranged between 11-63% with a mean diversity of 40% indicating a low to moderate diversity. The maximum similarity of 89% was observed between the accessions S1574 and S1576, both collected from the state of Bihar. The most similar accessions S1442 and S1439 obtained based on morphological data were similar by 76% as per ISSR data. The accessions S1408 collected from Chhattisgarh, S1527 collected from Lembucherra (Tripura) and S1572 collected from West Bengal were similar by only 37%. A similarity coefficient of 0.39 was observed among accessions from Chhattisgarh (S1408, S1574 and S1409) and Arunachal Pradesh (S1663), and Chhattisgarh (S1404) and West Bengal (S1572). *I. triloba* and *I. aquatica* were similar by 69%. The accession S1517 from Tripura was the most similar sweet potato accession to *I. triloba* (63%) than all the other accessions. S1512 from Tripura was similar to both *I. triloba* and *I. aquatica* by 57%. The accessions S1661 and S1662 both from Arunachal Pradesh were similar to *I. aquatica* by 57%. The low to moderate diversity observed in the sweet potato accessions may be because the accessions selected for the study included only the collections from eastern states and exchange of planting material between the farmers might be the reason for the high similarity between the accessions.

Mantel test

The Mantel statistic (r) value based on Pearson's correlation coefficient obtained as 0.0985 with a significance value (p) of 0.0003 indicated significant correlation between morphological and molecular marker information. Significant values obtained by Mantel test indicated that the morphological variability observed is genetically fixed and that the ISSR markers used could identify the variability.

Conclusion

In summary, the study indicates the existence of low to moderate genetic variability between sweet potato landraces/accessions collected from eastern states. The low variability observed might be because the collections selected for the study was restricted to eastern states only. However, the variability observed may be due to the cross-pollinating nature of the crop.

Acknowledgement

The authors are thankful to Dr Archana Mukherjee, Former Director, ICAR-CTCRI, and Dr V. Ravi, Director (Acting), ICAR-CTCRI, Sreekariyam, Thiruvananthapuram, for providing the facilities.

References

Agbore-Egbe, T. and Rickard, J.E. 1990. Evaluation of the chemical composition of fresh and stored edible aroids. *J. Sci. Food and Agri.*, **53** (4): 487-495.

Aguoru, C. U., Uhia, P. and Olasan, J. O. 2015. Varietal characterisation and taxonomic evaluation of sweet potato (*Ipomoea batatas*) using macro-and micro morphological evidence. *Libr. J.*, **2** (8): 117-121.

Allemann, J., Laurie, S.M., Thiart, S., Vorster, H.J. and Bornman, C.H., 2004. Sustainable production of root and tuber crops (potato, sweet potato, indigenous potato, cassava) in southern Africa. *South Afr. J. Botany.*, **70** (1): 60-66.

Chipungu, F., Changadeya, W., Ambali, A., Saka, J., Mahungu, N. and Mkumbira, J. 2017. Genetic and morphological diversity among sweet potato (*Ipomoea batatas* (L) Lam.) accessions from different geographical areas in Malawi. *Afr. J. Biotechnol*., **16** (22): 1285-1296.

CIP, AVRDC, IBPGR. [International Potato Center, Asian Vegetable Research and Developement Center, International Board for Plant Genetic Resources]. 1991. *Descriptors for Sweet Potato*. In: Huaman, Z. (ed.), International Board for Plant Genetic Resources, Rome, Italy. 52p.

Dellaporta, S. L., Wood, J., and Hicks, J. B. 1983. A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep*. **1** (4): 19-21.

Fajardo, D. S., La Bonte, D. R. and Jarret, R. L. 2002. Identifying and selecting for genetic diversity in Papua New Guinea sweetpotato *Ipomoea batatas* (L.) Lam. germplasm collected as botanical seed. *Gen. Resour. Crop Evol*., **49** (5): 463-470.

Fongod, A. G. N., Mih, A. M. and Nkwatoh, T. N. 2012. Morphological and agronomic characterization of different accessions of sweet potatoes (*Ipomoea batatas*) in Cameroon. *Int. Res. J. Agric. Sci. Soil Sci.*, **2** (6): 234-245.

Gepts, P., Nodari, R., Tsai, S.M., Koinange, E.M.K., Llaca, V., Gilbertson, R. and Guzman, P. 1993. Linkage mapping in common bean. *Annual report of the Bean Improvement Cooperative (USA)*.

