



## Genetic diversity analysis of banana using SSR markers

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### ABSTRACT

Banana, a crucial fruit crop thriving in tropical and sub-tropical regions, facing a notable challenge of elevated sterility across various cultivated banana genotypes. This sterility issue hampers the cultivation of diverse banana varieties. Therefore, a focused endeavour is crucial to thoroughly investigate the richness of diversity within both wild and cultivated banana genotypes, aiming to preserve it for future utilization. In the present study, 21 *Musa* genotypes were collected from different places of North-Eastern Region, and conserved in the field gene bank at Department of Horticulture, College of Agriculture, Lembucherra, Tripura during 2015-2022. Molecular characterization and DNA fingerprinting of these banana accessions were performed by employing 16 banana SSR markers. The number of alleles per locus ranged from 15 to 24 with an average 20.375. The expected heterozygosity (*He*) ranged from 0.564-0.967 with a mean of 0.852, and the polymorphic information content (PIC) varies from 0.638-0.945 (mean = 0.859). Genetic diversity analysis showed dissimilar groups, indicating possibility of involvement of different *acuminata* and *balbisiana* types in evaluation of banana in NE region. The outcome of the current study will be useful for designing banana crops against different stresses.

**Key words:** *Musa* spp., DNA fingerprinting, SSR markers, PIC.

### INTRODUCTION

Banana, belonging to the Musaceae family, is a popular fruit crop worldwide. The tropical region of Southeast Asia is recognized as the ancestral home of banana cultivation. India holds the top rank in global banana production, averaging an annual production of 31.5 million tonnes (Kumar *et al.* 6). Notably, bananas are an exceptional source of essential vitamins and minerals, making them a highly nutritious fruit. North-Eastern states of India have a huge range of physiographic and eco-climatic conditions and serve as the geographical gateway for different Indian flora and fauna. It is home for 17 crop species that represent 47% of the crop species diversity available in the country (Roy *et al.*, 13).

North-Eastern states of India, often called the seven sister states, serve as a significant hub for wild banana genotypes, showcasing vast natural diversity. The Indian wild *Musa* species predominantly thrives in North-East India, Eastern Ghats, Western Ghats, and the Andaman and Nicobar Islands (Hareesh *et al.*, 4). Southeast Asian species, known as *M. acuminata*, got hybridized with another species called *M. balbisiana* and produced bi-specific genomes, where *M. acuminata* contributed to the A genome and *M. balbisiana* contributed to the B genome. The genetic combinability between them led to the

development of a large spectrum of genomic groups ranging from diploid (AA and AB), triploid (AAA, AAB and ABB) to tetraploids (ABBB and AABB) (Uma *et al.*, 16).

Molecular markers serve as valuable tools in assessing genetic variation within different genotypes, significantly advancing the field of genetic analysis in crop plants. SSR markers have played a crucial role in the characterization of crop genotypes. SSR markers offer numerous advantages, such as cost-effectiveness, ability to detect multiple alleles, co-dominant inheritance, high reproducibility, widespread availability, extensive genome coverage, and high transferability to related species. SSR markers are highly beneficial for tasks such as mapping different traits, evolutionary studies, and marker-assisted selection (Ravishankar *et al.*, 11, 12).

So far, no systematic study has been conducted to characterize local and traditional banana genotypes of North-Eastern region. Hence, the current study was conducted to examine the diversity and genetic relatedness among the banana genotypes collected from Tripura and other NE states. Molecular data were analyzed and presented in the present study to understand the genetic relatedness among the collected *Musa* genotypes.

### MATERIALS AND METHODS

Twenty-one (21) banana genotypes were collected from the North-Eastern region and conserved in

