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Genetic diversity in cassava based on agronomical, physiological and EST-microsatellite markers under moisture stress conditions

B.R. Ajeesh, A.V.V. Koundinya*, K.M. Senthilkumar, Vivek Hegde[1] and Kalidas Pati[1]

Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute,
Thiruvananthapuram-695017, Kerala, India.

ABSTRACT

The genetic diversity of 23 cassava accessions was studied using agro-physiological and Expressed Sequence Tags (EST)-Microsatellite markers. The genotypes were classified into six clusters based on agronomical and physiological parameters. Cluster-2 had the highest number of genotypes (10), while clusters-5 and 6 had single accession, respectively. The high-yielding and highly drought-tolerant genotype 8S501 stood alone in cluster 5. It had high cluster mean values for the majority of the traits. The genotypes present in cluster-4 & 5 and cluster-1 & 2 were divergent and hybridisation between these two groups may result in superior genotypes for drought tolerance. The EST-Microsatellites obtained from the drought transcriptome of cassava showed that the mean number of alleles per locus was 1.81, with an average size of 217.7 bp. The locus, MeESSR47 (Thylakoid membrane phosphoprotein 14 kDa, chloroplast precursor, putative), had more heterozygosity (0.387) and Shannon's Index (0.575) among all the loci. Cumulatively, these results showed low polymorphism in the EST regions of cassava DNA. The dendrogram showed 4 clusters based on EST microsatellite diversity. The microsatellite-based cluster classification of genotypes did not follow the diversity based on agronomical traits under moisture stress conditions. A weak correlation revealed by the Mantel test also indicated no relation between agronomical and molecular diversity.

Key words: *Manihot esculenta*, Drought, Agro-physiological, EST-Microsatellites Polymorphism, Genetic diversity.

INTRODUCTION

Cassava or tapioca or *yucca* or *manioc* (*Manihot esculenta* Crantz.) is a starchy root crop that grows between 30° N and 30° S latitudes across the tropical parts of the world. Cassava is the 2nd most important starchy root/tuber crop after potato, feeding billions of people. Cassava can be grown on marginal soils with limited inputs and has diverse food, feed and industry applications. Cassava, in general, is said to be a relatively drought-tolerant crop when compared with cereals, vegetables, pulses, etc. However, the drought tolerance capacity of cassava is mainly attributed to its survival rather than tuber yield through destructive mechanisms such as leaf shedding, drooping and stomatal closure (Koundinya *et al.*, 6). So, there is a need to develop cassava varieties/hybrid clones that cope with water deficit stress and produce higher yields. The phenotypic characterisation for a particular trait and selection of genotypes is of utmost importance for any breeding programme. Estimating existing genetic diversity among the available germplasm facilitates the identification of superior and diverse genotypes and is essential for selecting parents in hybridisation programmes (Pillai, 10).

*Corresponding author: koundi.hortico@gmail.com
[1]Regional Centre, ICAR-Central Tuber Crops Research Institute, Bhubaneswar - 751019, Odisha, India

Cassava is a heterozygous and vegetatively propagated crop, and its improvement is highly limited to clonal selection within genotype collections of landraces and F_1 segregation progenies. The long cropping season of 10 months, along with protogyny and asynchronous flowering, hinders and delays the conventional breeding in cassava (Hegde *et al.*, 4). In such cases, molecular markers facilitate the early detection of the presence or absence of a specific allele(s). Transcriptome/mRNA sequencing analysis under stress conditions helps identify the differential expression of a set of genes. ESTs (Expressed Sequence Tags) are the cDNA copies obtained from mRNA sequences. The microsatellite repeats present in EST sequences are from the exonic regions of DNA. EST-Microsatellite/SSR markers help differentiate the individuals based on a particular trait. The literature survey confirms that EST-Microsatellite markers have yet to be used in cassava to estimate genetic diversity. With this background, the present work "Genetic Diversity in Cassava based on Agronomical, Physiological and EST-Microsatellite Markers under Moisture Stress Conditions" has been planned with an objective to study the extent of genetic diversity among various cassava genotypes for different agro-physiological traits and EST regions.

