Cytogenetical Investigations in Musaceae IV. Cytomorphology of an interspecific triploid hybrid of *Musa acuminata* Colla. $\times$ *M. rubra* Wall.¹

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Accepted September 17, 1987

Musa belongs to the family Musaceae, which has two genera and 31-36 species (Simmonds 1966). These species are used as vegetables, fruits, ornamentals and for extracting fiber. Interspecific hybridization has played a major role in the evolution of present day banana. A large number of diploid and triploid cultivars of banana have originated as a result of natural hybridization between *Musa acuminata* and *M. balbisiana*. However, except for a few reports on interspecific hybridization in banana (Bernardo 1957) no attempt has been made so far to produce artificial interspecific hybrids in *Musa* and to study their cytological behaviour to understand species relationship. Investigations were, therefore, conducted to raise interspecific hybrids of *Musa* and study these cytomorphologically. The present article describes cytomorphology of triploid interspecific hybrids obtained from cross *M. acuminata* $\times$ *M. rubra*.

Material and methods

The material for present investigations included two species of *Musa* viz. *M. acuminata* Colla. and *M. rubra* Wall. Suckers of these species were collected from Banana Research Station, Aduthurai and grown at IIHR Bangalore. For hybridization, flowers in the first hand of *M. acuminata* were emasculated one day before opening, bagged and were pollinated next morning with freshly collected pollen from *M. rubra* and again bagged with cloth bag. Same way all the female flowers in subsequent hands were emasculated, pollinated and bagged. Crossed fruits were allowed to mature and ripen on the plant. Seeds were extracted from these fruits and put for germination in saw dust, inside a mist chamber, soon after harvest. Seedlings were later transplanted to field and grown at a spacing of 2.5 M $\times$ 2.5 M. Observations were taken on various vegetative and fruit characters of parents and their hybrids.

For cytological studies, anthers from young male flower buds were fixed at appropriate time, in 1:3 acetic alcohol solution fortified with saturated aqueous solution of ferric chloride, for 24 hrs at 15°C and later transferred to 70% ethyl alcohol and stored at 5°C till use. Squash preparations were made using 2% acetocarmine solution. Pollen fertility was determined by stainability in acetocarmine solution. Photomicrographs were taken from temporary slides.

Observations

Morphology:

Out of fifty hybrids obtained from cross *M. acuminata* (2n=22) and *M. rubra* (2n=22), forty two were triploids (2n=33) and rest were higher polyploids. Various morphological features of parents and their hybrids have been described in Table 1. The two species differ strikingly in their growth habit; the underground part is corm (without any horizontal growth)

¹ I. I. H. R. Contribution No. 90/87.

Fig. 1. *M. acuminata* showing pendulous orientation of females inflorescence.

Fig. 2. *M. acuminata* $\times$ *M. rubra* hybrid showing horizontal orientation of female inflorescence.

Fig. 3. *M. rubra* showing upright orientation of female inflorescence.

Fig. 4-9. *M. acuminata* $\times$ *M. rubra* meiosis. 4, PMC at M_1 showing 11_{II} , 3_{III} and 5_I . $\times 1300$.

5, PMC at M_1 showing 12_{II} , 2_{III} and 3_I . $\times 1300$. 6, PMC at M_1 showing 5 univalents. $\times 1000$.

7, PMC at A_1 showing a lagging bivalent. $\times 1000$. 8, PMC at A_1 showing 33 chromosomes.

$\times 1000$. 9, PMC at M_2 showing 15 chromosomes. $\times 1300$.

in *M. acuminata*, rhizome (having lateral growth) in *M. rubra* and corm in the hybrid. *M. rubra* plants are shorter in height as compared to *M. acuminata*, the hybrid is of intermediate height. The angle of female inflorescence, which is pendulous in *M. acuminata* (Fig. 1), vertically up in *M. rubra* (Fig. 3) is horizontal in the hybrid (Fig. 2). Most of the other characters in hybrid are intermediate to the parents. The quantity of pollen produced is very less in *M. acuminata* but more in *M. rubra*, however, in hybrids, pollen quantity varies from plant to plant but fertility is very poor.

Table 1. Morphological characters of *Musa acuminata*, *Musa rubra* and their triploid hybrid *M. acuminata* × *M. rubra*

Sl. no.	Characters	<i>M. acuminata</i>	<i>M. rubra</i>	<i>M. acuminata</i> × <i>M. rubra</i> triploid
1	Plant height	1.86 M	0.9 M	1.45 M
2	Leaf length/width ratio	2.26	2.56	2.00
3	Angle of female inflorescence	Pendulous	vertically up	horizontal
4	No. of stomata/unit area	41–45	41–45	42
5	Length of stomatal aperture	22.5 μ	22.5 μ	25 μ
6	Width of stomatal aperture	12.5 μ	13.75 μ	12.5 μ
<i>Female flowers</i>				
7	Bract colour	dull purple	bright crimson	bright crimson
8	Length of female flowers	9.0 cm	6.0 cm	8.5 cm
9	Length of fused petal	5 cm	2.5 cm	3 cm
10	Length of free petal	2.6 cm	1.3 cm	2.5 cm
11	Length of stigma	0.5 cm	0.3 cm	0.5 cm
12	Length of style	3.5 cm	2.5 cm	3.5 cm
13	Length of ovary	5.0 cm	3.0 cm	4.4 cm
14	No. of locules	3	3	3
15	No. of ovules/locule	2	2	2
16	No. of staminodes	5	3	5
<i>Male flowers</i>				
17	Length of male flowers	4.7 cm	3.6 cm	6.0 cm
18	Length of fused petal	4.7 cm	2.7 cm	5.0 cm
19	Length of free petal	2.5 cm	1.1 cm	2.7 cm
20	Length of stamens	4.7 cm	2.7 cm	4.7 cm
21	Length of rud. stigma and style	4.1 cm	2.7 cm	4.7 cm
22	Pollen fertility	72.13%	98.69%	43.7%
23	Pollen size (diameter)	0.13 mm	0.1 mm	0.12 mm