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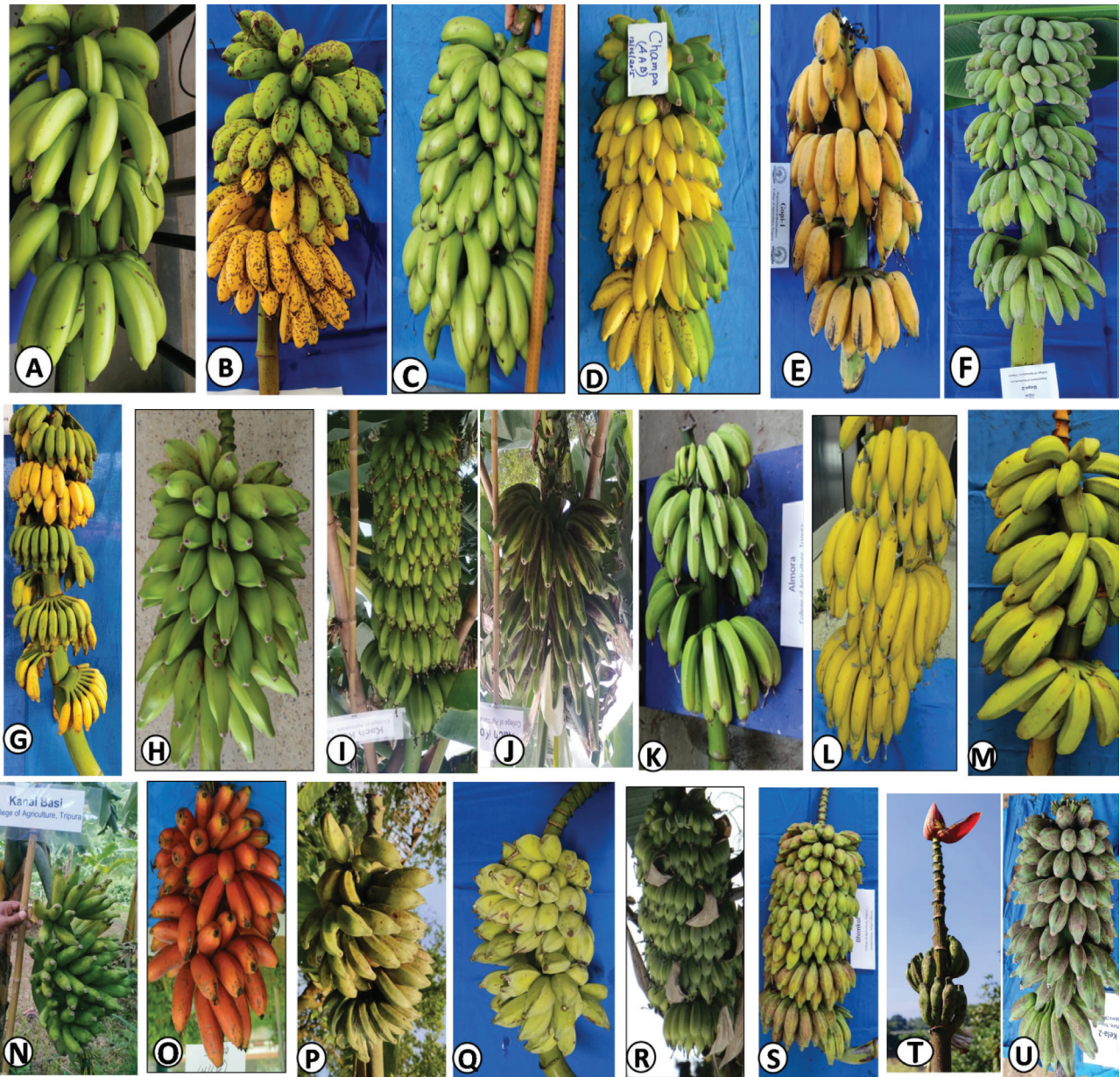
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germplasm bank at Dept. of Horticulture, College of Agriculture, Tripura (Table 1 and Fig.1). Fresh and young leaves of banana were used for total genomic DNA isolation using modified CTAB method described by Ravishankar *et al.* (9). The DNA quality and quantity were assessed through the utilization of a UV spectrophotometer (NABI, Microdigital) and agarose gel electrophoresis. Following DNA extraction, the DNA was diluted to a concentration of 20 ng/μl using sterile water and subsequently stored at 4°C. For PCR analysis, a total of 16 banana SSR markers were used and tailed with M13 sequences and labelled with fluorophores (Schuelke,

14). M13 tail was added for both forward primers (GTAAAACGACGGCCAGT) and reverse primers (GTTTCTT) at the 5' end. The PCR was conducted using a reaction volume of 20 μl. The PCR reaction mixture (20 μl) contained 4.0 μl of template DNA at a concentration of 20 ng/μl, 1 μl of each forward and reverse primer at a concentration of 5 pM, and 0.3 μL of *Taq*-DNA polymerase (3 U/μL) from Genei Pvt. Ltd. India. 2.5 μL *Taq* NA buffer A (with 15 mM MgCl<sub>2</sub>) (10X), 2 μL of dNTPs (1 mM), fluorescent probes 0.5 μL (5 pM) and 8.7 μL sterile distilled water. Fluorophores such as FAM, ATTO 550, ATTO565, and YAKAMA YELLOW, which were labelled at their

**Table 1.** Details of different local and traditional Banana genotypes including wild species collected from Tripura and other North-Eastern regions of India.

