Confirmation of Occurrence of Natural Tetraploid Banana in India

S Uma, S Sathiamoorthy and Nicolas Roux*
National Research Centre for Banana (NRCB), Trichy-620 017 (Tamil Nadu)

Exploration in North-eastern states of India has revealed the occurrence of a natural tetraploid of banana in India. Ploidy status was confirmed using flow cytometry while using morpho-taxonomic characterization, Bhat Manohar has been tentatively assigned the genomic status as ABBB. Being male and female fertile, this tetraploid has a good potential for use in breeding programmes.

Key words: Banana, Breeding, Characterization, Flow Cytometry, Genomic Status, *Musa*, Tetraploid

Banana and plantains (*Musa* spp.) are the oldest fruits cultivated by man. Evolution studies of present day bananas indicates its origin to Southeast Asia including Pacific Islands and India is the major centre of origin and diversification. Though South and South-east Asia are well recognized zones of *Musa* diversity, occurrence of large diversity for *Musa acuminata* ssp. *banksii, burmannicoides, siamea, microcarpa, malaccensis, truncata, zebrina* etc. in Malaysia, Thailand, and Indonesia are recorded in literature, while India and Myanmar have a wider diversity for *Musa balbisiana*. Due to movement and natural introgression among *acuminata* and *balbiasiana*, in Northeastern zones of India, a lot of variability occurs with respect to *acuminata- balbisiana* bispecific clones, especially for triploids like AAB and ABB (Singh *et al.*, 2001). Recent explorations conducted by NRCB, Trichy have revealed the occurrence of many unique clones from Northeastern India (Uma *et al.*, 1999-2000). Some of them exhibited tolerance to leaf spot diseases (Selvarajan, unpublished).

Materials and Methods

Three major explorations were conducted in North-eastern states of India including Assam, Arunachal Pradesh, Meghalaya, Mizoram and Tripura during 1998, 1999 and 2000. A total of 88 accessions were collected from various agroclimatic regions of North-eastern states.

The cultivar Bhat Manohar was collected during one of these explorations from the Namsai forest areas of Assam and Arunachal Pradesh border. Along with other accessions it was brought to NRCB field gene bank and planted under the prevailing wet-land conditions. It was evaluated for morphotaxonomic, other qualitative and quantitative traits like tolerance to Sigatoka leaf spot disease.

Assigning Tentative Genomic Status

Germplasm collected during such explorations were initially planted in 'Base Collection Block' where they were assigned tentative genomic groups using the Simmonds and Shepherd's (1955) fifteen character score card. In this, plant is scored from 1 to 5 as per descriptions available in Table 1. All the traits mentioned under *Musa acuminata* were given the score of 1 and those coinciding with *Musa balbisiana* were scored as 5. The variations between these two extreme characters were given the intermediary score based on experience. The total score ranged from 15-75 depending on the genomic groups. The total scores thus obtained were compared with the score card (Table 2) and corresponding genomic status was assigned to a particular accession.

Due to some of the lacunae like discontinuity and ambiguity with respect to score ranges in Simmond and Shepherd's score card, the modified score card developed by Silayoi and Chomchalow (1987) was referred. Hence at NRCB efforts were made to score 435 accessions belonging to various genomes and a modified score card has been proposed (Singh and Uma 1996). The trend of scoring for most of the accessions of a particular genome was used as the score range (Table 2).

Morphotaxonomy

Morpho-taxonomic characterization was carried out using IPGRI's (1996) banana descriptor for 117 traits.

Assignment of Ploidy Status

The ploidy status was determined using meiotic chromosome number and other morphological studies like stomatal density (no. of stomata per unit area),

* *FAO/IAEA, Agriculture and Biotechnology Laboratory Agency's Laboratories, A- 2444, Seibersdor, Austria.*

Table 1. Morpho-taxonomic scoring system for *Musa* genomic classification

S.No.	Character	*M. acuminata* Score 1	*M. balbisiana* Score 5
1.	Pseudostem colour	More or less heavily marked with brown black blotches	Blotches slight or absent.
2.	Petiolar canal	Margin erect or spreading with scarious wing below not clasping pseudostem	Margin inclosed, not winged below, clasping pseudostem.
3.	Peduncle	usually downy or hairy	Glabrous
4.	Pedicel	Short	Long
5.	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus.
6.	Bract curling	Bracts reflex and roll back after opening	Bracts lift but do not roll back.
7.	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder.	Broadly ovate, not tapering sharply.
8.	Bract apex	Acute	Obtuse
9.	Bract shoulder	Usually high (ratio less than 0.28)	Usually low (ratio more than 0.3).
10.	Bract colour	Red, dull purple or yellow outside and pink, dull and purple or yellow inside.	Distinctive brownish purple outside bright crimson inside.
11.	Colour fading	Inside bract colour fades to yellow towards base.	Inside bract colour continuous towards base.
12.	Bract scars	Prominent	Scarcely prominent
13.	Free tepal of male	Variably corrugated below flower	Rarely corrugated tip.
14.	Male flower colour	Creamy white	Variably flushed with pink.
15.	Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink.

Table 2. Modified score card for assigning tentative genomic groups

Genomes	Score card of		
	Simmonds & Shepherd (1982)	Silayoi and Chomchalow (1987)	Singh & Uma (1996)
AA/AAA	15-23	15-25	15-25
AAB	24-46	26-46	26-45
AB/AABB	49	59-63	59-49
ABB	59-63	59-63	59-65
ABBB	67	–	66-69
BB	70-75	70-75	70-75

stomatal measurements (length and breadth) and specific leaf weights.

