



Bharat Moni: A promising *Musa* cultivar of Assam

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ABSTRACT

Bharat Moni cultivar of *Musa* has been reported as indigenous to Assam, India which is less exploited so far. It is one of the most liked cultivars of banana due to its unique taste and flavour. Its male buds and the pseudostem are also eaten and liked by the local people. In the present study, the morphological and molecular characterization of Bharat Moni cultivar of *Musa* sp. collected from four districts, i.e., Biswanath, Charaideo, Dibrugarh and Lakhimpur of Assam, India were carried out. The morphological study was carried out as per the 'Descriptors for banana (*Musa* spp.) International Plant Genetic Resource Institute'. The molecular characterization was done using the genomic DNA isolated from its cigar leaves. Bharat Moni banana was found to be of AAA group as per the authentic score cards system and confirmed with phylogenetic tree analysis of the DNA sequence. Though it takes comparatively longer harvesting time (480-495 days), it fetches a higher market value and people's preference than other popular cultivars. The fruits of Bharat Moni are very sweet in taste and delicious, with a characteristic aroma and unique peel characteristic in terms of adherence to the pulp. The average yield was found to be 13-15 fruits per hand in a lax pattern. The average weight of a bunch with 5-7 hands of fruits was 13 to 14 kg and single hand weighed 1.7 to 1.9 kg approximately. It is the most preferred and promising banana cultivar of horticultural importance in Assam, India; thus, large-scale cultivation under optimum conditions may enhance state's economy considerably.

Key words: *Musa* spp., morphological characterization, molecular features, genome grouping.

INTRODUCTION

The name 'Banana' signifies a unique identity as a fruit consumed worldwide. To date, 82 species of banana with more than 1000 cultivars and varieties under the genus *Musa* have been reported (Gurumayum *et al.*, 5). North-eastern region (NER) of India is considered as a banana hub comprising about 27 species of wild *Musa*, contributing to 33% of total banana diversity. Banana, known for its antiquity, is an important fruit crop having great socio-economic significance, as it supports the livelihood of millions of people worldwide. *Musa* cultivars have immense economic importance, especially as a staple source of energy food for the rural poor in the tropical and subtropical regions of Asia, Africa and America. In developing countries, bananas rank fourth after cereals as important sources of food (Ahmed *et al.*, 1). Apart from effective sources of energy, banana is a good source of carbohydrates and potassium. The banana diversity (*Musa* spp.) in NER of India is notably rich including several traditional varieties such as Bharat Moni, Cheenichampa, Malbhog, Sabari, *etc.* known for their unique fruit qualities like flavour, aroma, taste, *etc.* Most of these resources from NER are still non-descript and mostly grow in home gardens and in the wild.

Bharat Moni, an indigenous *Musa* cultivar, is one of the important banana varieties of Assam, India. This banana cultivar is acquiring popularity in nearby states due to its unique features like its sweet taste, pleasant aroma and flavour (Rajappa *et al.*, 11). The fruits of this variety are thick and pulpy but comparatively shorter, with a thin yellow peel and yellowish pulp when ripe. In addition to fruits, their pseudo-stem and inflorescence are also sold in the market at a handsome price and consumed. Medhi (8) reported that Bharat Moni cultivar is a slow-growing species that takes a period of 491 days to harvest, with bunches weighing about 13.37 kg. The height of plant reaches typically to approx. 230 cm with girth as 46.67 cm, respectively. The Bharat Moni cultivar has shown a minimal distribution range, as reported only from Assam as a cultivated variety; thus, a systematic study is essential to explore its performance in and outside the state. Most of the seedless banana fruits are reported to be auto- and allopolyploid hybrids and cultivars of the two wild species, *Musa acuminata* with "AA" genome and *M. balbisiana* with "BB" genomes. Polyploidy and hybridization of A and B genomes have given rise to diploid (AA, AB, BB), triploid (AAA, AAB, ABB, BBB) and tetraploid (AAAA, AAAB, AABB, ABBB) bananas. The Bharat Moni cultivar of banana was earlier reported as ABB genome group by

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Mohapatra *et al.* (9), which was later revised by Uma *et al.* (17) to AAA. Very few reports mention a few morphological features of the Bharat Moni banana cultivar; however, no data on complete morphological and molecular characterization are available so far. Thus, the main aim of the present study is to present a complete report on the morphological and molecular characterization of the Bharat Moni cv. of *Musa* sp.

