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Studies of Ploidy Assessment in Some Synthetic Hybrids of Banana (*Musa* spp.)

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A B S T R A C T

Banana *(Musa* spp.) constitute a hybrid-polyploid complex and are classified according to different genome compositions such as AA, BB, AB, AAA, AAB, ABB, AAAA, ABBB, AAAB, AABB and AAABB. Knowledge of ploidy and exact genome compositions of the parental material is essential for *Musa* breeding. Flow cytometric analysis of nuclear DNA content was used to estimate ploidy levels. Twenty four Banana hybrids under phase-I and nineteen hybrids under phase-II evaluated and was done by flow cytometry analysis which enables rapid and precise measurements on whole cells, isolated nuclei or chromosomes in a monodisperse suspension. Studies found under phase-I that six hybrids diploids (AA and AB), five hybrids triploids (AAA and AAB), ten hybrids tetraploids (AABB) and three hybrids pentaploids (AAABB) were recorded and under phase-II found that one hybrid diploids(AB), three triploids (AAB) and rest of the hybrids tetraploids (AABB) were recorded.

Introduction

Bananas are among the largest herbs in the world. They are perennials with tall aerial shoots that arise from swollen, fleshy corms. Polyploidy in banana makes breeding a difficult process owing to complexities resulting from parthenocarpy and sterility. Besides, the degree of sterility is particularly high in edible cultivars, breeding of banana is complicated and time consuming Shepherd (1954, 1960). A minimum of two years is required to complete a seed-to-seed crop cycle. Even after thousands of crosses, very few viable seedlings were obtained from a limited percentage of seed set and each plant occupied $6m^2$ in the field for evaluation by Rowe (1984).

Stomata size was proportional to ploidy in banana, while stomatal density had the expected complementary relationship as reported by Rowe (1984), Simmonds (1948) and Borges (1971). A number of ploidy levels exist in *Musa* spp. by Tenkouano *et al.*, (2011). Knowledge of ploidy level in *Musa*

accessions is vital for breeding, conservation and tissue culture as they are affected by ploidy Suman *et al.,* (2012). Ploidy level influences fertility of banana. For instance, most triploids are sterile while diploids and tetraploids are fertile by Tenkouano *et al.,* (2011). Banana breeding usually involves the transfer of useful genes from diploids to triploids by carrying out 3x by 2x crosses. Such a cross can generate a variety of progeny with ploidy levels ranging from diploid, triploid, tetraploid, aneuploidy and hyperploids progeny by Pillay *et al.,* (2002). Ploidy level of banana determined primarily by morphological characteristics by Pillay *et al.,* (2003), Pillay *et al.,* (2006). The ploidy level is determined by other several methods, of which flow cytometry has screening a large number of accessions by Tenkouano *et al.,* (2011). In Jamaica breeding programme, Smith *et al.,* (1993) reported that the stomatal densities of two month old seedlings were employed to screen the progenies ploidy levels and genomic constitutions. Tetraploids derived from the diploid clone SH-3362 had a mean stomatal length of 26.9 μm as against 16.0μm in the diploid.

Currently, the genomic constitution of the new hybrids was assessed by morphological scoring method developed Simmonds and Shepherd (1955) and also referred to the scoring suggested by Simmonds and Shepherd (1987), Singh and Uma (1987, 1996). Flow cytometry enables rapid and precise measurements on whole cells, isolated nuclei or chromosomes in a monodisperse suspension. Van Duren *et al.,* (1996) used this technique to identify the *in vitro* induced tetraploids of SH-3362 banana clone. Since it involves determination of nuclear DNA, is more in the reliability of ploidy is more in detection by Dolezel (1997). Among the 24 hybrids evaluated by Das (2008), three were found to be pentaploids as confirmed by flow-cytometry.

Materials and Methods

The present study was taken up at the College orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Assessment of ploidy of hybrids

The ploidy status of the hybrids was assessed by the estimation of stomatal density and size at cellular level as postulated by Sathiamoorthy (1973). Ploidy levels of hybrids obtained from different cross combinations is a must in banana breeding because of potential production of diploid, triploid, tetraploid, hyperploid and aneuploid hybrids. Ploidy levels are estimated by phenotypic appearance and confirmed lither by root tip mitosis or stomatal density, size and number of *chloroplast* per guard cell pair. Sathiyamoorthy (1973) and Vandenhout *et al.,* (1995) classified banana clones diploids, triploids and tetraploids based on stomatal density and stomatal size, respectively.

