J. Hortl. Sci.
Vol. 3 (1): 30-34, 2008

Chromosome studies and karyotype analysis of some triploid banana (*Musa* species) cultivars of AAA genomic group

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ABSTRACT

Bananas are the highly evolved, oldest fruits known to mankind. The Cavendish group cultivars are popular commercial varieties. AAA genomic group cultivars are said to have evolved from the wild AA *Musa acuminata* species by natural hybridization and polyploidization and these vigorous triploids were selected by man for cultivation. Basic cytological studies on banana are comparatively few due to the plant's complex nature. In this report, karyo-morphological studies on five AAA Cavendish group cultivars i.e. Robusta, Dwarf Cavendish, Grand Naine, Gros Michel and Red banana are reported. All the five cultivars had similar karyotype, except cv. Robusta. Total chromosome length was highest in Red banana and lowest in cv. Gros Michel.

Key words: Cavendish banana, triploid, karyo-morphology

INTRODUCTION

Bananas a figure among the ancient fruits cultivated by man. It could be assumed that the fruit has evolved with civilization (Krishnamurthi and Seshadri 1958). Apart from its mention in Valmiki's Ramayana, Kautilya's Arthashastra and the ancient Tamil classic Silappadikaram, it also figures in the Indus Valley as early as 327 B.C. These evidences suggest an early existence of banana in India. Edible bananas were considered to be hybrids from two main species: *Musa acuminata* and *M. balbisiana* (Dodds and Simmonds, 1948). Edibility of mature fruits of diploid *M. acuminata* (AA) might have come about as a result of two mutation events, female sterility and parthenocarpy (Simmonds, 1962). With regard to cytological studies, meiotic data on 17 South Indian banana varieties by Agrawal (1983) revealed cytological abnormalities. However, meiotic studies in male sterile triploids did not exhibit chromosomal irregularities. (Agrawal, 1987). Chromosome counts of 20 Bihar banana cultivars were reported by Roy and Sharma (1951). Valsala Kumari and Nair (1990) reported chromosome number of 98 cultivars. Somatic chromosomes of 53 *Musa* land races and hybrids were reported by Osuji *et al* (1996) and 16 *Musa* species and land races by Dolezel *et al* (1998). Chromosome count and ploidy level was determined in 60 *Musa* accesssions, including plantains and somaclonal variants by Osuji *et al* (1996). However chromosome structure and morphology is not reported in any of these studies. Efforts have been made in the present study to understand karyo-morphology of AAA triploid cultivars.

MATERIAL AND METHODS

Plant material

Triploid AAA cultivars were derived from diploids which, in turn, could be the result of crosses between edible diploids and wild *M. acuminata* sub-species, resulting in a wide range of AAA phenotypes. These triploids are most vigorous, have larger fruits, and thus replaced the commercial AA diploids of Asia.

Robusta (Giant Cavendish): Also called Harichal, Bombay Green, Barjahaji, Peddapacha arti, etc., is similar to Dwarf Cavendish except for its height, bunch shape and fruit curvature. It is an important commercial banana variety. Robusta is semi-dwarf. The plants are about 2 - 2.5m tall, with blackish brown blotches on the pseudostem with a diameter of about 19 - 20 cm. Bunches are fairly large, with a length of 50-75 cm bearing 80-100 fruits in 7-10 hands. Female flowers (fruits) are sterile, whereas male flowers have partially fertile pollen.

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Dwarf Cavendish: Indian synonyms are Basrai and Jahaji. Basrai is cultivated for its dwarf stature and high yield, and is also less susceptible to wind damage. As per Simmonds (1962), it was introduced from South China to Mauritius in 1826 from where it would have come to the Indian subcontinent. Dwarf Cavendish is said to be a dwarf mutant of Robusta. The plants are short, with a height of 1 - 1.5m. The pseudostem has dark brown blotches. The girth of pseudostem varies between 17 and 19 cm diameter. Bunches are smaller than in Robusta, with 80-100 fruits per bunch. The bunch length was 40-45cm. The fruits are slightly curved, 60-80 fruits in 6-8 hands. Fruits are similar to Robusta but smaller.

Grand Naine: This cultivar is intermediate in height between Dwarf Cavendish and Robusta. It has increased commercial importance because of lower height, uniform bunch, finger shape and high yield. The cultivar Grand Naine is currently leading commercial highyielding cultivar allover the world.