MATERIALS AND METHODS

The study was carried out for three consecutive years, from 2017-18 to 2019-20, at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram. A total of 23 cassava genotypes were taken to assess the genetic diversity, including genotypes originating in different places and varieties released from ICAR-CTCRI. The genotypes were planted in a Randomized Block Design with three replications. In water deficit stress, the plants received rainfall for the first 3 months, which ranged from 979.3 to 980.6 mm. The stress was imposed from three months after planting up to 8 months after planting in open field conditions, during which the amount of rainfall recorded was very low (66 to 180 mm).

Observations were recorded on agronomical traits like yield per plant, harvest index, dry matter content and starch yield per plant (Sadasivam and Manickam, 13). Physiological parameters such as sprouting per cent, leaf area index-LAI, leaf retention index-LRI, closed stomata/100um^2, open stomata/100um^2, geometric mean productivity-GMP (Fernandez, 2), drought tolerant index-DTI (Fernandez, 2), susceptibility-SUS (Hossain *et al.,* 5), drought stability index-DSI (Fischer and Maurer, 3) and yield stability index-YSI (Bouslama and Schapaugh, 1) were also recorded on all the genotypes. DNA of the 23 genotypes was isolated from young apical leaves based on Saghai-Maroof *et al.* (14) method. Out of 25 primers used in this study, only 11 EST-SSR primers were amplified (Table 1). The primers were designed from the EST sequences obtained through drought transcriptome of cassava by Raji *et al.* (12). The data analysing software used were R, Darwin and GenExcel.

RESULTS AND DISCUSSION

There is a considerable variation among cassava germplasm for drought tolerance regarding tuber yield. Estimating genetic diversity among available germplasm is a prerequisite step for identifying superior clones and selecting parents for future hybridisation programmes.

The results indicated that all 23 genotypes were classified into six divergent clusters (Table 2). Cluster-2 had the maximum number of 10 genotypes, followed by clusters 1 and 3, each with 4 genotypes. Clusters-5 and 6 had a single genotype, while cluster - 4 had 3 genotypes.

All the low-yielding susceptible genotypes were present in the cluster-1. The genotypes in cluster-2 also had lower tuber yield and other drought-tolerant parameters except for two high-yielding genotypes, H-226 and 9S127. Their inclusion in cluster-2 and low-yielding genotypes could be due to similarities in other traits, such as LRI. The high-yielding and tolerant genotypes were grouped in clusters- 4 and 5. Cluster-6 had the highly susceptible genotype. The dendrogram (Fig. 1) clearly explained that the

Table 1. Primer sequences of 11 EST-Microsatellite markers.

S. No	Locus	Function	Forward Primer	Reverse Primer
1	MeESSR8	Ran GTPase binding protein, putative	ATTGAAATTGGCTTCCGTCA	AACCCCCACACCGTACAATA
2	MeESSR11	mads box protein, putative	CCCAAGGAATAAAGCCAGGT	GTTTCAGCCGAAGAACCAGT
3	MeESSR23	Ocs element-binding factor, putative	GCTGAGGTTCTGCTGGTTTC	CGGAGGATTTCACTGAGGAC
4	MeESSR34	DNA binding protein, putative	CCCCAGGTCCACTTCTCTTT	TCTTAATCTGTTGGCCCTCTG
5	MeESSR68	acetyltransferase complex ard1 subunit, putative	TTCATGATGTGGAGGCAAAG	GGCATTGGCTTGTTCTTCAT
6	MeESSR38	heat-shock protein, putative	CAGCAGTGGCAAATTCTTGA	AATCCCAAAGGCACACAAAC
7	MeESSR39	E3 ubiquitin ligase PUB14, putative	TCGTCGTGTGAGTTGTTTTCA	GGATCTTGGTTGCCGTAGAA
8	MeESSR47	Thylakoid membrane phosphoprotein 14 kDa, chloroplast precursor, putative	ACCCCGGTTTCTCGTCTAAT	CCCACCTCCATAGAGAACCA
9	MeESSR82	acyl-ACP thioesterase	CCGATCCTTTCTCCACTGAA	TAAGCAACTCATGGGCAGTG
10	MeESSR83	cytochrome P450, putative	TGGAACCTAACAATGGCGTA	TTGCCAAAACAAACTAATTCAAAA
11	MeESSR100	lipoic acid synthetase, putative	TGCCTTTCATCGTCATCATC	CGACATCACACGGAATGAAC