Stomatal density

The sample for stomatal study was taken from the centre portion of the third leaf. The sample leaves were cut into one centimetre^2 bits and boiled for two minutes in water and then transferred to 70 per cent ethanol, where it was kept for 24 hours to remove the chlorophyll. The sample was then washed with water and boiled in 70 per cent lactic acid for five minutes to soften the tissues. The treated sample bit was kept over a clean slide with the upper surface of lamina bit in contact with the slide. The tissues were gently scrapped with a sharp blade and the intervening fibers were removed carefully with a pointed needle, till the upper epidermis alone was in contact with the slide. The material was gently washed and mounted in glycerin and sealed with a cover slip and

examined under microscope of 45 x magnifications by Sathiyamoorthy (1973). The number of stomata per microscopic field (0.152 mm^2) was counted at least at ten different fields and the mean was arrived. The result was expressed as number of stomata per mm^2. Besides, the length and breadth of the stomata were also measured by using ocular micrometer (Plate 1 and 2). The size of the stomata was calculated by multiplying the length and breadth and was expressed in μm^2. The stomatal density and size of the hybrid seedlings were used to group the hybrids into diploids, triploids and tetraploids as indicated below:

Analysis of genome

The genomic constitution of the new hybrids was assessed by morphological scoring method (Table 1) by Simmonds and Shepherd (1955) *and* modified scoring (Table 2) by Singh and Uma (1996).

Young cigar leaves of selected hybrids were analysed for their ploidy level by measuring the size of the nuclear genome by this method. The cigar leaves were cut using sharp sterile blade up to 15-20 centimetres length from top, cleaned gently with sterile distilled water and wrapped with partially wetted sterilized whatman No.3 filter paper. The samples were then packed in zipped polyethylene cover and sent to the Laboratory of Molecular Cytogenetics and Cytometry, Czech Republic for ploidy analysis by Dolezel **(**1997**)**. Flow cytometry ploidy assay involved preparation of suspensions of intact nuclei from small amounts of leaf tissue and the analysis of fluorescence intensity after staining. Relative fluorescence intensity of stained nuclei was analysed using a partec ploidy analyser with a mercury arc lamp. The distribution of fluorescence intensities (relative DNA content) obtained after flow cytometric analyses are usually given as channel number. The ploidy screening, the instrument was calibrated using reference (standard) diploid (2x) with its peak set and other hand was used as the reference tetraploid (4x) with its peak set and other reference triploid (3x) with its peak set. The peaks of the unknown samples were determined by examining the position of their peaks relative to the reference accessions. Diploid banana (2x) nuclei were included in every sample as an internal reference standard.

Results and Discussion

Success of conventional breeding in banana is very limited due to sterility, parthenocarpy and varying ploidy levels. Commercial bananas are mostly triploids and are vegetatively parthenocarpic. Diploids are not suitable because of their reduced fruit size and vigour by Simmonds (1962).

Genome and ploidy assessment based on morphological characters

Among the 24 of phase-I hybrids scored for genome assessment, six were diploid (AA and AB), five triploid (AAA and AAB), ten tetraploid (AABB) and three pentaploids (AAABB) were recorded (Table-3 and 4). Out of 24 hybrids, one triploid, three tetraploid and three pentaploids were (Table 1) confirmed by Flow-cytometry test (Fig. 3, 4 and 5), which is indicated in * mark in the end of the table 3. Among the 19 of Phase-II hybrids evaluated, one diploid (AB), four triploids (AAB) and fourteen tetraploids (AABB) were identified.

Assessment of ploidy status by stomatal characters

Ploidy levels of the phase I and II hybrids were studied through morphological scoring Simmonds and Shepherd (1955) and Singh *et al.,* (1993) and stomatal density. Among the 24 of phase-I hybrids scored for genome

assessment, six were diploid (AA and AB), five triploid (AAA and AAB), ten tetraploid (AABB) and three pentaploids (AAABB) were recorded (Table- 3 and 4). Among the 19 hybrids evaluated, one diploid (AB), four triploids (AAB) and fourteen tetraploids (AABB) were identified (Table 5 and 6 and Fig. 2).

The various indirect methods of determining banana ploidy level, for example by estimating stomatal size and density by Vandenhout *et al.,* (1995) or measurement of pollen grain sizes by Tenkouano *et al.,* (1998). The ploidy status of newly developed hybrids was assessed based on microscopic measurements of density and size of stomata in the leaves of the respective hybrids. Among the 24 hybrids scored for genome assessment, with reference to the stomatal density, all confirmed with morphological and flow cytometry tests already conducted. However, the genome, H 511, recorded a stomatal density of 1711.29 which is below the level of tetraploid, confirmed through flow cytometry. Among the diploid which was, the stomatal density varied from 50.79 to 85.02/mm^2 while in triploid, it ranged from 32.51 and 47.26/mm^2 in tetraploids, it ranged from 13.15 to 17.42/mm^2 but in pentaploids, the range was from 5.02 to 7.89/mm^2 (Table 4 and Fig. 1). The stomatal density decreased with the increase in ploidy level. The mean stomatal length, breadth and size in hybrids were 28.05µm, 25.91µm and 726.89µm^2 respectively for diploids; 36.79µm, 33.95µm and 1252.43µm^2 respectively for triploids; 46.02µm, 41.82µm and 1921.66µm^2 respectively for tetraploids and 51.74 µm, 44.64 µm and 2311.60 µm^2 respectively for pentaploids (Table 3 and 4 and Fig. 2). Based on stomatal density, length, breadth and size, the hybrids were grouped into diploids, triploids and tetraploids. Similarly, phase-II hybrids (Table 5 and 6), the diploid H-03-06 recorded a stomatal density of 55.20 /mm^2 and in triploids, it ranged from 32.89 to 47.14/mm^2. Among the tetraploids, the hybrid H-03-05 registered the minimum number (12.15 stomata/mm^2), while the hybrid H-02-19 registered the maximum 29.20 stomata/mm^2. The stomatal size varied significantly with ploidy levels and a minimum of 493.74 µm^2 was recorded by the diploid hybrid H-03-06, while the maximum 2318.42µm^2 by the tetraploid hybrid H-02-19 (Table 5 and 6). Reliability of ploidy determination using stomatal measurements by correlating stomatal traits with chromosome counts in root tips of the hybrids 'Obino 1' Ewai × Calcutta 4 was carried out by Vandenhout *et al.,* (1995). Size and densities of stomata, which are negatively correlated, varied according to ploidy level. Diploid hybrids had an average of 29 stomata/mm^2 with an average size (length × width) of 1250µm^2, while tetraploids had an average of 15 stomata/mm^2 with an average size of 1840µm^2. In a similar observation reported by Elain Apshara (2000) observed stomatal densities namely 43.52/mm^2, 31.08/mm^2, 17.27/mm^2 and 10.50/mm^2 for diploid, triploid, tetraploid and pentaploid hybrids, respectively.

