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Mitotic instability in two wild species of bananas (*Musa acuminata* and *M. balbisiana*) and their common cultivars in Malaysia

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Abstract – Eumusa series of banana contains the majority of commercially important cultivars, which are interspecific hybrids of two wild species of bananas, *Musa acuminata* Colla (*AA* Group) and *Musa balbisiana* Colla (*BB* Group). Most of the banana cultivars are diploids or triploids. Occurrences of 'laggard' and 'bridge' during anaphase were considered as aberrant. Aberrant anaphase cells were observed in the root tip cells of wild *Musa acuminata* Colla (*AA* Group) (natural and tissue cultured materials), *M. balbisiana* Colla (*BB* Group) and the common local banana cultivars: Pisang Mas (*AA* Group), Pisang Berangan (*AAA* Group), Pisang Rastali (*AAB* Group), Pisang Raja (*AAB* Group), Pisang Awak (*ABB* Group) and Pisang Abu Nipah (*BBB* Group). Frequencies of aberrant anaphase cells were scored and statistically analyzed. Results indicated that somatically, 1) cultivated bananas were more stable than wild bananas, 2) triploids were more stable than diploids and 3) *M. balbisiana* or *B* genome was more stable than *M. acuminata* or *A* genome. In addition, environmental factors played a role in mitotic stability.

Key words: Banana cultivars, bispecific origin, genomic constitution, laggard, mitotic instability, ploidy level, wild banana.

INTRODUCTION

A botanist, Sulpiz Kurz recognized the bispecific origins of banana cultivars in 1865 (SIMMONDS 1962). Eumusa series of banana contains the majority of commercially important cultivars, which are interspecific hybrids of two wild species of bananas, *Musa acuminata* Colla (*AA* Group) and *M. balbisiana* Colla (*BB* Group). Most of the banana cultivars are diploids or triploids. There are numerous reports on the cytological effects of these interesting genomic features. DODDS (1943) described male cytology of certain edible diploids and observed a high degree of chromosome association at first metaphase of banana meiosis. WILSON (1946) examined the meiosis of five triploid cultivar clones and found generally low association and great variations in pairing between cells within one sample and between samples from different places. However, it is not ploidy level alone that plays a significant role in meiotic chromosome association, but also the combinations of the interspecific hybrid genomes. For example, DODDS (1943) found that *AB* genome (Ney poovan) has a lower and variable chromosome pairing in comparison to *AA* genomes (Sucrier, Palembang, Bandé, Pisang lilin, and Tongat) among the diploids. WILSON's (1946) experiment showed that *AAB* genome (French Plantain) has a higher frequency of association among other triploids (*AAA*-Gros Michel, Pisang Masak Hijau,

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and Dwarf Cavendish; *ABB*-Bluggoe). Mitotic study vis-à-vis these topics in banana are relatively poor. However chromosome instability in mitosis for other plants is widely reported. Chromosome instability in somatic tissue within an individual plant contributes to intraplant variations in some morphological characters in grasses (JALAL and NIELSEN 1967; NIELSEN 1968). In certain specific somatic tissues, somatic reduction was observed in apogeotropic roots in Cycads (STOREY 1968a) and endomitotic variations in ploidy levels as in roots of *Chlorophyton elatum* (STOREY 1968b). Somatic mitosis in *Cymbopogon flexuosus* exhibits a significant degree of chromosome instability (LAVANIA 1987). TAN (1977) demonstrated that the octoploid of *Bromus inermis* is somatically more stable than the lower ploidies by examining the frequency of abnormal anaphase in the root tip cells of three different ploidies of *Bromus inermis* clones. First anaphases are predominantly regular in bananas (SIMMONDS 1962). However, lagging (usually dividing) univalents (relicts from metaphase pairing failure) and bridges occur with low frequencies in many clones. Chromosomal fragments were observed but not always accompany these aberrations. SIMMONDS and DODDS (1949) surveyed 31 clones of which 20 clones showed no bridges; four with bridges and fragments; six with bridges only; and one with both kinds of bridge. Frequencies of the abnormalities per cell ranged from 0.01 to 0.19. The present paper describes the mitotic instability in *Musa acuminata* Colla (*AA* Group) and *M. balbisiana* Colla (*BB* Group) and their common cultivars in Malaysia with different ploidy levels and genomic constitutions by examining the frequencies of abnormal anaphases in their root tip cells.