Red Banana: it is also known as Lalkela or Chen kadali and is called Red banana because of its very distinctive colour. The plants too have magenta red pigmentation on the pseudostem and leaf petioles. They are vigorous but poor yielders with a long crop-duration, take about eighteen months from planting to harvest, are tall with 2.5 - 3 m height. The pseudostem has a diameter of 20-25cm. Bunches are small, 30-35 cm long with fewer fruits of about 40-45 per bunch of 4-5 hands. The pulp is deep yellow in colour. The male axis is long and anthers have very little, but fertile, pollen.

Gros Michel: Big, very vigorous plants, bear heavy bunches with large fruits. It differs from other Cavendish group bananas in strong colouration of its upper sheaths and midribs. It was the most popular clone in Central and South America. Due to its high susceptibility to *Fusarium* wilt disease, it was replaced by other Cavendish cultivars in mid 1960's. The plants were tall, with a height of 2.5 - 3 m. The pseudostem had a few blotches of brownish black colour, bunches were of medium size with a length of 40-45cm with 70-80 fruits in 6-7 hands. It was found to be both male and female fertile and was widely used in breeding programs.

Cytological studies

Root apices of all the accessions were collected from healthy suckers planted in pots. Root tips were washed thoroughly and pre-treated with 0.003M 8-hydroxyquinone for two hours at 14-16^{o}C. The pre-treated root tips were fixed in Carnoy's-II fixative viz., 6:3:1 of Absolute alcohol : glacial acetic acid : Chloroform. The fixed materials were transferred to 70% alcohol after 24 hours of fixation, and stored.

The stored root tips were rinsed with distilled water and hydrolysed with 1 N HCl at 60^{o} C for 5 minutes. The hydrolysed root tips were transferred to Lilee's (1951) or Schiff's reagent after a rinse in distilled water, and stored in the dark for $1^{1}/_{2}$ to 2 hours. Schiff's reagent stains actively dividing meristematic tissue to a deep magenta colour. The stained tips were squashed with a drop of 1% aceto-carmine after removing the root cap. The slides were sealed with gum mastic, and observed under microscope on the same day or the next day. The slides were scanned for well spread metaphase chromosomes; such cells were selected for karyo-morphological studies. Three to five slides per accession were prepared and several cells were observed to ascertain chromosome number.

It was observed that chromosomes were very small in size, sticky in nature and needed hard tapping to obtain better chromosome spread. Hence, it was difficult to obtain good picture of wellspread plates. Increase in hydrolysis time resulted in poor staining and moderate quality pictures.

Chromosomes were measured manually from camera lucida drawings. The measurements were recorded in millimeters (mm) and converted to micrometers (mm), using a conversion factor. Observations were recorded on short arm, long arm and satellites separately. Homologus chromosomes were paired based on their arm length and arranged in decreasing order of total chromosomal length. The total length of the complement was calculated in microns by adding the average length of each chromosome pair.

Levan's (1964) system was followed for description of karyotypes and their classification, which was done based on the arm ratio (long arm/short arm). The chromosomes were categorized as follows:

1. SAT-m : Chromosome with median centromere having satellite
2. SAT-sm : Chromosome with sub-median centromere having a satellite
3. SAT-st : Chromosome with sub-terminal centromere having a satellite
4. m : Chromosome with median centromere

5. sm : Chromosomewith sub-median centromere

6. st : Chromosomewith sub-terminal centromere

In AAA triploids (autopolyploids), the pairing was done in triplets, i.e., a set of three chromosomes formed a complex.

RESULTS AND DISCUSSION

The present investigations included five cultivars of the genomic group AAA, viz Robusta, Dwarf Cavendish, Gros Michel, Grand Naine, and Red Banana. The chromosome number in all the cultivars was found to be 2n=3x=33 and reported to be triploid in nature (Dolezel, 2004).

The karyotypic formula of Robusta was 2n=3x=3SAT-m+21m+9sm. It consisted of three types of chromosomes. There were 3 chromosomes with the median centromere and satellite of 0.42 mm length. Twenty-one chromosomes (7 triplets) had the median centromere and 9 chromosomes had the sub-median centromere. The range of chromosome length was 1.47 to 2.49 mm. Absolute chromosome length was 21.87 mm (Table 1).

The karyotypic formula of cultivar Dwarf Cavendish was 2n=3x=3SAT-m+27m+3sm. It consisted of three types of chromosomes. There were three chromosomes with the median centromere and satellite of 0.42 m. The 27 chromosomes had median centromere and 4 chromosomes had sub-median centromere. The length of chromosomes measured 1.54 to 2.31 mm and absolute length was 20.56 mm (Table 2).

The karyotypic formula of Gros Michel was 2n=3x=3SAT-m+27m+3sm. It consisted of three types of chromosomes. There were 3 chromosomes with satellite measuring 0.42 mm in length and median a centromere.