Assessment of ploidy by using flow-cytometry

The hybrids viz., H 504, H 511, H 534, H 537, H 540, H571 and H 573, which were found deviating from the scale and score, were referred to Dr. Jaroslav Dolezol, Laboratory of Molecular cytogenetics and cytometry, Institute of Experimental Botany, Czech Republic for flow cytometry analysis to fix the ploidy levels. The result of flow-cytometry analysis revealed that one triploid, three tetraploid and three pentaploid progenies (Table 4). The ploidy of individual plant was estimated based on the ratio of peaks corresponding to G1 nuclei of *Musa* sample and reference standard (2x) (Fig. 3, 4 and 5).

Stomatal density

Ploidy	Stomatal density (No. of stomata/mm^2)	Stomatal size (µm^2)
Diploids	40.00 – 50.00	1250.00
Triploids	30.00 – 40.00	1250-1840
Tetraploids	9.00 - 15.20 Sathiamoorthy (1973)	1840.00 Vandenhout *et. al.*, (1995)

Table.1 Taxonomic scoring of banana cultivars by Simmonds and Shepherd (1955)

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

Table.2 Modified scoring by Singh and Uma (1996)

Genomes	Score card	
	Simmonds and Shepherd (1955).	**Singh and Uma** (1996).
AA/AAA	15 – 23	15 – 25
AAB	24 – 46	26 – 45
AB	49	46 – 49
ABB	59 – 63	59 –65
ABBB	67	66 – 69
BB/BBB	-	70 –75

Flow- cytometry analysis

Table.3 Genome assessment of banana hybrids under phase I evaluation

S.N	Hybrids	Parentage	Genome	Scoring	Chromosome no.	Ploidy
1	H 504***	H-03-09 x PL	AAABB	51.0	55	5X
2	H 508	ANK x PL	AA	21.0	22	2X
3	H 511**	H-02-34 x Ykm-5	AABB	56.0	44	4X
4	H 515	Mano x ANK	AAA	22.0	33	3X
5	H 516	ANK x PL	AA	23.0	22	2X
6	H 529	H-03-16 x ANK	AABB	52.0	44	4X
7	H 530	H-03-13 (OP)	AABB	53.0	44	4X
8	H 531	Poovan x PL	AAB	28.0	33	3X
9	H 532	H-201 x Mano	AAB	29.0	33	3X
10	H 534*	H-03- 13 x Rose	AAB	38.0	33	3X
11	H 537**	(H-201x PK) x Rose	AABB	52.0	44	4X
12	H 540***	(H-201 x PK) x Rose	AAABB	54.0	55	5X
13	H 542	H-02-34 x ANK	AABB	55.0	44	4X
14	H 547	H-02-23(OP)	AABB	53.0	44	4X
15	H 548	H-02-23(OP)	AABB	56.0	44	4X
16	H 556	H-04-06 x Ykm-5	AABB	59.0	44	4X
17	H 563	H-201 x PL	AB	44.0	22	2X
18	H 564	H-201 x PL	AB	46.0	22	2X
19	H 571**	H-04-05 x Ykm-5	AABB	63.0	44	4X
20	H 572	H-03-35 (OP)	AAB	28.0	33	3X
21	H 573***	H-03-12 x Rose	AAABB	61.0	55	5X
22	H 576	H-201(OP)	AB	46.0	22	2X
23	H 579	Mano x Rose	AA	25.0	22	2X
24	H 589	H-03-19 (OP)	AABB	57.0	44	4X

PL– Pisang Lilin; ANK – Anaikomban; PK-Peykunnan; OP- Open Pollinated; Mano- Manoranjitham
AA/ AAA-15-25; AAB-26-45; AB-46-49; ABB-59-65; ABBB-66-69
(* Triploid, ** Tetraploid, *** Pentaploid- Flow cytometry tested)