MATERIALS AND METHODS

For the collection and morphological characterization of Bharat Moni cv. of *Musa* sp., several locations in Assam were surveyed. Samples were collected from five places in four districts of Assam namely, Biswanath (Rajgarh), Charaideo (Kathiakhunda), Lakhimpur (Rangpuria and Khoga) and Dibrugarh (Namrup) (Fig. 1). The complete morphological characterization had been carried out by following the 'Descriptors for banana (*Musa* spp.) - International Plant Genetic Resource Institute (IPGRI)' (6).

The genome group classification of the Bharat Moni *Musa* cultivar was carried out based on the modified genome scorecard (Singh and Uma, 15) system. For genome classification, 15 important morphological characters of banana cultivars were

used. There are mainly two types of banana genome: AA genome (*Musa acuminata*) and BB genome (*Musa balbisiana*). Score 1 was credited for characters resembling *M. acuminata*, and 5 for characters resembling *M. balbisiana* (Table 1). The fresh cigar leaves of Bharat Moni plant were used for DNA isolation and amplification. The leaves were collected in cetyltrimethylammonium bromide (CTAB) storage buffer following standard methodologies (Saghai-Marouf *et al.*, 12) for further use. The isolation of DNA was carried out using TENS buffer as lytic reagent following simplified SDS method (Chiong *et al.*, 3) from the cigar leaves of banana. The sample was treated with PCI and centrifuged (13000 rpm) for 10 min. The supernatant was treated with enzyme RNase and precipitated with ammonium acetate and chilled absolute ethanol by centrifuging (10000 rpm for 8 min). The precipitate was dehydrated with 70% ethanol and again centrifuged (10000 rpm) for 5 min. The precipitate so obtained was dried in room temperature and dissolved in TE buffer and for further analysis, stored at 4°C. The genomic DNA obtained in the above step was subjected to PCR of ITS region using BIO RAD T100 thermal cycler with reaction mix of total 25 µl volume [2.5 µl 10x Taq. buffer with MgCl₂, 0.5 µl dNTPs (10mM), 0.5 µl of ITS1 and ITS4 10 pmol each, 1 µl of 1u/µl Taq. Polymerase, 2 µl genomic DNA and sterile water].

The Sanger method was used to sequence the PCR product at Eurofins Genomics India Pvt. Ltd., Bengaluru, India.

RESULTS AND DISCUSSION

The detailed morphological characters of Bharat Moni banana cultivar (Fig. 2) carried out as per the 'Descriptors of Banana (*Musa* spp.)' revealed the following: The root system of Bharat Moni banana was comprised of rhizome, suckers and fibrous roots. Its fibrous roots have an approx. 2 feet deep penetration which is less dense.

The pseudo-stem height of the cultivar measured between 2.2 to 2.6 m. It is dull green from the outside, while the underlying pseudo-stem was green with pink-purple pigmentation and had watery sap. The petiole base bear sparse blotches of brown-black, and the suckers were close to the parent and 3-5 in number. The height of the suckers was between 1/4th to 3/4th of the parent plant. The leaf habit of the plant was intermediate, with the central leaf erect and the others half drooping. The petiole canal of the third leaf was wide, having erect margins. The petiole margin was winged and undulating, and the colour of the petiole margin was green. The petiole margin edge had a colour line along it. The length and width of the leaf blade ranged from 153-178 cm