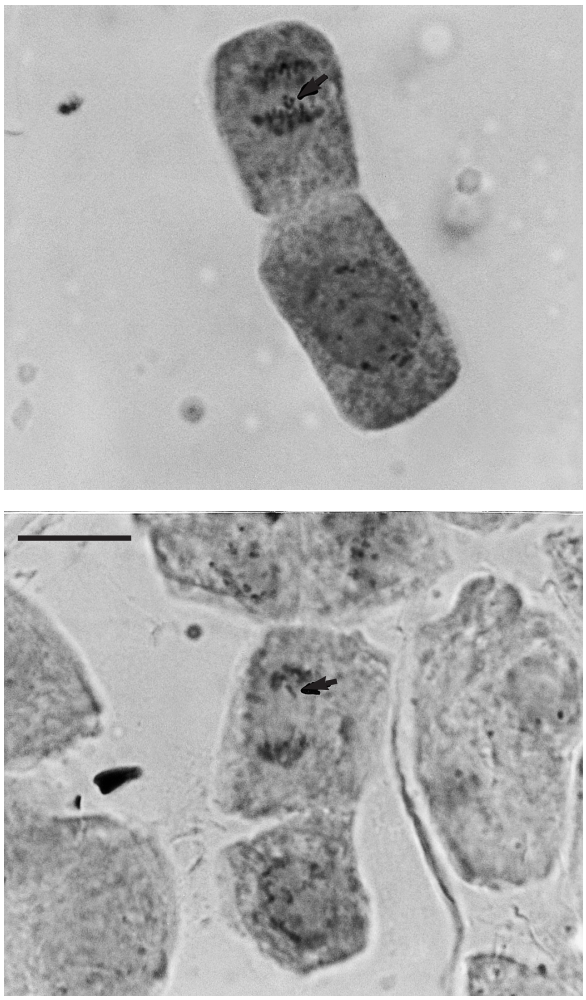

Fig. 1 – Anaphase cells with lagging chromosomes from banana root tip at 10 x 100 magnification.
Bar (15mm) = 10 µm

MATERIALS AND METHODS

Plants

Wild *Musa acuminata* Colla (*AA* Group) (abbreviated in this paper as NA) was obtained around the University of Malaya whereas *Musa balbisiana* Colla (*BB* Group) (NB) was obtained from the banana farm of Malaysian Agricultural Research and Development Institute (MARDI). Tissue cultured *Musa acuminata* Colla (*AA* Group) (CA) was obtained from the tissue culture laboratory, Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya. Local banana cultivars: Pisang Mas (*AA* Group) (MS), Pisang Berangan (*AAA* Group) (BR), Pisang Rastali (*AAB* Group) (RS), Pisang Raja (*AAB* Group) (RJ), Pisang Awak (*ABB* Group) (AW), and Pisang Abu Nipah (*BBB* Group) (AB) were obtained from a local farm in a Malay village in Tanjung Karang, Selangor, Malaysia.

Sampling

Banana plants always grow in clumps due to their asexual propagation. For natural plants, four clumps of plants per type were identified and 4-10 root tips were collected from each clump of plants. The root tips were prepared into 4-12 slides. Four slides with the highest mitotic activity were examined. For tissue culture materials, four culture bottles of plants were identified and 4-10 root tips were collected from a

Table 1 – Frequencies of aberrant anaphase cells observed in wild bananas and their local cultivars in Malaysia. Root tip specimens were obtained from four different clumps of plants for each banana type.