Table.4 Assessment of ploidy in phase I hybrids by stomatal characters

S. No	Hybrids	Parentage	Genome	Ploidy	Stomatal density (no./ $mm^{2)}$	Stomatal length (µm)	Stomatal Breadth (µm)	Stomatal size($µm^2$)
1	H-504	H-03-09 x PL	AAABB	5X	6.16	51.44	45.62	2346.69
2	H-508	ANK x PL	AA	2X	85.02	25.70	26.4	678.48
3	H-511	H-02-34 x Ykm#5	AABB	4X	20.13	45.72	37.43	1711.29
4	H-515	Mano. x ANK	AAA	3X	34.59	37.20	34.30	1275.96
5	H-516	ANK x PL	AA	2X	83.60	25.80	25.40	655.32
6	H 529	H-03-16 x ANK	AABB	4X	21.47	45.35	41.98	1903.79
7	H 530	H-03-13 (OP)	AABB	4X	24.27	45.66	42.20	1926.85
8	H-531	Poovan x PL	AAB	3X	37.26	37.20	35.25	1311.30
9	H-532	H-201 x Mano.	AAB	3X	35.00	37.10	34.60	1283.66
10	H-534	H-03- 13 x Rose	AAB	3X	30.08	38.84	35.41	1375.32
11	H-537	(H-201 x PK) x Rose	AABB	4X	22.51	49.19	46.55	2289.79
12	H-540	(H-201 x PK) x Rose	AAABB	5X	5.02	56.25	44.65	2511.56
13	H 542	H-02-34 x ANK	AABB	4X	16.44	46.20	41.40	1912.68
14	H-547	H-02-23(OP)	AABB	4X	23.39	46.35	41.76	1935.38
15	H-548	H-02-23(OP)	AABB	4X	28.45	45.27	42.48	1923.07
16	H-556	H-04-06 x Ykm#5	AABB	4X	13.89	44.73	41.98	1877.77
17	H 563	H-201 x PL	AB	2X	50.79	29.67	24.49	726.62
18	H 564	H-201 x PL	AB	2X	51.45	28.95	26.55	768.62
19	H 571	H-04-05 x Ykm#5	AABB	4X	25.08	44.47	41.92	1864.18
20	H 572	H-03-35 (OP)	AAB	3X	32.51	38.60	36.20	1397.32
21	H-573	H-03-12 x Rose	AAABB	5X	7.89	47.54	43.68	2076.55
22	H 576	H-201 (OP)	AB	2X	73.39	26.20	25.90	678.58
23	H 579	Mano. x Rose	AA	2X	77.63	31.95	26.72	853.70
24	H 589	H-03-19 (OP)	AABB	4X	28.95	47.26	39.53	1868.19

PL – Pisang Lilin; ANK – Anaikomban; PK- Peykunnan; OP- Open pollinated; Mano – Manoranjitham

Table.5 Genome and ploidy assessment in phase II hybrids through morphological scoring (Sucker to Harvest)

S.N	Hybrids	Parentage	Genome	Mark scored	Ploidy
1	H-02-19	KAR x RED	AABB	60	4X
2	H-02-23	KAR x RED	AABB	59	4X
3	H-02-26	KAR x RED	AABB	63	4X
4	H-02-34	KAR x RED	AABB	62	4X
5	H-03-05	Peykunnan (OP)	AABB	59	4X
6	H-03-06	H-02-32 x PL	AB	49	2X
7	H-03-13	Peykunnan x EV	AABB	56	4X
8	H-03-16	Peykunnan x PL	AABB	62	4X
9	H-03-17	Peykunnan x PL	AABB	58	4X
10	H-03-19	Peykunnan x EV	AABB	60	4X
11	H-04-05	H-02-32 x PL	AABB	47	4X
12	H-04-06	H-02-32 x PL	AABB	35	4X
13	H-04-10	Peykunnan (OP)	AAB	30	3X
14	H-04-12	Pisang Saba x PL	AABB	62	4X
15	H-04-21	H-02-10 x PL	AAB	44	3X
16	H-04-24	Peykunnan (OP)	AABB	61	4X
17	NPH-02-01	H 201 x ANK	AAB	42	3X
18	H-510	Poovan (OP)	AABB	61	4X
19	H-531	Poovan x PL	AAB	28	3X

AA/ AAA-15-25; AAB-26-45; AB-46-49; ABB-59-65; ABBB-66-69

ANK – Anaikomban; EV – Erachivazhai; PL – Pisang Lilin; OP- Open pollinated; KAR-Karpooravalli; RED- Red banana