Table 1. Somatic chromosome measurement in cv. Robusta (AAA)

Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Position of centromere	Arm ratio
1	1.61	0.88	2.49	Sub-Median	1.83
2	1.16+0.42	0.84	2.42	SAT-Median	1.28
3	1.30	1.05	2.35	Median	1.24
4	1.09	1.01	2.10	Median	1.08
5	1.40	0.67	2.07	Sub-Median	2.09
6	1.26	0.74	2.00	Sub-Median	1.70
7	1.05	0.84	1.89	Median	1.25
8	0.95	0.84	1.79	Median	1.13
9	1.05	0.70	1.75	Median	1.50
10	0.95	0.59	1.54	Median	1.61
11	0.84	0.63	1.47	Median	1.33

Table 2. Somatic chromosome measurement in cv. Dwarf Cavendish (AAA)

Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Position of centromere	Arm ratio
1	1.40	0.91	2.31	Median	1.54
2	1.05	1.05	2.10	Median	1.00
3	1.26	0.84	2.10	Median	1.50
4	1.33	0.67	2.00	Sub-Median	1.99
5	1.05	0.84	1.89	Median	1.25
6	0.80+0.42	0.63	1.85	SAT-Median	1.27
7	0.95	0.84	1.79	Median	1.13
8	0.91	0.84	1.75	Median	1.08
9	0.95	0.67	1.62	Median	1.42
10	0.91	0.70	1.61	Median	1.30
11	0.84	0.70	1.54	Median	1.20

Table 3. Somatic chromosome measurement in cv. Gros Michel (AAA)

Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Position of centromere	Arm ratio
1	1.37	0.74	2.11	Sub-Median	1.85
2	0.80 +0.42	0.77	1.99	SAT-Median	1.04
3	1.05	0.91	1.96	Median	1.15
4	0.84	0.84	1.68	Median	1.00
5	0.84	0.74	1.58	Median	1.14
6	0.88	0.67	1.55	Median	1.31
7	0.88	0.63	1.51	Median	1.40
8	0.84	0.63	1.47	Median	1.33
9	0.84	0.63	1.47	Median	1.33
10	0.74	0.49	1.23	Median	1.51
11	0.67	0.49	1.16	Median	1.37

Also 27 chromosomes had the median centromere. Length of the chromosomes was 1.16 to 2.11 mm. The absolute length was 17.71 mm (Table 3).

The karyotypic formula of cv. cultivar Grand Naine was found to be 2n=3x=3SAT-m+27m+3sm. It consisted of three types of chromosomes. There were three chromosomes with satellite of 0.42 mm length and a median centromere. It had 27 chromosomes with a median centromere and three chromosomes with a sub-median centromere. The length of chromosomes varied between 1.26 and 2.52 mm and the absolute length was 21.23 mm (Table 4).

Cultivar Red Banana had karyotypic formula of 2n=3x=3SAT-m+27m+3sm. It had three types of chromosomes. One set of three chromosomes had satellite of 0.42 mm and a median centromere. Twenty-seven chromosomes had a median centromere and three chromosomes were with a sub-median centromere. Chromosomes varied in their length between 1.55 and 2.52 mm. The absolute length was 23.04 mm (Table 5).

Table 4. Somatic chromosome measurement in cv. Grand Naine (AAA)

Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Position of centromere	Arm ratio
1	1.37	1.16	2.53	Median	1.18
2	1.64	0.88	2.52	Sub-Median	1.86
3	1.09	1.05	2.14	Median	1.04
4	0.84+0.42	0.84	2.10	SAT-Median	1.00
5	1.09	0.91	2.00	Median	1.20
6	1.22	0.77	1.99	Median	1.58
7	1.05	0.84	1.89	Median	1.25
8	0.91	0.80	1.71	Median	1.14
9	0.95	0.67	1.62	Median	1.42
10	0.84	0.63	1.47	Median	1.33
11	0.63	0.63	1.26	Median	1.00

Table 5. Somatic chromosome measurement in cv. Red Banana (AAA)