Confirmation of ploidy status

The confirmation of ploidy was done using Flow Cytometry. For this, 10 cm^2 leaf bits were collected from the freshly and fully opened 3rd leaf from the top. Care was taken to collect the leaves without blemishes or spots caused by major and minor microbes. Such leaf samples were folded in wet paper and sealed in a polybag with labels. The sample were analysed at Vienna, Austria for ploidy using cytometry. The standard cultivars used as reference clones were, Pisang Lilin (2x), Rasthali (3x) and FHIA-01 (4x).

Sample Preparation for Ploidy Analysis Using Flow Cytometry

Ploidy level was analysed with a PA-I flow cytometer (Partec, Munster, Germany). Samples were prepared according to Dolezel *et al.*, (1997) with minor modifications. Briefly 20-30 mg of leaf tissue was chopped with a sharp razor blade in a plastic pertridish containing 0.5 ml Partec HR-A buffer. The sample was filtered through a 50um nylon mesh. Chicken red blood cell nuclei (CRBC) were prepared according to Dolezel *et al.* (2000) and added to the suspension of released nuclei as internal reference-standard. To stain nuclear DNA, 2ml Partec HR-B solution containing DAPI (4',6-diamidino-2-phenylindole) was added to the suspension of released nuclei. To avoid rapid tissue oxidation, 2µl/ml β mercaptoethanol was added to the HR-B solution just before use. The gain of the instrument was adjusted so that the Go/G1 peak of CRBC nuclei was approximately on channel 100. The relative DNA content of *Musa* accessions was then calculated by comparing peak positions of CRBC nuclei and nuclei of the sample. In each sample, a total of 5000-10000 nuclei were analysed. For each plant at least 8 measurements were made with 4

replicates and 2 repetitions. On observation, the accession of interest Bhat Manohar, collected from Namsai forest ranges exhibited very interesting morphological traits unlike other diploid and tetraploid clones.

Results and Discussion

Accession Bhat Manohar was evaluated for 15 characters as given by Simmond and Shepherd score card over three seasons (Table-1). The average score obtained was 68.8 out of the total score of 75. This was compared with the score card by Simmonds and Shepherd (1955) which, however, could not be used for allotting its genomic status specific into any particular group. It was then compared with the score card of Silayoi and Cham Chalow (1987) which too failed to provide a clear cut genomic status. Finally, the score card developed by Singh and Uma (1996) aided the classification of this accession tentatively under ABBB which has a score range of 66-69. The tetraploidy status of the accession was interesting since there are very few reports on the occurrence of banana tetraploids in nature (Simmonds, 1962). Klue teparod (4X), first reported natural tetraploid was later proved to be a triploid (3X) by Jenny *et al.* (1997). In general, tetraploids are less favoured in nature to their gigantic stature and long durations. Bhat Manohar has exhibited partial resistance to Sigatoka leaf spot disease with a YLS (Youngest Leaf Spotted) of more than 11.0 at shooting.

To confirm the tentative assignment of genomic and ploidy status, other preliminary tests were conducted like meiotic studies for chromosomal count, stomatal density, stomatal length, leaf thickness and specific leaf weights. Average leaf thickness of Bhatmanohar measured to be 3.91 as against 3.68-3.71 for triploids, and 3.23-3.38 for the diploids.

The per cent dry weight (83.2) was at par with other known synthetic tetraploids like FHIA-03(82.2), while triploids exhibited a range from 76.45 to 77.82 and diploid from 71.78-79.74. The stomatal density exhibited a lowest of 3.91 at par with other tetraploids (3.82 for FHIA-01 and 3.89 for FHIA-03) while stomatal density was as high as in diploids 40.5 to 43.6 followed by 35.7 to 38.3 in triploids. By nature, tetraploids exhibit a lower stomatal density (Simmonds, 1962) but comparatively larger stomata in size. This was also confirmed by the present observations of 32.3 μ in Bhat Manohar as against 28.4-29.1 μ in tetraploids and the smallest stomata were recorded in diploids (23.8-24.5 μ).

Chromosomal studies revealed the presence of more than 33 chromosomes but since the size of the chromosomes was very small, a final conclusion could not be reached. Hence, the use of Flow cytometry was sought. The results of Flow cytometry (Fig. 1), calculating the relative DNA content by comparing with peak positive of CRBC nuclei and nuclei of the sample confirmed the tetraploidy status of Bhat Manohar.

With all the preliminary and confirmatory tests, ploidy status of Bhat Manohar is determined to be 4x (tetraploid). Based on the morpho taxonomic scoring in comparison with Singh and Uma's score card (2000), the genomic status is tentatively assigned as ABBB. Though there was earlier reports of natural tetraploids, from Papua New Guinea, the genomic status was reported to be AAAB and AABB and Bhat Manohar is the first report of a natural banana tetraploid with ABBB genomic status. Doubts are also raised about the occurrence of pure natural tetraploid of *acuminata* and *balbisiana* origin (Horry *et al.* 1997).

In nature, tetraploids have been less preferred in the course of evolution with no satisfactory reason attached to it. No doubt that the tetraploids are one of the stepping stones in the evolution of present day commercial banana of tetraploidy status. The importance of tetraploids in target breeding was also envisaged as early as 1962 by Simmonds. Though lot of efforts have been diverted in developing a synthetic tetraploid, less emphasis is given for the search for natural tetraploids in the zones of evolution. Many pathogens are also expected to undergo co-evolution in such zones. This situation might have lead to the survival of only such tetraploids which have acquired resistant genes through introgression. Such tetraploids are very useful in breeding for improved triploids and secondary tetraploid. More attention is needed for cartography of natural habitats of wild germplasm, identification of tetraploids from potential co-evolution zones their collection and exploitation in genetic improvement programs.

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