Sl. No.	Genotype/ Cultivar local name	Genome group	Place of collection/Locality	State	Geographical coordinate	
					Latitude	Longitude
1.	Sabri	AAB	S. K. Para, Dhalai dist., Tripura	Tripura	23°58.809'N	91°58.862'E
2.	Malbogh	AAB	Kahikuchi, Guwahati, Assam	Assam	26°06'19.7N	91°35'20.2E
3.	Martaman	AAB	Sachindra Nagar, Jirania, West Tripura dist, Tripura	Tripura	23°50.413'N	91°27.589'E
4.	Champa	AAB	Naradanagar, Dhamcherra, Tripura	Tripura	24°14.642'N	92°16.619E
5.	Gopi-1	ABB	Birgang, Amarpur, Gomati dist., Tripura	Tripura	23°31.753'N	91°39.968E
6.	Gopi-2	ABB	Sri Rampur, Dhalai dist., Tripura	Tripura	24°08.634'N	91°47.344'E
7.	Manipur (Gopi type)-1	AA	Iroisemba, Manipur	Manipur	24°50.352'N	93°55.736'E
8.	Kach Kela -1	ABB	College tilla, Agartala, Tripura	Tripura	23°49.312'N	91°17.425'E
9.	Kach Kela -2	ABB	Khasiamangal, Khowai dist., Tripura	Tripura	23°48.668'N	91°38.938'E
10.	Kach Kela -3	ABB	Unnokoti Tample, North Tripura dist.	Tripura	24°19.030'N	92°04.068'E
11.	Almora	ABB	Almoa, Uttarakhand	Uttarakhand	NA	NA
12.	Tripura Cavendish	AAA	Vanghmun, Jampui hill, Tripura	Tripura	23°58.577'N	92°16.731'E
13.	Raj Laxmi	AAB	Old Jay Ram Para, Gandacherra, Dhalai dist, Tripura	Tripura	23°35.542'N	91°54.674'E
14.	Kanai Basi	AA	Mahadeb para, South Taranagar, Mohanpur, Tripura	Tripura	23°57.373'N	91°22.394'E
15.	Red Banana	AAA	Netaji Palli, Mora pukur, Belonia, South Tripura	Tripura	23°15.126'N	91°28.290'E
16.	<i>Musa flaviflora</i> (wild seeded)		Mandai, West Tripura dist., Tripura	Tripura	23°48.889'N	91°33.048'E
17.	BB type -(Ram Kela-wild seeded)	BB	Kamting bari, Boromura, Tripura	Tripura	23°49.015'N	91°33.473'E
18.	BB type -(Attikol)- Manipur-2	BB	CAU Campus, Iroisemba, Manipur	Manipur	24°45.832'N	94°03.261'E
19.	BB type-(Bhimkol-wild seeded)	BB	Tuisinda, Teliamura, Tripura	Tripura	23°46.745'N	91°37.199'E
20.	Ornamental ( <i>Musa ornata</i> )		Don Bosco, West Tripura	Tripura	NA	NA
21.	Manahar Kela-2		Jorhat, Assam	Assam	NA	NA



**Fig. 1.** Pictorial view of harvested bunch of different banana genotypes: A. Sabri; B. Malbogh; C. Martaman; D. Champa; E. Gopi-1; F. Gopi-2; G. Manipur-1 (Gopy type); H. Katch Kela-1; I. Katch Kela-2; J. Katch Kela-3; K. Almora; L. Tripura Cavendish; M. Raj Laxmi; N. Kanaibasi; O. Red Banana; P. *M. faviflora*; Q. Ram Kela; R. Aitta kela 1; S. Bhimkol; T. *Musa ornata*; U. Manahar Kela-2.