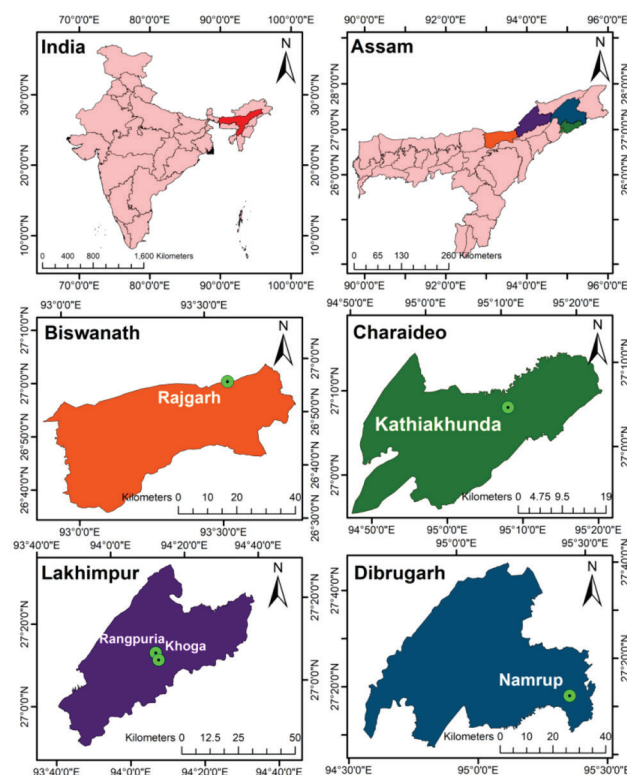


Fig. 1. The sites of Bharat Moni banana sample collection.