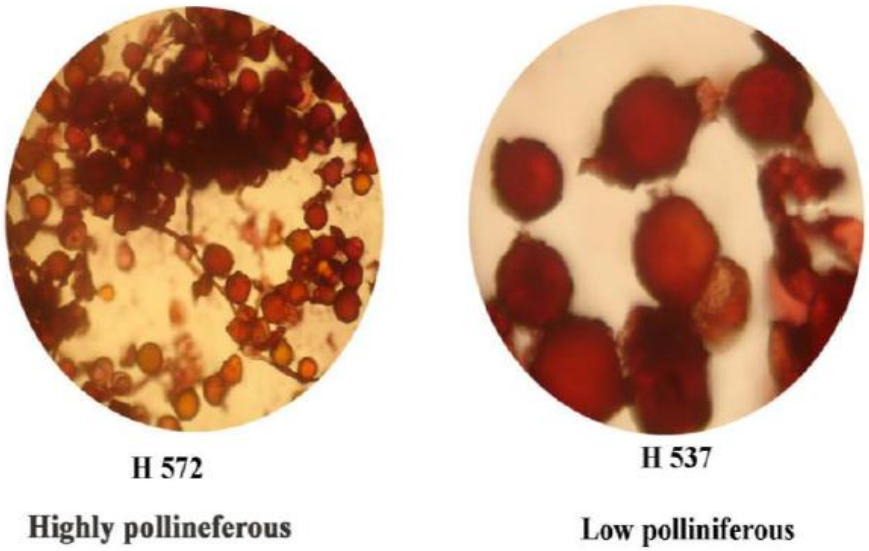
Table.6 Assessment of ploidy in phase II hybrids by stomatal characters

S.N	Hybrids	Parentage	Genome	Stomatal density ($mm^{2)}$	Stomatal length (µm)	Stomatal Breadth (µm)	Stomatal size ($µm^2$)
1	H-02-19	KAR x RED	AABB	29.20	48.20	48.10	2318.42
2	H-02-23	KAR x RED	AABB	22.10	35.00	35.00	1225.00
3	H-02-26	KAR x RED	AABB	28.15	41.00	41.00	1681.00
4	H-02-34	KAR x RED	AABB	23.00	31.80	31.60	1004.88
5	H-03-05	Peykunnan (OP)	AABB	12.15	42.20	37.70	1590.94
6	H-03-06	H-02-32 x PL	AB	55.20	23.40	21.10	493.74
7	H-03-13	Peykunnan x EV	AABB	19.12	43.10	41.05	1795.12
8	H-03-16	Peykunnan x PL	AABB	18.60	41.90	35.15	1472.79
9	H-03-17	Peykunnan x PL	AABB	12.70	38.45	31.86	1225.02
10	H-03-19	Peykunnan x EV	AABB	20.70	39.55	34.94	1381.88
11	H-04-05	H-02-32 x PL	AABB	13.15	44.10	41.65	1836.77
12	H-04-06	H-02-32 x PL	AABB	16.44	39.60	36.75	1455.30
13	H-04-10	Peykunnan (OP)	AAB	32.89	38.60	31.86	1229.80
14	H-04-12	Pisang Saba x PL	AABB	13.15	42.58	34.60	1473.27
15	H-04-21	H-02-10 x PL	AAB	47.14	38.45	32.77	1260.01
16	H-04-24	Peykunnan (OP)	AABB	13.18	39.55	36.90	1459.40
17	NPH-02-01	H 201 x ANK	AAB	36.20	25.55	23.60	602.98
18	H-510	Poovan (OP)	AABB	23.28	39.35	36.70	1444.15
19	H-531	Poovan x PL	AAB	37.00	37.20	35.25	1311.30
SEd				0.913	0.589	0.542	26.166
CD(.05 %)				1.853	1.194	1.099	53.073
CD(.01%)				2.484	1.601	1.474	71.165

ANK – Anaikomban; EV – Erachivazhai; PL – Pisang Lilin; OP- Open pollinated; KAR-Karpooravalli; RED- Red banana