5's end, were purchased from the M/s Eurofins facility in Bengaluru. The amplification process was carried out using the T100TM Thermal Cycler from BIO-RAD, California, USA. The PCR program was initiated with an initial denaturation step at 94°C for 3 min. This was followed by 35 cycles, with each cycle consisting of denaturation at 94°C for 30 min., annealing at either 50 or 60°C (as indicated in Table 2) for 1 min, and extension at 72°C for 1 min. Lastly, a final extension step was performed at 72°C for 6 min.

After a successful PCR, the products from four different primers with four different fluorescent dyes were multiplexed. Then, multiplexed PCR products were resolved using an automated ABI 3730 DNA analyzer (Applied Biosystems, USA) at M/s Eurofins facility, Bengaluru, for estimation of fragment size (bp). The precise length of the PCR product fragments was determined using peak Scanner software from Applied Biosystems, USA. The fragment size data in bp (base pairs) was used for the calculation of

**Table 2.** Genetic diversity analysis of 16 SSR makers using 21 Nos. of *banana* genotypes collected from Tripura and other North-Eastern region of India.

Sl. No.	Primer ID	Forward and Reverse sequence (5'-3')	T <sub>m</sub> (°C)	No. of alleles per locus (K)	H <sub>o</sub>	H <sub>e</sub>	PIC	PI	Reference
1.	CL-DI-11 F	GCATTTCTCCCCATGTATTTGT	55	24	0.429	0.967	0.942	0.016	
	CL-DI-11 R	ATGTAAACCGAGACCGAGTCAT							
2.	CL-DI-13 F	GGTGATGAAAGATGCTGTGCTA	60,55	22	0.524	0.854	0.927	0.029	
	CL-DI-13 R	CAACAGAAAGGGTAGGAGATGG							
3.	CL-DI-19 R	CCATTGCTAAGGGATTTAAGG	60,55	22	0.143	0.772	0.867	0.065	
	CL-DI-13 F	ATGGAGGAGTAGTTCCTTCGAC							
4.	CL-DI-31 F	GTTGCACAAATGGAACACTACGAG	55	19	0.095	0.962	0.835	0.097	
	CL-DI-31 R	CGTCTTGGGAGGAAGTAGAAGA							
5.	CL-DI-42 F	TCAGACATTGATACGGCTTCTG	55	21	0.333	0.863	0.737	0.068	
	CL-DI-42 R	ACATTAAGCAGCAGAGACACCA							
6.	CL-DI-43 F	AAGATGGCTGAAGACGAACAAT	55	20	0.286	0.564	0.638	0.041	
	CL-DI-43 R	CGCAGCTTATACCAATACATC							
7.	CL-DI-44 F	TCCTCCGTCCTTTCACATATC	55	20	0.333	0.763	0.737	0.064	
	CL-DI-44 R	TAGCTGTGGAGGCTAAGGTTTC							
8.	CL-DI-49 F	TGTTTAATACTCCCATGTTGCG	60,55	18	0.091	0.662	0.835	0.058	
	CL-DI-49 R	TTCTTCTACTACCAAGCGTCA							
9.	CL-T-6 F	ACGTTGCCGTTGTTCTCTACAT	60,55	22	0.286	0.567	0.742	0.074	
	CL-T-6 R	GGTGAGGGGAGGACATGATATA							
10.	CL-T-13 F	TCCTGAAGTACCCAGTAGAGGC	60,55	22	0.714	0.965	0.939	0.061	
	CL-T-13 R	GCCACTTATATGAAAATGCTGC							
11.	CL -T-20 F	CTCTCGAATCCTGACCCTAGC	55	20	0.381	0.962	0.935	0.062	
	CL -T-20 R	GAGAAGACAGGGACAAAGGGAT							
12.	CL-T-32 F	CGCGGAAGAAGAAGGAGA	55	15	0.238	0.933	0.904	0.064	
	CL-T-32 R	TACTTCCGCTCTTCAGATCCA							
13.	BK-DI-F36 F	CAGGTTATCATTTTCCTCGGAT	55	22	0.286	0.969	0.943	0.074	
	BK-DI-F36 R	TCATTTGCGAGCTATGTCTGTT							
14.	BK-DI-F44 F	AGATCCAGAAGGTGTTGGACAT	55	21	0.190	0.971	0.945	0.076	
	BK-DI-F44 R	CATTGACTTGTCTTGGCTGTGA							
15.	BK-DI-F57 F	ATCTTGACACTGCACTCCCTTC	55	18	0.350	0.946	0.917	0.062	
	BK-DI-F57 R	AGATGCTCTCGCGTTTACAGTC							
16.	BK-TRI-F7 F	GTGCAAGAAGAGGTAGGTGGAG	55	20	0.280	0.914	0.914	0.064	
	BK-TRI-F7 R	GGTGAGTGCATCTGAAGTAGCC							