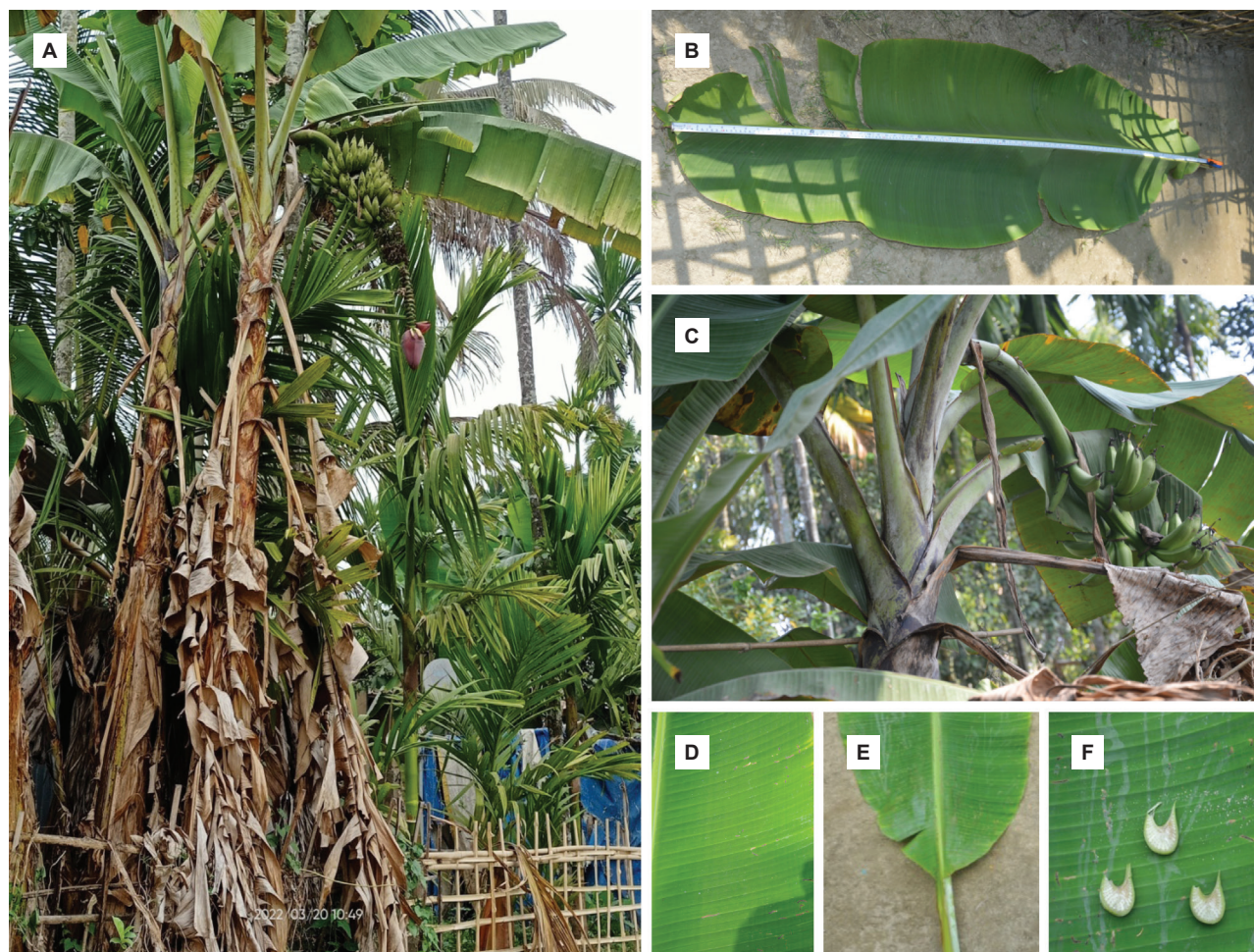


Fig. 2. Morphological characterization of different parts of Bharat Moni cultivar of *Musa* sp. [A- Complete plant, B- Leaf lamina, C- Blotch pattern, D- Leaf corrugation, E- Leaf base, F- Petiole canal].

× 58-69 cm, respectively, with very little wax. The upper surface of the leaf was dark green, while the lower surface was medium green in colour with leaf corrugation of a few strips. The insertion point of the leaf blades on the petiole was symmetric; they are symmetrically pointed in shape at the leaf base. The colour of the outer surface of the cigar leaf was green. The inflorescence characteristics of Bharat Moni are presented in Fig. 3.

The banana bunch includes the fruits, rachis and the male bud. The bunch position was slightly angled from the pseudo-stem and cylindrical in shape with a lax appearance so that one could put his hand in between two banana hands. The peduncle was green and hairless, bearing one empty node. The bunch of fruits had 5-7 hands bearing 13-15 fruits on the third hand. Rachis was present with persistent male bud, while its position was also angled with a few hands of neutral flowers. The male bud of Bharat Moni banana cultivar was rounded in shape and normal type. The

size of the male bud at the time of harvest ranged from 18-28 cm; the male bract was ovate, lifting one at a time. The bract base shape was medium with an intermediate apex. Bract imbrication was the older bracts overlaps at the apex of bud. The bract external face was purple-brown in colour, and the internal face was orange-red; it used to have tinted with yellow and a discoloured line in the apex. The bract was revolute and had very little wax with moderate grooving.

The female flowers appeared cream in colour with rust-coloured spots on the compound tepal. A well-developed lobe of the compound tepal was yellow. The oval-shaped free tepal had a triangular apex and was translucent white. The white-coloured filament bears an undeveloped anther. The outward-angled style was cream in colour without any pigmentation. The shape of the ovary was arc-like without any sign of pigmentation, bearing a four-rowed ovule arrangement. The fruit development from female flowers takes about 35 to 40 days.

Table 1. Scoring for genome grouping of Bharat Moni cultivar of *Musa* sp. against the characters with *M. acuminata* and *M. balbisiana*.

Character	<i>Musa acuminata</i>	<i>Musa balbisiana</i>	Bharat Moni	Genome score
Pseudo-stem colour	More or less heavily marked with brown or black blotches	Blotches very slight or absent	Sparse blotches, Brown black	1
Petiole canal	Margin erect or spreading with scarios wings below, not clasping pseudo-stem	Margin enclosed not winged but clasping pseudo-stem	Erect with wide margin, winged and undulating	1
Peduncle	Usually downy or hairy	Glabrous	Hairy	1
Pedicel	Short	Long	Short	1.5
Ovule	Two regular rows in each loculus	Four irregular rows in each loculus	Two ovules per locule	1
Bract shoulder	Usually high (ratio<0.28)	Usually low (ratio >0.30)	Ratio 0.27	1
Bract curling	Bracts reflex and roll back after opening	Bracts do not reflex	Revolvute	1
Bract shape	Lanceolate or narrowly ovate tapering sharply from the shoulder	Broadly ovate, not tapering sharply	Ovate	2.2
Bract apex	Acute	Obtuse	Acute	1
Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish purple outside; bright crimson inside	Purple brown outside; orange red inside	2.5
Colour fading	Inside bract colour usually fades to yellow towards the base	Inside bract colour usually continuous to base	Bract apex tinted with yellow	1
Bract scars	Prominent	Scarcely prominent	Very prominent	1
Free tepal of male flower	Variably corrugated below tip	Rarely corrugated	More or less smooth	2
Male flower colour	Creamy white	Variantly flushed with pink	Yellow	1.5
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink	Orange	1
Total Genome score				19.7