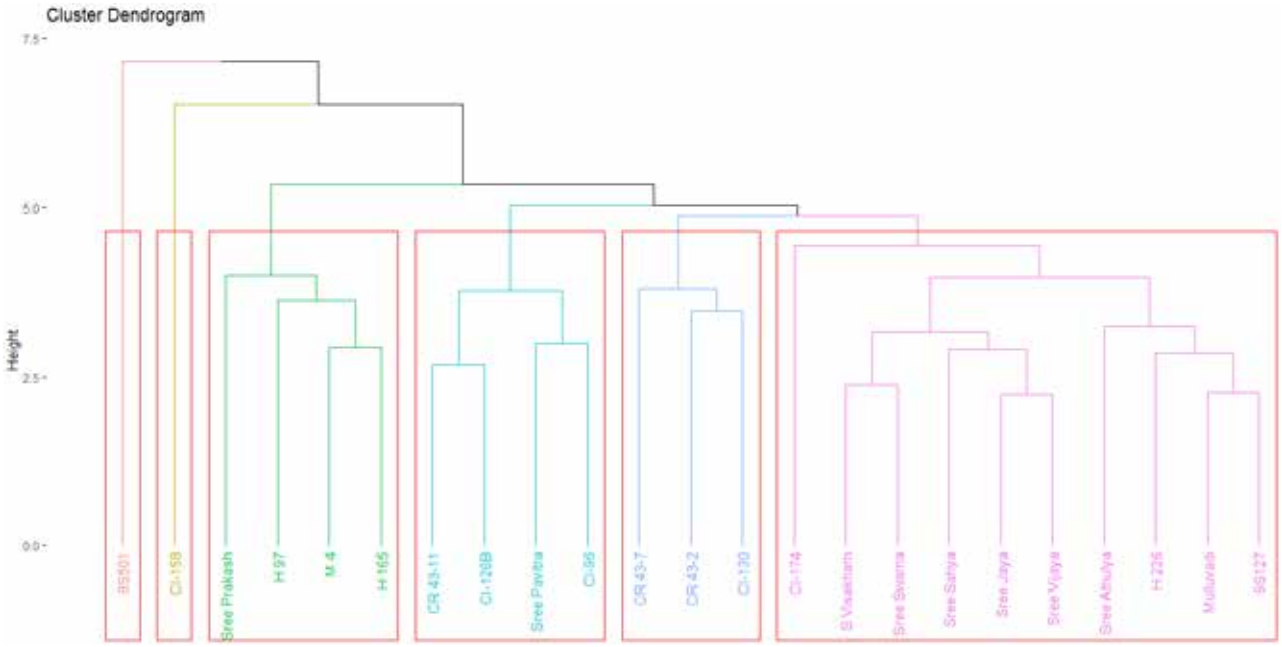

Fig. 1. Dendrogram showing cluster classification of genotypes based on agronomical and physiological traits.

Table 2. Clustering of genotypes based on agronomical and physiological traits under moisture stress conditions.

Cluster Number	No. of genotypes	Genotypes
1	4	M 4, H 97, H 165, Sree Prakash
2	10	H 226, Sree Jaya, Sree Vijaya, Sree Visakham, Sree Sahya, Sree Athulya, Sree Swarna, Mulluvadi (Local cultivar), 9S127 (Selfed progeny), CI-174 (Indigenous collection)
3	4	Sree Pavitra, CR 43-11(South American), CI-96 (Indigenous collection), CI-126B (Indigenous collection)
4	3	CR 43-7(South American), CR 43-2(South American), CI-130 (Indigenous collection)
5	1	8S501(Selfed progeny)
6	1	CI-158 (Indigenous collection)

drought-tolerant genotype in cluster-5 (8S501) was highly divergent among all the accessions, followed by the susceptible CI-158 (Cluster-6). Similarly, Pillai (10) also classified cassava genotypes into clusters.