Musa acuminata Colla (*AA* Group) (Tissue Culture)				*Musa acuminata* Colla (*AA* Group)				*Musa balbisiana* Colla (*BB* Group)			
Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4
0.28	0.22	0.12	0.22	0.18	0.10	0.08	0.12	0.12	0.10	0.14	0.08
0.26	0.08	0.13	0.16	0.26	0.06	0.06	0.10	0.08	0.10	0.10	0.06
0.28	0.14	0.13	0.16	0.26	0.08	0.08	0.15	0.10	0.06	0.04	0.14
0.20	0.26	0.13	0.10	0.18	0.08	0.04	0.23	0.04	0.12	0.08	0.10
Pisang Mas (*AA* Group)				Pisang Berangan (*AAA* Group)				Pisang Raja (*AAB* Group)			
Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4
0.08	0.06	0.08	0.14	0.02	0.02	0.08	0.06	0.06	0.04	0.08	0.02
0.14	0.08	0.08	0.12	0.02	0.06	0.06	0.06	0.08	0.08	0.04	0.02
0.04	0.10	0.08	0.06	0.06	0.06	0.10	0.04	0.04	0.04	0.04	0.06
0.14	0.06	0.08	0.12	0.04	0.04	0.08	0.06	0.06	0.04	0.12	0.04
Pisang Rastali (*AAB* Group)				Pisang Awak (*ABB* Group)				Pisang Abu Nipah (*BBB* Group)			
Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4	Clump 1	Clump 2	Clump 3	Clump 4
0.06	0.12	0.08	0.08	0.08	0.06	0.08	0.06	0.02	0.04	0.04	0.02
0.06	0.06	0.06	0.04	0.04	0.04	0.06	0.08	0.02	0.02	0.02	0.04
0.08	0.12	0.04	0.06	0.02	0.06	0.04	0.08	0.04	0.02	0.04	0.02
0.04	0.10	0.08	0.04	0.08	0.04	0.04	0.04	0.02	0.02	0.02	0.02

culture bottle of plants. Root tips squashed were made up to 4-12 slides and four slides with the highest mitotic activity were examined.

Slide Preparation

Fresh root tips were fixed in 3:1 alcohol/acetic acid solution overnight. Root tips were hydrolysed in 1N HCl at 60ºC for 15 minutes and then stained with the Feulgen solution at room temperature for one hour (DARLINGTON and LACOUR 1962). The root tips were then squashed in a drop of aceto-carmine. Slides were put in saturated alcohol chambers overnight. They were then mounted in euparal and put in petri dishes with one or two drops of alcohol overnight. The slides were then dried on hot plates at 45ºC for three days.

Aceto-carmine seemed to stain unspecifically and Feulgen staining seemed not solidly dark in the case of banana root tips. The counter-staining method as suggested above solved the problem as the Feulgen solution stained the chromosome specifically and acetic carmine solution enhanced the Feulgen staining.

Scoring

Large numbers of anaphase cells were observed from the non-pretreated root tips. Anaphase cells with bridges or laggards were scored as aberrant (Fig. 1). 50 anaphase cells per slide were scored and the frequen-

Table 2 – Maximum, minimum, and means of frequencies of aberrant anaphase cells observed in wild bananas and their local cultivars in Malaysia.

Banana Plant	Abbreviation	Frequency of Aberrant Anaphase Cells		
		Maximum	Minimum	Mean
Musa acuminata Colla (*AA* Group)(Tissue Culture)	CA	0.28	0.08	0.179375
Musa acuminata Colla (*AA* Group)	NA	0.26	0.04	0.12875
Musa balbisiana Colla (*BB* Group)	NB	0.14	0.04	0.09125
Pisang Mas (*AA* Group)	MS	0.14	0.04	0.09125
Pisang Berangan (*AAA* Group)	BR	0.10	0.02	0.05375
Pisang Raja (*AAB* Group)	RJ	0.12	0.02	0.05375
Pisang Rastali (*AAB* Group)	RS	0.12	0.04	0.07000
Pisang Awak (*ABB* Group)	AW	0.08	0.02	0.05625
Pisang Abu Nipah (*BBB* Group)	AB	0.04	0.02	0.02625