Plate 1. Stomatal variation in Banana Hybrids

Plate 2. Palynological studies of Banana bybrids



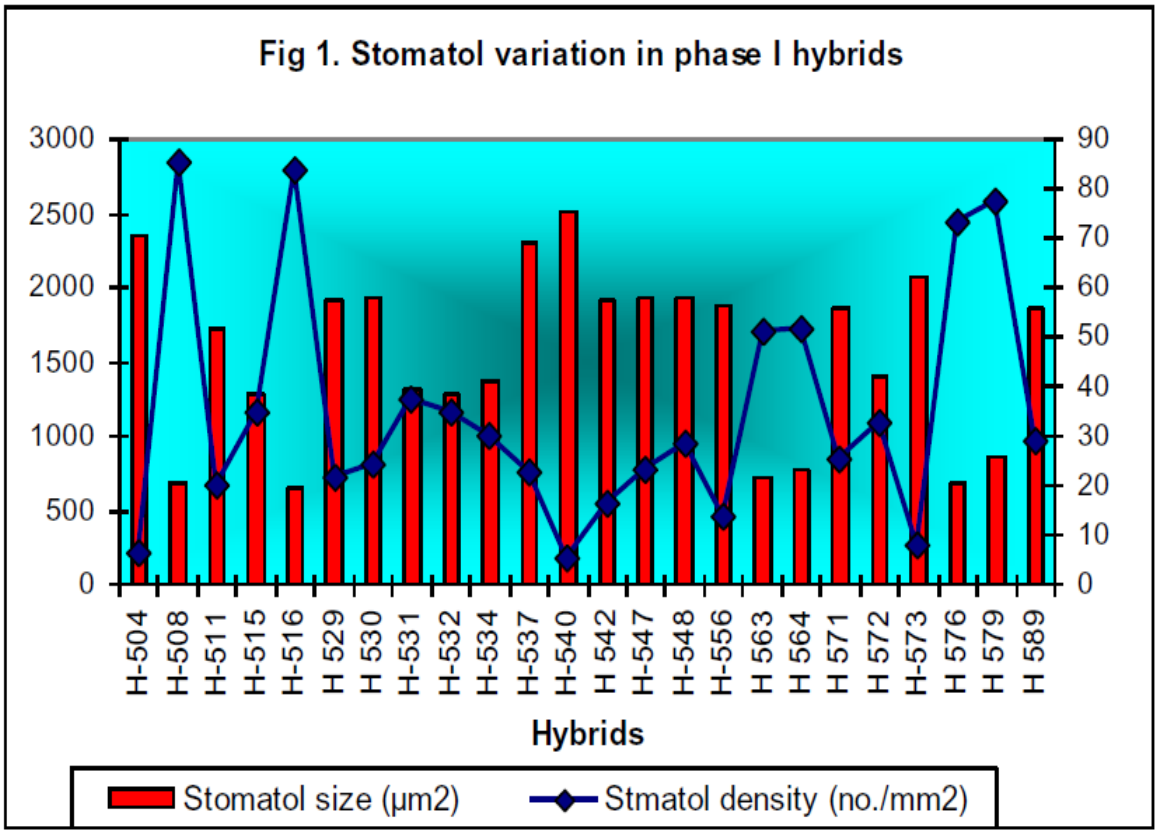


Fig 1. Stomatol variation in phase I hybrids
3000
2500
2000
1500
1000
500
0
90
80
70
60
50
40
30
20
10
0
H-504
H-508
H-511
H-515
H-516
H 529
H 530
H-531
H-532
H-534
H-537
H-540
H 542
H-547
H-548
H-556
H 563
H 564
H 571
H 572
H-573
H 576
H 579
H 589
Hybrids
Stomatol size (µm2)
Stmatol density (no./mm2)