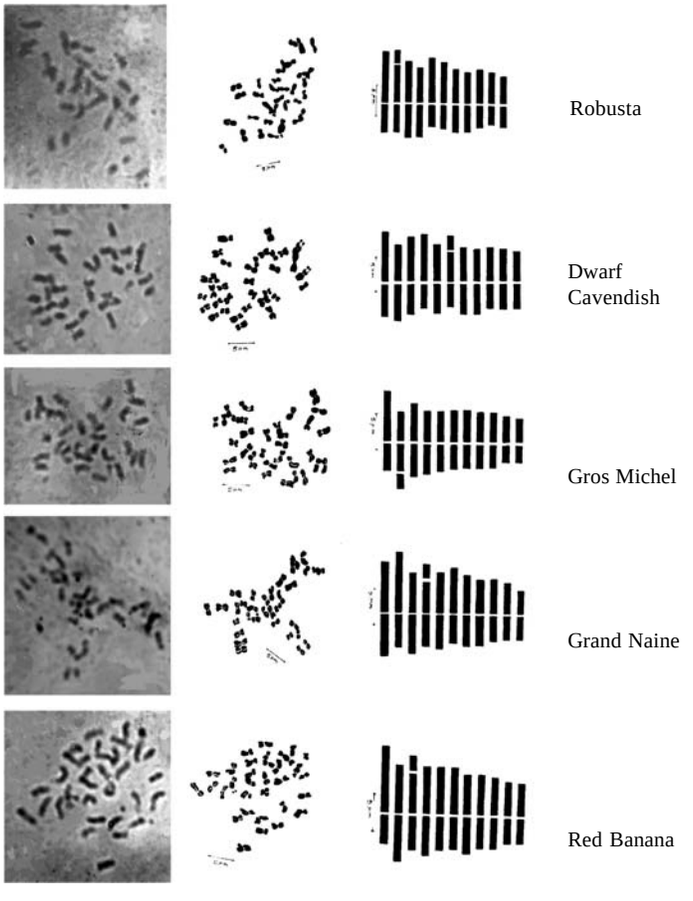
Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Position of centromere	Arm ratio
1	1.75	0.77	2.52	Sub-Median	2.72
2	1.26	1.22	2.48	Median	1.03
3	1.05+0.42	0.91	2.38	SAT-Median	1.16
4	1.26	1.05	2.31	Median	1.20
5	1.26	0.91	2.17	Median	1.38
6	1.26	0.88	2.14	Median	1.43
7	1.09	1.01	2.10	Median	1.08
8	1.09	0.84	1.93	Median	1.30
9	1.01	0.84	1.85	Median	1.20
10	0.91	0.70	1.61	Median	1.30
11	0.88	0.67	1.55	Median	1.31

Table 6. Comparative karyo-morphological data of five cultivars of AAA group bananas

Sl. No	Acc. Name	Karyotypic Formula	SAT-Size (mm)	Range of chromosome length (mm)	Absolute length (mm)
1	Robusta	3SAT-m+21m+9sm	0.42	1.47-2.49	21.87
2	Dwarf Cavendish	3SAT-m+27m+3sm	0.42	1.54-2.31	20.56
3	Gros Michel	3SAT-m+27m+3sm	0.42	1.17-2.32	20.24
4	Grand Naine	3SAT-m+27m+3sm	0.42	1.26-2.53	21.23
5	Red Banana	3SAT-m+27m+3sm	0.42	1.55-2.52	23.04

Comparative karyo-morphological data on five AAA group cultivars are given in Table 6. All the 4 cultivars except Robusta, had similar karyotypes but varied in their absolute length. Robusta differed from the others in having 9 chromosomes with a sub-median centromere. Cultivar Red Banana had the highest absolute length of 23.04 mm and cultivar Gros Michel had lowest absolute length of 17.71 mm. All the cultivars differed in their morphology and also showed variations in molecular profiles (Rekha *et.al*, 2001).

Differences in the absolute chromosome size between related species or genera might reflect different amounts of gene duplication. Other differences are generally due to structural changes, as stated by Stebbins (1971). Stebbins (1950) also opined that polyploidy, combined with hybridization had a major influence on evolution of higher plants, its effects being conservation. Polyploidy stabilizes new genotypes evolved through hybridization by reducing the amount of genetic segregation. Such a phenomenon would have taken place in the evolution of bananas, which are able to tolerate a wide range of environmental conditions. Wang *et.al(*1993) studied the karyotypes of banana group AAA and reported that three sets of chromosomes of group AAA were all that homologous. In



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the present studies, chromosomes were paired based on arm length and centromere position where high similarity was observed. The chromosomes were found to be highly homologous and, also, there was no set pattern of chromosome complement. Variations were observed within and between groups which could also be due to structural differences in the chromosome complement, and may have resulted in the evolution of new cultivars. To conclude, morphological variations observed in the above cultivars appears to be a part of the evolutionary process in this important, vegetatively propagated crop.

ACKNOWLEDGEMENT

The authors are thankful to Head and the staff of Department of Botany, Karnatak University, Dharwad, for their kind help during the above study.

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(MS Received 15 November 2007, Revised 17 April 2008)