The cluster mean values are presented in Table 3. The mean values of genotype 8S501, which was the sole genotype in cluster-5, had high mean values for all traits except for LAI, susceptibility and DSI. Cluster-6 had the highest mean values for LAI and lower susceptibility values. It also had the second-highest mean values for dry matter content and YSI. Cluster-4, comprising of the genotypes CR 43-7, CR 43-2 and CI-130, had the second highest mean values for the majority of the traits such as sprouting per cent, yield per plant, harvest index, starch yield per plant, GMP, DTI and DSI. Cluster-3 had second highest mean values for LAI, closed stomata/100um^2 and open stomata/100um^2 and LRI. The lowest susceptibility and highest DSI values were possessed by the genotypes present in cluster-1.

The perusal of the cluster mean values indicated that cluster-5, followed by cluster-4, had the highest mean values for most of the agronomical and physiological traits, suggesting the genotypes in these clusters could be selected for better performance in the moisture stress conditions. The lowest mean values for most traits were found in cluster-1 followed by cluster-2. So, the genotypes present in cluster-4 & 5 and cluster-1 & 2 were divergent and hybridisation between these two groups can be done to exploit the heterosis. Pillai (10) also identified the genotypes present in diverse clusters based on tuber yield and yield components to use them as parents in the breeding programme.

Table 3. Cluster mean values of the 6 six clusters based on agronomical and physiological traits under moisture stress conditions.

Character	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6
Sprouting per cent (%)	36.59	42.24	39.11	51.48	58.89	5.56
LAI	0.11	0.27	0.87	0.53	0.5	1.05
Closed stomata/100um^2	20.08	42.05	49.79	36.45	50.33	38.00
Open stomata/100um^2	15.66	10.20	16.35	15.12	19.67	11.50
Yield per plant (kg)	0.22	0.61	0.43	0.75	1.29	0.36
Harvest Index	0.24	0.35	0.25	0.40	0.56	0.24
Dry matter (%)	16.75	20.28	18.01	19.94	25.98	20.74
starch yield (g) /plant	19.96	52.10	27.66	77.48	115.4	30.0
GMP	0.42	0.91	0.79	1.37	1.75	0.41
DTI	0.10	0.44	0.32	0.97	1.54	0.08
Susceptibility	0.61	0.76	1.03	1.79	1.09	0.10
DSI	1.98	1.46	1.87	1.88	1.22	0.55
YSI	0.25	0.45	0.30	0.29	0.54	0.79
LORI	0.10	0.19	0.73	0.33	0.76	0.54

LAI-Leaf Area Index-LAI, GMP- Geometric Mean Productivity, DTI-Drought Tolerant Index, DSI-Drought Stability Index-DSI, YSI-Yield Stability Index, LRI-Leaf retention index

Molecular markers facilitate the detection of the presence or absence of a particular locus in the early stages of crop growth. Compared with the normal microsatellite markers, the EST-Microsatellites provide more information as they are part of the expressed sequences, and variation in these regions corresponds with the phenotypic expression. A total of 20 alleles of 11 loci were amplified among 23 cassava genotypes. The mean number of alleles per locus was 1.81, with an average size of 217.7 base pairs (bp). The allelic information is presented in Table 4. The loci MeESSR 8 (Ran GTPase binding protein, putative), 11 (mads box protein, putative), 23 (Ocs element-binding factor, putative) and 34 (DNA binding protein, putative) were found to be monomorphic with a single allele of size 175, 250, 225, 200 and 150 bp respectively. The locus MeESSR 38 (heat-shock protein, putative) had four alleles of various sizes viz., 240, 250, 260 and 280 bp. Remaining loci such as MeESSR 68 (250 and 275 bp), 39 (175 and 190 bp), 47 (145 and 150 bp), 82 (240 and 260 bp), 83 (200 and 210 bp) and 100 (176 and 203 bp) were having two alleles each.

Previously, Raghu *et al.* (11) and Sreelekha *et al.* (15) identified more alleles with UTR SSR markers, suggesting that when compared with the UTR SSR markers, the EST-SSR markers are less variable.

The allele size ranged from 145 to 280 bp. The difference between the smallest and the largest allele was found to be high for MeESSR38 at 40 bp, and the lowest difference was found for MeESSR47 (Thylakoid membrane phosphoprotein 14 kDa, chloroplast precursor, putative) at 5 bp. Ndung'U *et al.* (8) also discovered the smallest difference between the highest and lowest values of allele size was 10 bp at locus SSRY13, and the largest difference (40 bp) was detected at locus SSRY 35.