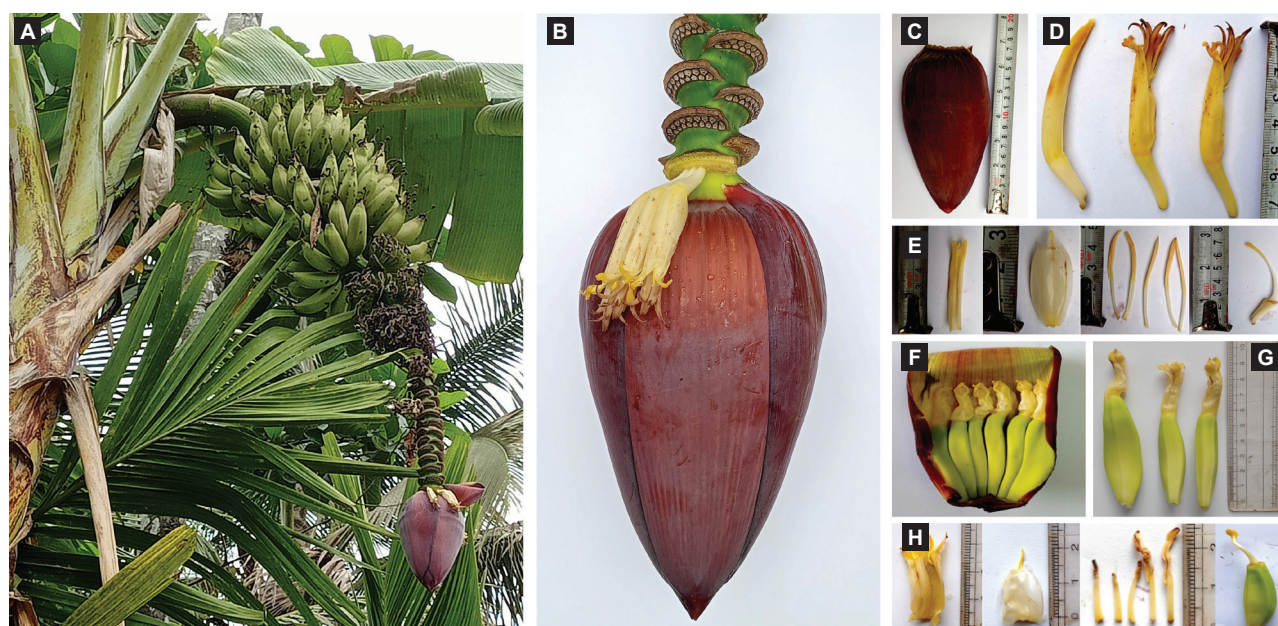


Fig. 3. Parts of the inflorescence of Bharat Moni cultivar of *Musa* sp. [A- Complete bunch, B- Male bud, C- Bract, D- Complete male flower, E- parts of male flower, F- Bract with female flower, G- Complete female flower, H- parts of female flower].

The dominant colour of the male flower was cream, just like the female flower. The colour of the compound tepal of male flowers was cream with spots of rust colour. The compound tepal lobe was yellow with a well-developed lobe. The oval-shaped free tepal was translucent white with a smoother appearance and a well-developed triangular apex. White filaments with cream-coloured anther bear cream pollen sac. The primary colour of the style was cream without any pigmentation and straight. The arch-shaped ovary was also cream-coloured with no pigmentation and had a four-rowed ovule arrangement. The male flowers fall after the bract.

The length of the fruit ranged from 15–18 cm and was straight in shape, curved towards the stalk in position. The apex of the fruit was lengthy and pointed with a persistent style as remains of flower relicts. The fruit pedicel length was 9-14 mm, and the pedicel was very partially fused. The colour of the immature fruit peel was dark green, which turn yellow with maturity. The fruit peel was adherent to the pulp, and not easy to peel. There was no visible sign of a crack in the fruit peel, and the colour of the matured fruit pulp was yellowish cream. No seed was observed in the fruit, and the predominant taste was sweet, with a soft pulp texture. The bunch of fruit weighed 13 to 14 kg, and the single hand weighed 1.7 to 1.9 kg (Fig. 4). Genome grouping was carried out based on morphological characterization. Table 1 shows the scoring details against the contrasting characters considered for the genome group. From the modified scorecard system

(Table 2), the total genome score for the Bharat Moni cultivar was obtained as 19.7; thus, it came under the AAA genome group as per all three scorecard systems.