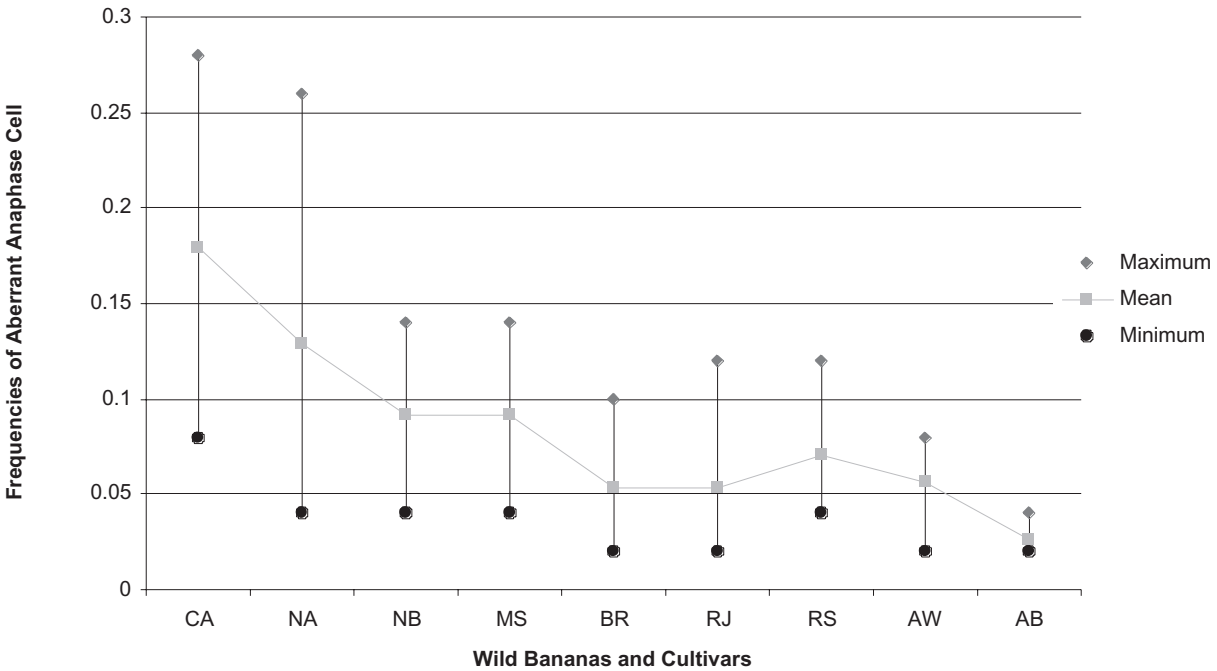

Fig. 2 – Maximum, minimum, and means of frequencies of aberrant anaphase cells observed in wild bananas and their local cultivars in Malaysia. Abbreviations: CA= *Musa acuminata* Colla (*AA* Group)(Tissue Culture); NA= *Musa acuminata* Colla (*AA* Group); NB= *Musa balbisiana* Colla (*BB* Group); MS= Pisang Mas (*AA* Group); BR= Pisang Berangan (*AAA* Group); RJ= Pisang Raja (*AAB* Group); RS= Pisang Rastali (*AAB* Group); AW= Pisang Awak (*ABB* Group) and AB= Pisang Abu Nipah (*BBB* Group).

cies of aberrant anaphase cells of the sample (50 cells) were calculated. The sample size of 50 scored cells per slide was determined by calculating and plotting cumulative or running frequencies according to principle of running mean (KERSHAW 1964).

Analyses

Mitotic instability was expressed in terms of the percentages or frequencies of aberrant anaphase cells in the samples. Arcsine-transformation was done on the data sets to stabilize the variance and improve the normality (ZAR 1984). The arcsine-square root transformation was calculated from P' = arcsine (square root of P), where P is the percentage or frequency to be transformed and P' is the transformed percentage or frequency. Transformed data were submitted to a nested analysis of variance (nested ANOVA) with the null hypotheses tested being: 1) There is no difference in mitotic instability among banana types and 2) there is no difference among clumps/culture bottles in affecting mitotic instability. Plant clump/culture bottle (random-effect factor) was nested within each type of banana (main factor). If the nested ANOVA-test was significant (i.e. at least one pair was different), Duncan's New Multiple Range Test (DMRT) (DUNCAN 1955) was carried out to determine which pairwise comparisons were different. Computer software Statistica™ Version 5 facilitated all statistical tests.