Notably, the PIC value represents the strength of markers to evaluate polymorphism in a population (Norhayati *et al.,* 9). The higher the polymorphism information content (PIC) value, the more informative the marker is (Raghu *et al.*, 11; Lyimo *et al.*, 7). In the present experiment, very low PIC values were recorded, indicating that the markers used were less informative. The overall PIC values ranged from low (0.00) to medium (0.312), with an average value of 0.149, indicating a moderate level of polymorphism for these 11 loci. Being monomorphic and having a single allele, the loci MeESSR 8, 11, 23 and 34 exhibited a PIC value of 0.00. The loci MeESSR83, followed by MeESSR82 and MeESSR100, had low PIC values of 0.146, 0.175, and 0.200. MeESSR47 (0.312) observed the highest PIC values, followed by MeESSR39 (0.280). Despite four alleles, the PIC of the locus MeESSR38 was moderate at 0.250. PIC values, in general, are highly dependent on the number of detected alleles and their distribution frequency. But, contrastingly, the locus MeESSR38 with a maximum number of alleles (4) had a moderate PIC, while the MeESSR47 with 2 alleles had the highest PIC. Raghu *et al.* (11) and Lyimo *et al.* (7) observed the highest PIC of SSR markers in cassava.

Table 4. Allelic information of 11 loci among 23 cassava genotypes.

S. No.	Locus	No. of alleles	Allele size (bp)	Frequency of allele	PIC	He	Shannon's index (I)
1	MeESSR8	1	175	1.000	0.000	0.000	0.000
2	MeESSR11	1	250	1.000	0.000	0.000	0.000
3	MeESSR23	1	225	1.000	0.000	0.000	0.000
4	MeESSR34	1	200	1.000	0.000	0.000	0.000
5	MeESSR68	2	250	0.800	0.269	0.320	0.500
			275	0.200			
6	MeESSR38	4	240	0.022	0.250	0.268	0.548
			250	0.848			
			260	0.022			
			280	0.109			
7	MeESSR39	2	175	0.214	0.280	0.336	0.519
			190	0.786			
8	MeESSR47	2	145	0.262	0.312	0.387	0.575
			150	0.738			
9	MeESSR82	2	240	0.891	0.175	0.194	0.244
			260	0.109			
10	MeESSR83	2	200	0.913	0.146	0.159	0.296
			210	0.087			
11	MeESSR100	2	176	0.864	0.207	0.235	0.398
			203	0.136			
Mean		1.81	217.7	0.550	0.149	0.173	0.280

Similar to PIC, the heterozygosity (He) and gene diversity or Shannon's index (I) values of the loci MeESSR8, 11, 23 and 34 were zero because of the monomorphic nature of the loci (Table 4). The overall mean heterozygosity value, which is the probability that two randomly selected alleles in a given accession are different, of 0.173 indicates significantly less heterozygosity in the population for these loci. Moderate to lower heterozygosity values indicated that these alleles tend to have homozygosis in the population but not to fixation as their frequencies were moderate to high except for the 210 bp allele of MeESSR83, 240 and 260 bp alleles of MeESSR38. The locus MeESSR83 (0.159) had less heterozygosity, and the locus MeESSR47 had more heterozygosity (0.387) among all the loci. From these values, it was understood that the loci MeESSR68 (0.320), MeESSR38 (0.268), MeESSR39 (0.336), MeESSR82 (0.194), MeESSR83 (0.159) and MeESSR100 (0.235) had average heterozygosity. Sreelekha *et al.* (15) and Ndung'U *et al.* (8) found similar values using SSR markers in cassava.

Shannon's information index (I) indicated the gene diversity among the screened individuals. The loci MeESSR68, MeESSR38, MeESSR39 and MeESSR47 had the 'I' value of more than 0.500, suggesting high gene diversity for these loci. However, the mean I value of 0.280 among all the loci suggested the low gene diversity among the 23 cassava genotypes used in the study. The loci MeESSR8, MeESSR11, MeESSR23 and MeESSR34 had a gene diversity of 0.00, indicating all the genotypes under study were similar to this particular locus. The locus MeESSR47 had more diversity (0.575), followed by MeESSR38 (0.548) among all the loci. Sreelekha *et al.* (15) and Ndung'U *et al.* (8) found low to moderate I value in cassava.