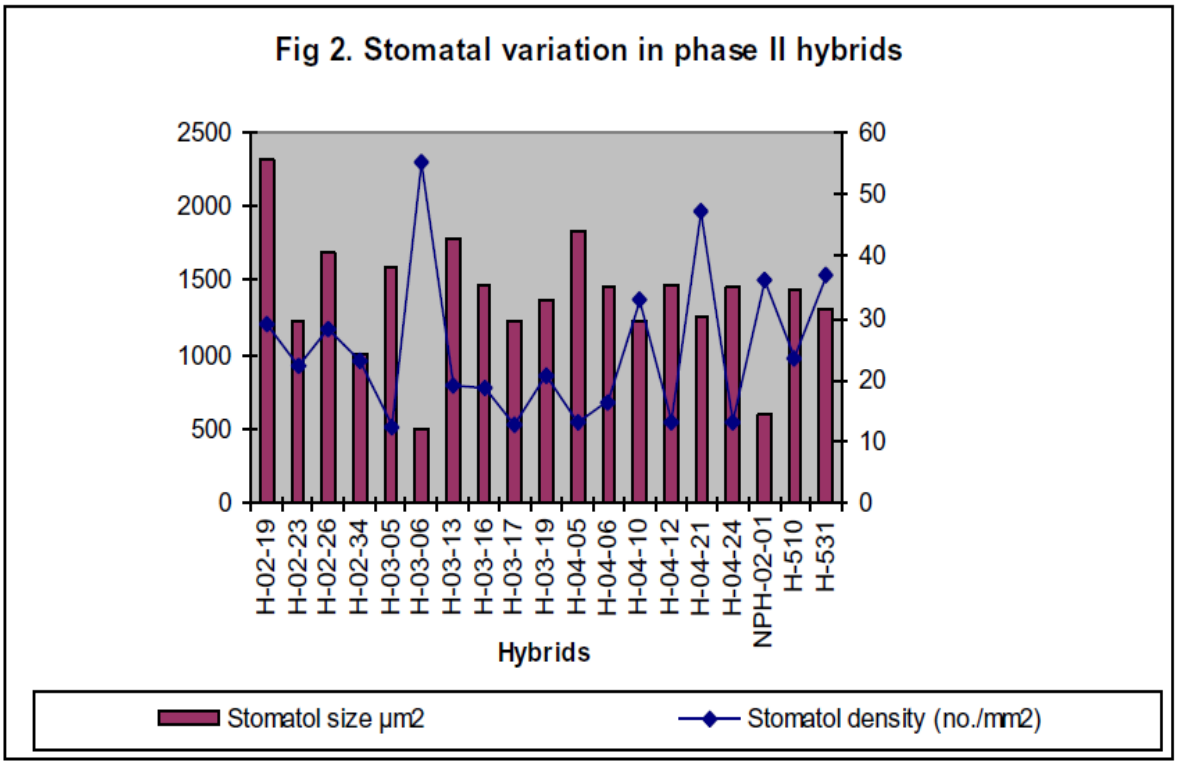


Fig 2. Stomatal variation in phase II hybrids
2500
2000
1500
1000
500
0
60
50
40
30
20
10
0
H-02-19
H-02-23
H-02-26
H-02-34
H-03-05
H-03-06
H-03-13
H-03-16
H-03-17
H-03-19
H-04-05
H-04-06
H-04-10
H-04-12
H-04-21
H-04-24
NPH-02-01
H-510
H-531
Hybrids
Stomatol size µm2
Stomatol density (no./mm2)

Fig:3 H 504 (AABBB)

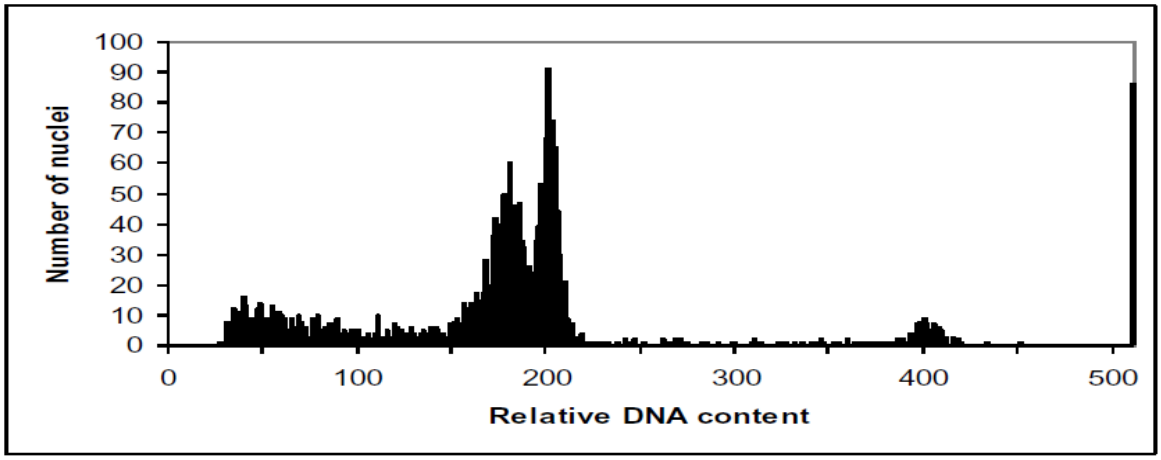


Fig: 4 H 511 (AABB)

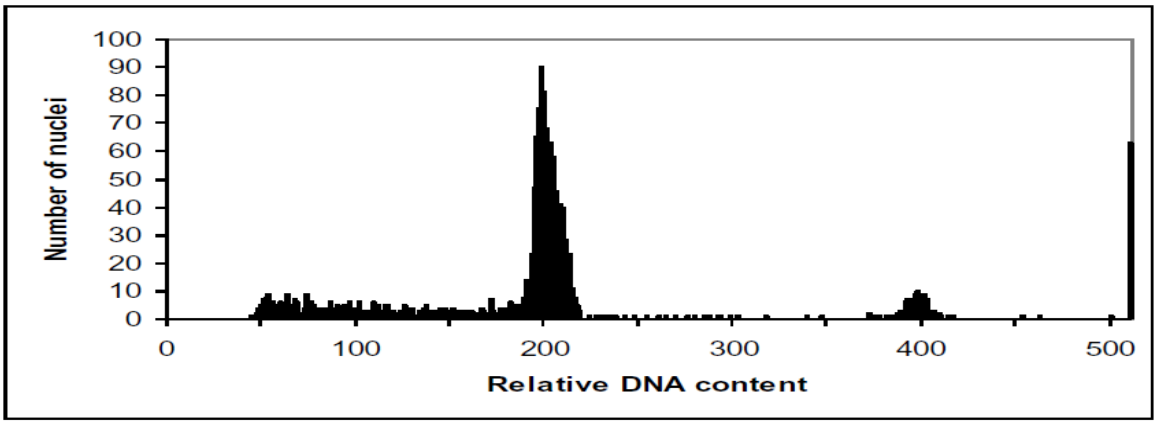


Fig:5 H 534 (AAB)