Hernandez, T.P. and Miller, J.C. 1964. Further studies on incompatibility in the sweet potato. Proc. Amer. Soc. Hort. Sci. **85**: 426–429.

Huaman, Z. 1999. Sweet potato Germplasm Management (Ipomoea batatas).Training manual, International Potato Centre, Lima, Peru. 218p.

Katayama, K., Kobayashi, A., Sakai, T., Kuranouchi, T. and Kai, Y., 2017. Recent progress in sweetpotato breeding and cultivars for diverse applications in Japan. Breeding science., **11**(3): 16-29.

Kauri, H. W., Ateka, E. M., Amata, R., Nyende, A. B., Muigai, A. W. T., Mwasame, E. and Gichuki, S.T. 2010. Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. *Int. J. Agric. Biol.*, **12** (1): 33-38.

Khalik, K. A., El-Sheikh, M. and El-Aidarous, A. 2013. Floristic diversity and vegetation analysis of wadi Al-Noman, Mecca, Saudi Arabia. *Turkish J. Bot.*, **37** (5): 894-907.

Koussao, S., Gracen, V., Asante, I., Danquah, E. Y., Ouedraogo, J. T., Baptiste, T. J. and Vianney, T. M. 2014. Diversity analysis of sweet potato (*Ipomoea batatas* [L.] Lam*)* germplasm from Burkina Faso using morphological and simple sequence repeats markers. *Afr. J. Biotech.*, **13** (6): 729-742.

Li, Q., Liu, Q.C., Zhai, H., Ma, D.F., Wang, X., Li, X.Q. and Wang, Y.P., 2008. Genetic diversity in main parents of sweetpotato in China as revealed by ISSR markers. *Acta Agronomica Sinica*, **34** (6): 972-977.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res*., **27**: 209-220.

Moulin, M. M., Rodrigues, R., Gonçalves, L. S. A., Sudré, C. P. and Pereira, M. G. 2012. A comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces *(Ipomoea batatas* (L.) Lam.). *Acta Sci. Agron.*, **34** (2): 139-14.

Naik, S.K. and Chand, P.K. 2006. Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. *Scientia Horticulturae*, **108** (3):247-252.

Ozias-Akins, P. and Jarret, R.L. 1994. Nuclear DNA content and ploidy levels in the genus Ipomoea. *J. Ameri. Soci. Horti. Sci.*, **119** (1): 110-115.

Prakash, C.S., He, G. and Jarret, R.L., 1996. DNA marker-based study of genetic relatedness in United States sweet potato cultivars. *J. Ameri. Soci. Horti. Sci.*, **121** (6): 1059-1062.

Purseglove, J. W. 1991. Tropical crops: Dicotyledons. Longman Scientific and Technical Co published in the United States with John Wiley and Sons Inc., New York

Rao, N. K. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotech.*, **3** (2): 136-145.

Reed, B. M., Okut, N., D'Achino, J., Narver, L. and DeNoma, J. 2003. Cold storage and cryopreservation of hops (*Humulus* L.) shoot cultures through application of standard protocols. *CryoLetters*, **24** (6): 389-396.

Rohlf, F. J. 1998. *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System Version 2.02*. (Reprint, 2004). Exeter Publishing Ltd, Setauket, New York, USA, 44.

Sneath, P. H. A. and Sokal, R. R. 1973 *Numerical Taxonomy*. WH Freeman and Company, San Francisco. pp: 100-308.

Tsegaye, E., Dechassa, N., and Sastry, E. D. 2007. Genetic variability for yield and other agronomic traits in sweet potato. *J. Agron.*, **6** (1): 94-99.

Veasey, E. A., Borges, A., Rosa, M. S., Queiroz-Silva, J. R., Bressan, E. D. A. and Peroni, N. 2008. Genetic diversity in Brazilian sweet potato (*Ipomoea batatas (L.)* Lam., Solanales, Convolvulaceae) landraces assessed with micro satellite markers. *Genet. Mol. Biol*., **31** (3): 725-733.

Westman, A. L. and Kresovich, S. 1997. Use of molecular marker techniques for description of plant genetic variation. *Biotechnol. Agri.* pp: 9-48.

Yada, B., Tukamuhabwa, P., Wanjala, B., Kim, D. J., Skilton, R. A., Alajo, A., and Mwanga, R. O. 2010. Characterization of Ugandan sweet potato germplasm using fluorescent labelled Simple Sequence repeat markers. *Hort. Sci.*, **45** (2): 225-230.