Cytology: *Musa acuminata* and *M. rubra* are diploid having $2n=22$ chromosomes. However, majority of the hybrids obtained from the cross have $2n=33$ chromosomes. Meiosis in *M. acuminata* is characterized by the presence of high frequency of bivalents, unequal distribution of chromosomes and presence of laggards at anaphase I (Tables 2, 3). In *M. rubra* also, at metaphase I, high frequency of bivalents was observed, apart from this about 86% pollen mother cells showed presence of 1–3 B chromosomes which paired among themselves and separated equally to the two poles. Pollen fertility was 98.6% (Tables 2, 3). The triploid hybrids of *M. acuminata* × *M. rubra* have $2n=33$ chromosomes (Fig. 8). At meiotic metaphase I high frequency of bivalents followed by univalents and trivalents was observed (Figs. 4, 5). Five univalents were very commonly observed (Fig. 6). At anaphase I, 1–5 lagging bivalents were seen (Fig. 7) and chromosomes separated irregularly to the two poles

(Tables 2, 3). At anaphase II equal number of chromosomes moved to two poles (Fig. 9). Stickiness of chromosomes was observed at metaphase I. Pollen fertility was 43.70%.

Discussion

The two species *Musa acuminata* and *M. rubra* have striking differences in their growth habit, inflorescence orientation and leaf shape. Baker (1893) has placed *M. acuminata* in sub-genus Eumusa of *Musa* and *M. rubra* under sub-genus Rhodochlamys on the basis of number of flowers to a bract and bract colour. Morphologically, triploid hybrids are more like *M. acuminata* rather than *M. rubra*. The occurrence of triploids in the cross of these diploid species suggests formation of unreduced female gametes in *M. acuminata* (since *M. rubra* invariably produces haploid pollen grains) which on fertilization with normal haploid male

Table 2. Table showing chromosomal associations in PMCs of *Musa acuminata*, *M. rubra* and their hybrid

<i>Musa</i> species/hybrid	2n chr. no.	Univalents per PMC	Bivalents per PMC	Trivalents per PMC
<i>Musa acuminata</i> *	22	3.67	9.17	—
<i>M. rubra</i> **	22	0.2	10.21	0.46
<i>M. acuminata</i> × <i>M. rubra</i> (3n)	33	5.0	11.00	2.00

* Agarwal 1988.

** Agarwal 1983.

Table 3. PMCs showing chromosomal distribution at anaphase I in *M. acuminata*, *M. rubra* and their hybrid

<i>Musa</i> species/ hybrid	Chromosome distribution at anaphase I							Laggards/PMC		
	7-15	9-13	10-12	11-11	15-18	16-17	Others	Range	% PMCs showing laggards	Pollen fertility
<i>M. acuminata</i> *	7.7	7.7	7.7	30.77			46.15	0-4	46.15	72.13%
<i>M. rubra</i> **				98.00			2.00	0-2	86.00	98.69%
<i>M. acuminata</i> × <i>M. rubra</i> (3n)					9.00	18.00	73.00	0-8	72.72	43.70%

* Agarwal 1988.

** Agarwal 1983.

gametes from *M. rubra* produce triploid ($2n=33$) hybrids. This is further confirmed by the dominance of *M. acuminata* characters in the hybrid. The phenomenon of restitution nuclei formation has played an important role in the evolution of present day triploid (AAB and ABB) bananas, which are considered to have evolved by hybridization of restitution nuclei forming cultivars of *M. acuminata* with *M. balbisiana* pollen and vice versa (Simmonds 1966). This phenomenon of unreduced female gamete formation has also been made use of, in breeding improved triploid and tetraploid banana varieties (Row and Richardson 1975). Meiotic studies in the PMCs of *Musa acuminata* did not reveal the formation of unreduced male gametes (Agarwal, 1988) indicating thereby that meiosis leading to the formation of male gametes do not necessarily reflect the events taking place during formation of female gametes. Similar observations on variation in male and female meiotic divisions have been made by Darlington and LaCour (1940) in *Lilium*. They, however, considered it to be due to differences in living space available for the chromosomes.

The presence of eleven bivalents at metaphase I in the PMCs of triploid hybrids apparently suggests the complete bivalent formation of *M. acuminata* chromosomes and their non homology with *M. rubra* chromosomes. Attempts are underway to obtain diploid hybrids of *M. acuminata* × *M. rubra*, the pairing behaviour of chromosomes in this will further throw light on species relationship. The restitution nuclei forming clone of *M. acuminata*, identified during the present hybridization programme, however, cannot be used for breeding purposes as it lacks the desirable fruit characters and quality necessary for incorporation in the improved hybrid.

Summary

Cytomorphology of interspecific hybrids of *Musa acuminata* × *M. rubra* showed $2n=33$ chromosomes. The hybrid had intermediate morphological characters. The occurrence of triploids in diploid × diploid cross suggested formation of unreduced female gametes in *M. acuminata*. The absence of unreduced male gamete formation in the same indicated differences in male and female gametogenesis in same species. Formation of 11 bivalents per PMC in hybrids apparently suggests complete pairing of all *M. acuminata* chromosomes and lack of homology with *M. rubra* chromosomes.

Acknowledgements

The author is thankful to Director, Indian Institute of Horticultural Research (ICAR), Bangalore for providing necessary facilities to carry out the work.

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