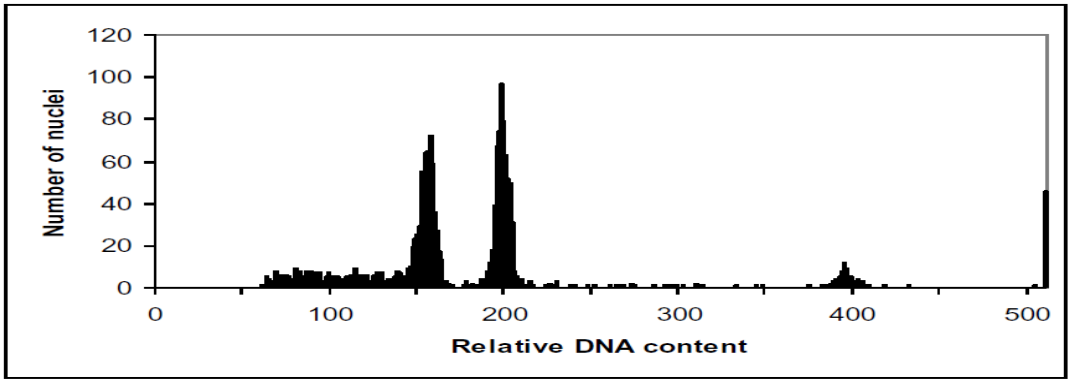


Fig: 3, 4 and 5. Flow cytometry analysis of selective banana hybrids for ploidy confirmation

Banana taxonomists have always assigned ploidy levels to different accessions on the basis of morphological traits such as leaf orientation, and biochemical aspects by Mustafa (2013). The ploidy of plants with large chromosomes can easily be determined by chromosome counting but bananas present a challenge due to its small chromosomes which are always hard to spread out during squash preparations by Dolezel *et al.*,(1998), Pillay and Tenkouano (2011). Flow cytometry is a user-friendly technique, considering the fact that it is faster and reproducible for screening large number of accessions.

Ploidy and parthenocarpy assessment of hybrids

Ploidy level of banana hybrids was fixed through morphological scoring as described by Simmonds (1952) and Singh *et al.,* (2001). Besides, stomatal density and flow cytometry analysis of nuclear DNA by Dolezel *et al.*, (1998) were the other tools used in recent years. Among the three methods, flow cytometry analysis is considered as the recent and reliable because, it is precise and rapid method when other methods were inconclusive. Precision is more because of the analysis of the nuclear DNA, which is not affected by the environmental factors. In the present investigation, ploidy was fixed using stomatal density, morphological scoring and flow-cytometry. Among the 24 hybrids, in phase-I, 6 were found to be diploid (AA and AB), 5 triploids (AAA and AAB), 10 tetraploids (AABB) and 3 pentaploids (AAABB) (Table 3 and 4). The pentaploid hybrids obtained in this investigation were resulted from the cross between tetraploids (AABB) as female and diploids (AA) as male parent. Classification based on stomatal density agrees with the earlier reports of Sathiamoorthy (1987). The doubtful hybrids were subjected to flow-cytometry analysis for confirmation of ploidy. The origin of pentaploids might be through a fusion of unreduced gametes from the tetraploid parent with reduced gametes from the diploid parent and the frequency of occurrence of unreduced gametes is genotype-dependent. Result of different ploidies of the selected hybrids as compared with nuclei isolated from diploid hybrid (2x) used as internal reference standard reveals that H 504, H 540 and H 573 are clear pentaploids. Determination of nuclear DNA increased the reliability of ploidy and easy detection of mixiploids by Dolezel *et al.,* (1997). Occurrence of pentaploids in 4n x 2n cross was also earlier reported by many workers.

Hands of hybrids of phase I evaluation were bagged to study the female fertility/ parthenocarpiness. Among the hybrids evaluated in phase I generation, fifteen were found to be parthenocarpic and the rest viz., H 511, H 529, H 530, H 537, H 542, H 547, H 548, H 556 and H 571 were non parthenocarpic (Table 3 and 4). However, some of the parthenocarpic hybrids when pollinated artificially produced seed. Elain Apshara (2000) also observed similar results. Selection and utilization of parents with parthenocarpic pedigree might have contributed for enhanced parthenocarpy in the present investigation.

It also confirmed the role of dominant genes in controlling parthenocarpy by Simmonds (1953). Using flow cytomery, previous studies have shown inconsistencies in ploidy levels of banana accessions whose ploidy was determined based entirely on morphological traits by de Jesus *et al.,* (2013), *Dolezel et al.,* (1994), Irish *et al.,* (2009), Nsabimana *et al.,* (2006). Karamura *et al.,* (2016) studies the ploidy level of 120 banana accession in the ex situ germplasm collection centre for the East and Central Africa through the flow cytometric analysis of the nuclear DNA content was used to determine the ploidy level of the accessions. Flow cytometry provides a rapid way of determining ploidy levels in this crop.

Out of 24 hybrids taken for Phase I evaluation, six diploids (AA and AB), five triploids (AAA and AAB), ten tetraploids (AABB) and three pentaploids (AAABB) were found. Among the 19 phase II hybrids evaluated, one diploid (AB),

four triploids (AAB) and fourteen tetraploids (AABB) were observed based on stomatal characters and morphological scoring and flow-cytometry studies. Knowledge of the ploidy of bananas is valuable for banana breeding schemes as it involves interploidy crossed leading to several possible ploidy levels in the progeny. Flow cytometry provides a rapid way of determining ploidy levels in Banana.

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