RESULTS AND DISCUSSIONS

The frequencies of aberrant anaphase cells observed in wild bananas and their local cultivars in Malaysia are shown in Table 1. A range of 8-28% of aberrant anaphase cells were observed in tissue cultured wild *Musa acuminata* Colla (*AA* Group), whereas natural wild *M. acuminata* Colla (*AA* Group) had 4-26% of aberrant anaphase cells. Frequencies of aberrant anaphase cells observed in *M. balbisiana* Colla (*BB* Group) were in the range of 4-14%. While for local banana cultivars, these were: Pisang Mas (*AA* Group) 4-14%, Pisang Berangan (*AAA* Group) 2-10%, Pisang Rastali (*AAB* Group) 4-12%, Pisang Raja (*AAB* Group) 2-12%, Pisang Awak (*ABB* Group) 2-8%, and Pisang Abu Nipah (*BBB* Group) 2-4% (Table 2 and Fig. 2). The nested ANOVA indicated that percentages or frequencies of aberrant anaphase cells (mitotic instability) among the banana types ($P << 0.01$) and plant clumps/culture bottles were significantly different ($P << 0.01$) (Table 3). The DMRT results showed five homogenous groups based on mitotic instability. AB (*BBB*), NA (*AA*) and CA (*AA*) were significantly different from each other and all others. RJ (*AAB*), BR (*AAA*), AW (*ABB*), and RS (*AAB*),

Table 3 – Results of nested analysis of variance for determining the differences among groups, which were wild bananas and their cultivars tested and among subgroups, which were different clumps of plants for a type. From the results, there were differences among the banana types and plant clumps in affecting the mitotic instability.

	df Effect	MS Effect	df Error	MS Error	F	*P*-level
Groups	8	337.5483	108	8.79168	38.39406	3.19E-28***
Subgroups	27	31.38673	108	8.79168	3.570049	1.36E-06***

*: significant at 0.05 level; **: significant at 0.01 level; ***: significant at 0.001 level.

which formed one homogenous group, were not significantly different among themselves but significantly different from other groups. MS (*AA*) and NB (*BB*), which formed another homogenous group, were not significantly different between themselves but significantly different from other groups (Table 4).

Wild and Cultivated bananas

Cultivated bananas had comparatively higher mitotic stability than wild bananas (Fig. 2). Generally, cultivars had lower frequencies of aberrant anaphase cells, which ranged from 2.6 to 9.1% (mean) whereas those wild bananas ranged from 9.1 to 12.9% (mean) (Table 2). In a particular case, cultivated MS (*AA*) (mean frequencies of aberrant anaphase cells, 9.1%) was significantly more stable ($P < 0.01$) than wild NA (*AA*) (mean frequencies of aberrant anaphase cells, 12.9%) somatically although they have the same genomic constitution and ploidy level. The tested cultivars are all sterile and producing no seed, but wild bananas are segregating populations since they are seed producers. One of the possible reasons is that sterile plants asexually propagate and thus their genomes may be more homogenous. After all, they might have gone through process of selection and only those that were more stable in mitosis might have obtained higher chance of survival. The fertile plants produce more variations because they sexually and asexually propagate. In addition, fertile plants might cross among different species and subspecies. Thus, their genomes may be heterogeneous and this might lead to mitotic instability.

Ploidy level

TAN (1977) found that the octoploid of *Bromus inermis* is somatically more stable than the lower ploidies by examining the frequency of abnormal anaphase in the root tip cells of three different ploidies of *Bromus inermis* clones. The same conclusion could be drawn for bananas. Mean frequencies of aberrant anaphase cell in

Table 4 – Results of Duncan's New Multiple Range Test (DMRT), of which was used to make pair-wise comparison. Abbreviations: CA= *Musa acuminata* Colla (*AA* Group)(Tissue Culture); NA= *Musa acuminata* Colla (*AA* Group); NB= *Musa balbisiana* Colla (*BB* Group); MS= Pisang Mas (*AA* Group); BR= Pisang Berangan (*AAA* Group); RJ= Pisang Raja (*AAB* Group); RS= Pisang Rastali (*AAB* Group); AW= Pisang Awak (*ABB* Group) and AB= Pisang Abu Nipah (*BBB* Group).