Ravishankar et al. (10)

polymorphic information content (PIC), probability of identity (PI), expected heterozygosity (He), observed heterozygosity (Ho) and number of alleles per locus using CERVUS software ver.3.0 (Kalinowski *et al.*, 5). The SSR genotypic data were utilized for cluster analysis employing the UPGMA (Unweighted Pair Group with Arithmetic Mean) method and the construction of a Neighbor-joining (NJ) tree by using a simple matching (SM) dissimilarity matrix. The

analysis was conducted using DARwin software version 6.0.10 (Perrier and Jacquemoud Collet, 8), and in population structure analysis, the number of subgroups (K) was set from 2 to 10. For each K, 10 iterations were performed. The project parameters consisted of a burn-in period of 10,000, followed by 100,000 Monte Carlo Markov Chain (MCMC). The  $\Delta K$  value was calculated using the method described by Evanno *et al.* (3), which involves assessing the rate

of change in the log probability of the data between successive K values.

## RESULTS AND DISCUSSION

Genetic analysis of 16 SSR markers has been generated by screening 21 North-Eastern banana genotypes, which detected a total of 326 alleles among the studied genotypes with an average value of 20.375 alleles per locus. In an earlier study, a similar range of observations was also found by Christelova *et al.* (2), where 21.5 alleles per locus were detected by using 19 SSR markers.

The North-Eastern region of India is widely recognized as a significant hotspot for plant biodiversity, particularly known for its rich biodiversity of citrus, mango, and banana species (Sankaran and Dinesh, 15). The region is renowned for hosting a wide variety of cultivars and wild relatives of several fruit crops, making it a vital hub for their genetic resources and their conservation efforts. In our current study, we observed a remarkably high level of polymorphism information content (PIC) for the SSR markers used in the study. The PIC values ranged from 0.638-0.945, with an average of 0.859. The average PIC value of 16 SSR markers was greater than 0.5, indicating a higher discriminating power of the analyzed loci. A similar range of PIC values was also reported by earlier researchers (Christolova *et al.*, 2; Workneh *et al.*, 17).

In the present study, the expected heterozygosity (He) ranged from 0.138-0.934 with an average of

0.852, and the average observed heterozygosity (Ho) was 0.309. For all the markers used, the observed heterozygosity showed low values to the expected heterozygosity indicating a higher level of homozygosity. The observed lower range of heterozygosity in the studied banana genotypes may be due to the low level of outcrossing. Oriero *et al.* (7) and Quain *et al.* (9) have reported the average observed heterozygosity of 0.75 among 40 *Musa* germplasm, which was higher than the value detected in the present study.

Cluster analysis of the SSR data showed three distinguished groups in UPGMA method, based on their close genetic relatedness (Fig. 2). The cluster I consisting of nine genotypes were of BB-type (Atikol), BB-type (Bhimkol), BB-type (Ramkela) with BB genome, Kach Kela-1, Kach Kela-2, Kach Kela-3, Gopi-1, Gopi-2 and Almora with ABB genome, Likewise Cluster II had eight genotypes *viz.*, Sabri, Maratman, Champa, Malbhog, Raj Laxmi with AAB genome, Manipur-1, Kanai Basi with AA genome and Mizo Cavendish with AA genome. Similarly, cluster III had four genotypes, namely, Manhar Kela-2 and Red Banana, and it also included *M. flaviflora* and ornamental banana species. Similarly, the population structure analysis revealed that  $\Delta K$  was 2.4 for K3. Therefore, the optimal  $\Delta K$  for K3 showed that the best-fit model for the sampled 21 banana genotypes revealed three clusters denoted with three different colours (blue, red and green) (Fig. 3a). These

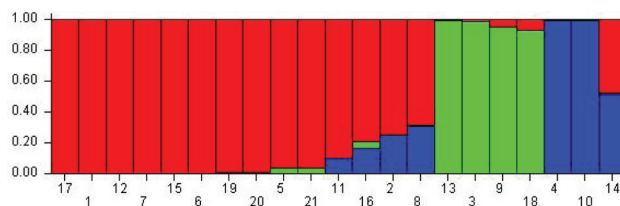


Fig. 2. Dendrogram of *Musa* genotypes derived by hierarchical UPGMA method.