The isolated genomic DNA appeared as sharp band with a ratio of absorbance at 260 and 280 nm approx. 1.7 purity. The DNA was sequenced by Sanger method at Eurofins Genomics India Pvt. Ltd., Bengaluru, India and analysed further. The data was analysed using National Center for Biotechnology Information - Basic Local Alignment Search Tool (NCBI-BLAST) program as described by Zhang *et al.* (18). The sequenced data showed a percentage identity of 99.14 with *Musa* ABB group and 96.11 with *M. acuminata*. The accession number assigned by GenBank for the sequence submitted is PP869179.

Table 2. Modified score card system for assigning tentative genomic groups to banana.

Genome	Score card		
	Simmonds and Shepherd (14)	Silayol and Chomchawlo (13)	Singh and Uma (15)
AA/AAA	15-23	15-25	15-25
AAB	24-46	26-46	26-45
AB	49	-	46-49
ABB	59-63	59-63	59-65
ABBB	67	-	66-59
BB/BBB	-	70-75	70-75



Fig. 4. Bunch of fruits of Bharat Moni cultivar of *Musa* sp. [A- Fruit bunch, B- matured fruit hand, C- ripe fruit hand, D- Single fruit, E- T.S. of ripe fruit, F- L.S. of ripe fruit].

The Maximum Parsimony method was used to infer the evolutionary history. Tree #1 out of 9 most parsimonious trees (length = 0) is shown. The consistency index is 0.994302 (0.993197), the retention index is 0.998174 (0.998174), and the composite index is 0.992486 (0.991383) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 4). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 10) with search level 1 in which the initial trees were received by the random addition of sequences (10 replicates). This analysis involved 12 nucleotide sequences. There were a total of 1015 positions in the final dataset. Evolutionary history analyses were conducted in MEGA11 (Tamura *et al.*, 16). The tree constructed in MEGA 11 was edited with the help of the online tool 'Interactive Tree Of Life' (iTOL) (Letunic and Bork, 7), a web-based tool that offers display and annotation of phylogenetic trees. In the phylogenetic tree Bharat Moni grouped with *M. acuminata* ITS 1 with a higher bootstrap of 0.86, confirming this to be triploid AAA type. The phylogenetic tree (Fig. 5) also revealed that the selected cultivar Bharat Moni is different from the other popular cultivars of the region.

The Bharat Moni cultivar of banana is an indigenous variety of Assam, India and is known for its unique sweet taste and pleasant aroma of the fruit. The local people identify it by the presence of reddish pigmented petiole with sparse blotching as compared to Cheenichampa cultivar. In the present study, it was found that the height and girth of the Bharat Moni cultivar ranged from 2.2 to 2.6m and 45-49cm, respectively. Earlier, Medhi (8) also reported a similar height and circumference of 2.3 m and 46.67cm, respectively. However, some other popular banana cultivars reported to have plant

height and plant girth viz., Cheenichampa, Malbhog, Kachkal 2.53m and 49.35 cm; 2.28 m and 48.50 cm and 2.78 m and 51.17 cm, respectively. In another evaluation study conducted by Baruah *et al.* (2) in Assam, it was recorded that the highest pseudo-stem height (272.25 cm) was found in Champa (Mysore), followed by Kachkal (271.25 cm). During this study, it was observed that the Bharat Moni banana is slow-growing and takes a more extended time period of about 480-495 days to harvest the fruit, which is in a similar line to the reports of Medhi (8), *i.e.*, 491 days. In contrast, Cheenichampa and Malbhog take lesser time of 376 and 351 days, respectively. The bunch weight of Bharat Moni banana ranged from 13-14 kg, in similarity with Medhi (8), who also reported it as 13.37 kg as compared to 14.19 kg and 15.35 kg in Malbhog (Silk) and Kachkal (Cooking type) cultivars, respectively and Cheenichampa and Bhatmanohar as 12.5 kg and 12 kg (Baruah *et al.* 2). The fruit peel character after ripening of the fruit is found to be unique in Bharat Moni banana. It is not easy to peel, unlike other popular cultivars. The pedicel of the fruit adheres to the peel after ripening, which makes it difficult to detach easily. As a result, pulp also comes out along with the peel, resulting in a shorter shelf life of ripe fruits. The number of fruits per hand is also found to be much lesser in Bharat Moni (5-7) in comparison to other cultivars like Malbhog (6-8), Jahaji (6-8) and Cheenichampa (9-11) *etc.*