	CA	NA	AB	NB	BR	RJ	RS	MS	AW
CA	—	0.000165***	1.62E-05***	4.54E-05***	2.02E-05***	1.67E-05***	2.84E-05***	5.65E-05***	2.37E-05***
NA	0.000165***	—	1.67E-05***	0.006645**	2.37E-05***	2.02E-05***	4.87E-05***	0.005024**	2.84E-05***
AB	1.62E-05***	1.67E-05***	—	2.37E-05***	0.00051***	0.000439***	2.86E-05***	2.02E-05***	0.00018***
NB	4.54E-05***	0.006645**	2.37E-05***	—	0.000225***	0.000235***	0.035934*	0.992277	0.000645***
BR	2.02E-05***	2.37E-05***	0.00051***	0.000225***	—	0.979472	0.071757	0.000247***	0.691841
RJ	1.67E-05***	2.02E-05***	0.000439***	0.000235***	0.979472	—	0.077835	0.000258***	0.693431
RS	2.84E-05***	4.87E-05***	2.86E-05***	0.035934*	0.071757	0.077835	—	0.044851*	0.131225
MS	5.65E-05***	0.005024**	2.02E-05***	0.992277	0.000247***	0.000258***	0.044851*	—	0.00077***
AW	2.37E-05***	2.84E-05***	0.00018***	0.000645***	0.691841	0.693431	0.131225	0.00077***	—

AB	RJ	BR	AW	RS	MS	NB	NA	CA
(0.02625)	(0.05375)	(0.05375)	(0.05625)	(0.07000)	(0.09125)	(0.09125)	(0.12875)	(0.179375)

*: significant at 0.05 level; **: significant at 0.01 level; ***: significant at 0.001 level.

triploids ranged from 2.6 to 7%, whereas diploids were from 9.1 to 12.9% (Table 2). Triploid bananas were somatically more stable than diploids bananas (Fig. 2). This could be explained by the reason suggested above that cultivated bananas had higher mitotic stability since most of the triploids used in this study are sterile and most of the diploids used are fertile.

Genome

Mitotic stability of *Musa acuminata* or *A* genome was less stable than *M. balbisiana* or *B* genome since the mean frequency of aberrant anaphase cells in NA (*AA*) was significantly higher ($P << 0.01$) than NB (*BB*) (Fig. 2 and Table 4). Mean frequency of aberrant anaphase cells in NA (*AA*) was 12.9% whereas NB (*BB*) was 9.1%. Results also showed that among the triploids, those genomic constitutions, which contain *A* genome showed lower stability in mitosis. BR (*AAA*), RJ (*AAB*), RS (*AAB*), and AW (*ABB*) were not significantly different from one another and their mitotic instabilities were about 5.4- 7% (mean) (Table 2). AB (*BBB*) was the most stable somatically compared to BR (*AAA*), RJ (*AAB*), RS (*AAB*), and AW (*ABB*) as it showed the highest mitotic stability of them all (2.6%).

Environment

Variability in chromosome number and morphology is more common in somatic cell cultured *in vitro* than in natural environment (BAYLISS 1980; LARKIN and SCOWCROFT 1981). This is one of the possible reasons for somaclonal variation occurring in tissue culture. *In vitro* environment affected mitotic instability in bananas, as the mean frequency of aberrant anaphase cells was significantly higher in CA (*AA*) ($P << 0.01$) than in NA (*AA*) (Fig. 2 and Table 4). Root tip specimens from different clumps (which were from different sites) of plants within a banana type were significantly different ($P << 0.01$) (Table 3). Thus, environment factors seemed to be important in determining mitotic stability even in the natural environment.

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