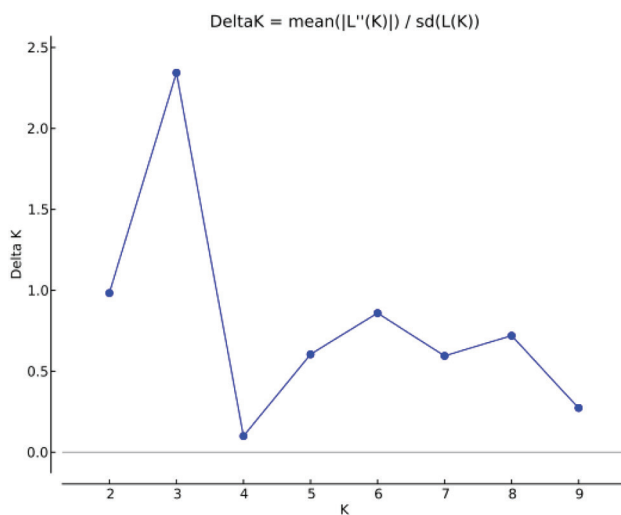
21 genotypes formed a clear separation of three groups, between different local and traditional banana genotypes, collected from North-East region. The structure analysis revealed that among the groups, cluster-I had maximum (66%) genotypes distributed across Tripura, Assam, Manipur and Uttarakhand and also included two different species of the banana such as *M. ornata* (ornamental) and *M. flaviflora* (wild-seeded). The cluster I (Fig. 3a red colour) had genotypes with genome AAB, BB, AAA, AA and ABB; Cluster II (green colour) had BB, AAB and ABB type, whereas Cluster III (blue colour) had AAB and ABB genome along with a genotype Kanai Basi with AA genome found as admixture.

Population structure analysis using a molecular data set obtained from 16 SSR markers, we found that cluster I had around nine (9) banana genotypes (Fig. 3a) from Tripura, Uttarakhand and Manipur, having BB and ABB genomes. In contrast, cluster II consisted of eight banana genotypes from Tripura, Assam, and Manipur, which have genomes of AAB, AAA, and AA. Cluster III consisted of *M. flaviflora* and ornamental species of banana collected from Tripura. In an earlier study structure analysis, Biswas *et al.* (1) also showed the distinct clustering of the 50 banana germplasm accessions according to their genomic background. In the current investigation, the UPGMA cluster analysis failed to group the genotypes based on their genome constitution (AB, AAA, AAB and ABB) or geographical origin. Regardless of their genomic constitution or geographical origin, the genotypes were found to be intermingled in all clusters irrespective of their genome constitution. The current findings agree with the earlier findings of Workneh *et al.* (17).

The absence of distinct clustering based on geographical origins among the banana genotypes could be attributed to the introductions of cultivars followed by their migration to areas beyond their initial origins. The main objective of the present investigation was to primarily study the genetic variation among the banana genotypes collected from the North-Eastern region of India. SSR marker-based surveillance among the studied genotypes revealed the existence of a significant amount of genetic diversity in the local and traditional banana genotypes of the North-Eastern region of India. Hence, comprehending the genetic variation among and within genomic groups plays a vital role in unravelling evolutionary history. The outcome of this research will offer valuable insights for future breeding and conservation approaches concerning diverse local and traditional banana varieties found in the North-Eastern Region of India.



**Fig. 3a.** Bar plot of the POPULATIONSTRUCTURE analysis showing the partitioning of 21 banana genotypes into ( $K = 3$ ) three colored segments that designate the population's estimated membership fraction in to the inferred subgroups. Red colour (cluster I), green colour (cluster II), blue colour (cluster III).



**Fig. 3b.** Structure harvester analysis of banana genotypes showing; Delta K high probability of three (Delta K = 3).

## AUTHORS' CONTRIBUTION

Conceptualization of research (SCD, PD, RS and US); Designing of the experiments (SCD, PD); Contribution of experimental materials (SCD, PD); Execution of field/lab experiments and data collection (SCD, PD, AK, PP, SS, DP); Analysis of data and interpretation (PD, PP); Preparation of the manuscript (PD, AS, DP, SCD).

## DECLARATION

The authors declare that they do not have any conflict of interest.

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