For a better interpretation of diversity among these 23 cassava accessions for 11 EST-microsatellite loci, an unweighted pair grouping by unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was done based on distance matrices. The dendrogram (Fig. 2), with a similarity index ranging from 0.0 to 0.1, showed the 4 major diversity clusters from the 23 cassava accessions. The fourth cluster was further classified into 4 sub-clusters, namely 4a, 4b, 4c and 4d. Previously, Raghu *et al.* (11) and Sreelekha *et al.* (15) also classified cassava accessions into clusters.

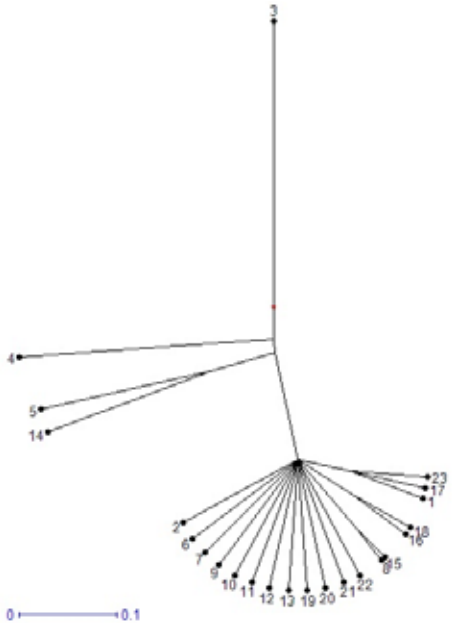

Fig. 2. Dendrogram showing cluster classification of genotypes based on EST-Microsatellite markers.

A perusal of the dendrogram and cluster classification table (Table 5) suggested that the genotype H-165 in cluster-1 was highly distant, followed by H-226 in cluster-2 and CR 43-7 and Sree Jaya of Cluster-3. Cluster- 4 had a maximum of 19 genotypes, suggesting that these 19 were almost similar to the 11 amplified loci, which were further classified into 4 sub-clusters. The genotypes in these sub-clusters are more identical to each other, such as Sree Sahya and CR 43-11 (cluster 4b), CR 43-2 and 9S127 (cluster 4c) and M4, 8S501 and CI-174 (cluster 4d).

No relationship was found when this grouping of genotypes based on EST-Microsatellite markers

Table 5. Cluster classification of genotypes based on EST-Microsatellite markers.

Cluster Number	No. of genotypes	Genotypes
1	1	H-165
2	1	H-226
3	2	Sree Jaya, CR 43-7
4a	12	H-97, SreeVijaya, Sree Visakham, Sree Swarna, Sree Athulya, Sree Pvithra, Sree Prakash, Mulluvadi, CI-96, CI-126B, CI-130, CI-158
4b	2	Sree Sahya, CR 43-11
4c	2	CR 43-2, 9S127
4d	3	M4, 8S501, CI-174

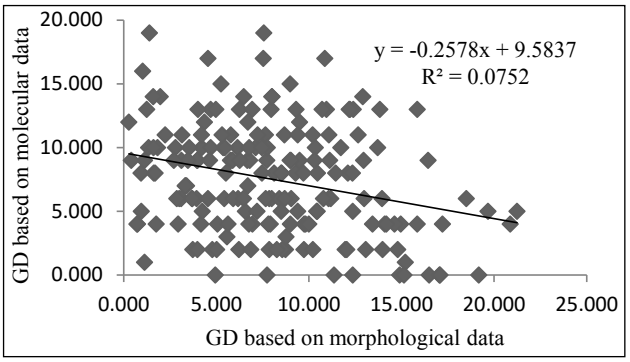

Fig. 3. Mantel test between genetic distances among the genotypes based on agro-physiological traits and EST-Microsatellites.

was studied, along with their yields under moisture-stress conditions. Because the high-yielding 8S501 (1.02 kg/plant) and low-yielding M4 (0.260 kg/plant) were grouped in the same cluster. Since moisture stress tolerance is polygenic and influenced by many factors, it is difficult to differentiate the genotypes based on 11 loci. It could be said from the above points that EST-Microsatellites are less diverse than normal microsatellites because the exonic regions of DNA are highly conservative in nature. The Mantel test also revealed no significant correlation between morphological and molecular genetic distance, as evidenced by the low R^2 value of 0.0752. This means they explained only 7.5% of the variation, and the remaining 92.5% was unexplained (Fig. 3).