Similarly, the number of hands developed in a fruit bunch is also lesser. The pleasant and sweet taste of the Bharat Moni banana makes it a very popular and the most preferred banana variety amongst local people; however, its lesser number of fruits per bunch and slow-growing rate make it commercially less exploited. Further studies on increasing the fruit yield, as well as lessening the harvesting time, may enhance the commercial viability of this popular cultivar. The fruit bears persistent style as flower relicts at the

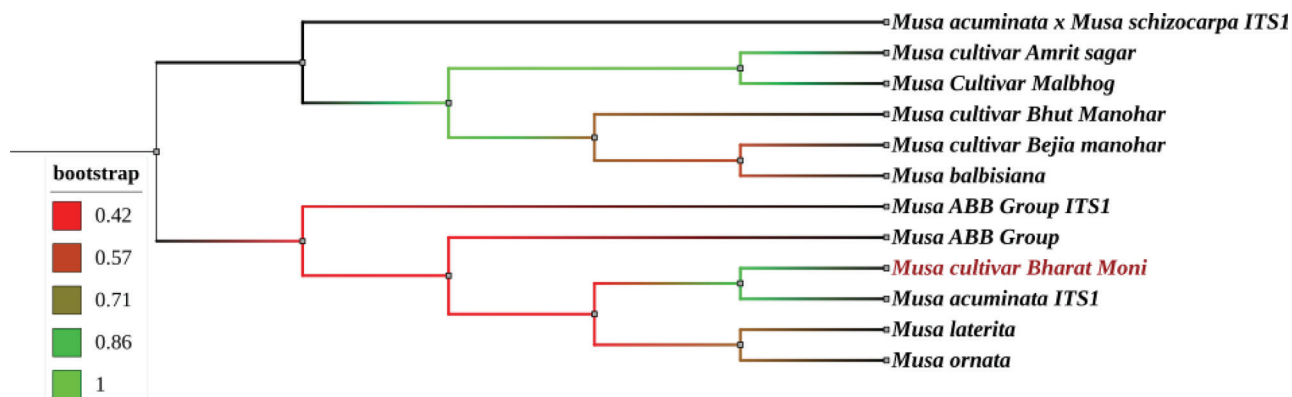


Fig. 5. Cladogram of Bharat Moni banana cultivar as generated in MEGA11.

apex, unlike Malbhog and Chenichampa. The present study confirms that Bharat Moni cultivar comes under the AAA genome group of banana cultivars as per the scorecard system and the taxonomic description is very similar to those reported by Uma *et al.* (17) and contradictory to the reports of Mohapatra *et al.* (9). The molecular characterization study confirms that Bharat Moni cultivar of *Musa* sp. showed the maximum percentage identity with the ABB group of *Musa* sp. (99.14 %) and 96.11 with *M. acuminata* and the phylogenetic tree indicated its separate origin. In the phylogenetic tree Bharat Moni grouped with *M. acuminata* ITS 1 with a higher bootstrap of 0.86, confirming this to be triploid AAA type. The present study is the first detailed documentation on the morphological and molecular characterization of Bharat Moni cultivar of *Musa* sp. The peel character of the fruit while ripening makes it unique and different from others. The cultivar belongs to the AAA genome group as per the scorecards, which was also confirmed by the phylogenetic tree analysis with the Maximum Parsimony method of the sequence. The survey revealed that Bharat Moni cultivar of banana is one of the most liked cultivars of the region due to its very sweet unique taste and pleasant aroma. Thus, large-scale cultivation under optimum conditions may affect and enhance the socio-economic status of the farmers and the state as a whole; therefore, it needs to be explored for large-scale cultivation with a faster growth rate. Consequently, it may be concluded that the Bharat Moni cultivar of banana is an up-and-coming horticultural crop of the region.

AUTHORS' CONTRIBUTION

Conceptualization of research (KS, BJS); Design of the experiment (KS, BJS, SSS); Contribution of experimental materials (KS); Execution of field/lab experiments and data collection (BJS, KS); Analysis of data and interpretation (BJS, KS); Preparation of the manuscript (BJS, KS).

DECLARATION

The authors declare that there is no conflict of interest.

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