AUTHORS' CONTRIBUTION

Conceptualisation of Research (AVVK, VH, KMS), Designing of the experiment (AVVK, BRA, KMS), Execution of field/lab experiments and data collection (ABR, AVVK, SKM), Analysis of data and interpretation of results (AVVK, KP), Preparation of manuscript (AVVK, VH, KMS).

DECLARATION

The authors do not have any conflict of interest.

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REFERENCES

1. Bouslama, M. and Schapaugh, W.T. 1984. Stress tolerance in soybean. Part 1: Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci.* **24**:933-37.

2. Fernandez, G.C.J. 1992. Effective selection criteria for assessing plant stress tolerance. In: *Adaptation of food crops to temperature and water stress.* Kuo, C. G. (Ed.). Asian Vegetable Research and Development Center, Shanhua, pp. 257-70.

3. Fischer, R.A. and Maurer, R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* **29**:897-902.

4. Hegde, V., Koundinya, A.V.V., Sheela, M.N., Visalakshi Chandra, C. and Mukherjee, A. 2019. Storage of Cassava Pollen for Conservation of Nuclear Genetic Diversity and Overcoming Hybridisation Barriers. *Indian J. Hortiic.***76**:104-11.

5. Hossain, A.B.S., Sears, A.G., Cox, T.S., and Paulsen, G.M. 1990. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci.* **30**:622-27.

6. Koundinya, A.V.V., Hegde, V., Sheela, M.N. and Visalakshi Chandra, C. 2018. Evaluation of cassava varieties for drought tolerance under water deficit stress conditions. *J. Root Crops* **44**:3-9.

7. Lyimo, L.D., Tairo, F.M. and Rweyemamu, C.L. 2012. Morphological and molecular characterisation of Cassava (*Manihot esculenta* Crantz) landraces using SSR markers. *Int. J. Integr. Biol.* **2**:71-78.

8. Ndung'u, J.N., Wachira, F.N., Kinyua, M.G., Lelgut, D.K., Njau, P., Okwaro, H. and Obiero, H. 2014. Genetic diversity study of Kenyan cassava germplasm using simple sequence repeats. *Afr. J. Biotechnol.* **13**:926-35.

9. Norhayati, I.S., Nasarudin, N.S., Firdaus, A.R.S., Fairuz, M.Y.M. and Azwan, J.M. 2018. Characterisation of 13 polymorphic simple sequence repeat (SSR) markers for cassava (*Manihot esculenta* Crantz). *J. Trop. Agri. Food Sci*. **46**:17-23.

10. Pillai, S.V. 2002. Variability and genetic diversity in exotic germplasm of cassava (*Manihot esculenta* Crantz) *Indian J. Genet.* **62**:242-44.

11. Raghu, D., Senthil, N., Saraswathi, T., Raveendran, M., Gnanam, R., Venkatachalam, R., Shanmugasundaram, P. and Mohan, C. 2007. Morphological and simple sequence repeats (SSR) based fingerprinting of south Indian cassava germplasm. *Int. J. Integr. Biol.* **1**:141-48.

12. Raji, A.A., Anderson, J.V., Kolade, O.A., Ugwu, C.D., Dixon, A.G. and Ingelbrecht, I.L. 2009. Gene-based microsatellites for cassava (*Manihot esculenta* Crantz): prevalence, polymorphisms, and cross-taxa utility. *BMC Pl. Biol.* **9**:118.

13. Sadasivam, S. and Manickam A. 1991. *Biochemical methods.* New Age International Publishers, Chennai p 270.

14. Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. L. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci.* **81**:8014-18.

15. Sreelekha, S., Kumar, S. and Pillai, S.V. 2010. Assessing Genetic Diversity of Indian cassava: A comparison of old and new collections using microsatellite markers. *Asian Australas. J. Plant Sci. Biotechnol.* **4**:43-52.

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