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Morphological Characterization and Karyotype Analysis of Abaca (*Musa textilis* Nee) and its Hybrids with *Musa balbisiana* Colla

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To determine if progenies from the cross between *Musa textilis* Nee (2n = 20) and *Musa balbisiana* Colla (2n = 22) produced at the Institute of Plant Breeding (IPB), University of the Philippines Los Baños are true hybrids, abaca cultivars (Abuab and Inosa), a banana cultivar (Pacol), and their back crosses (BC_1, BC_2, and BC_3) were characterized morphologically and cytogenetically. Agronomic characters and fiber quality assessment through ANOVA and pairwise analysis revealed significant differences in tensile strength and agronomic parameters, namely, fiber quality and percent fiber recovery, except for leaf sheath number and girth measured at the top. This confirmed the recovery of recurrent parent genome (abaca) until the third generation of backcrosses. Moreover, this study reports the successful optimization of cytogenetic techniques. The most favorable time of root tips collection was from 10:00 a.m. to 10:30 a.m. due to the high number of dividing cells observed compared to other time slots tried. Two-hour cold shock pre-treatment resulted in considerably larger chromosomes, and higher number of well-spread prometaphase cells that helped in the construction of karyograms. Chromosome characteristics based on chromosome count and relative length were determined and compared among plant samples. Comparative karyotyping revealed a diploid chromosome number of 2n = 20 for abaca cultivars and hybrids. Inosa, another cultivar of *M. textilis* Nee, was observed to have a diploid chromosome number of either 2n = 20 or 2n = 22, in contrast to an earlier report of 2n = 17 to 2n = 23.

Key Words: karyotype, chromosomes, hybrids, mitosis, *Musa textilis* Nee

INTRODUCTION

Abaca (*Musa textilis* Nee), a humid tropical plant with succulent stalks, is indigenous to the Philippines (Kligman 2006). It belongs to the Family *Musaceae* (Valmayor et al. 2002) and is known worldwide as a source of the strongest natural fiber (FIDA 2012) referred to as Manila hemp, due to its great strength and its resistance to the action of sea water. The Philippines supplies 85% of the world's need for the fiber, generating an average of US$80 million a year (Palacio 2005). Abaca is grown practically all over the Philippines, in about 56 provinces (PRDP 2014), except in the northernmost part of the country.

Even before World War II, the Philippines had already gained a monopoly of abaca fiber exports in the world trade. Abaca fiber is used as raw material for cordage, fiber crafts and pulp for the production of specialty paper products such as security papers, tea bags, cigarette papers, meat and sausage casings, non-woven and other thin printing papers (Bajet and Magnaye 2002). In 1801, Nee claimed that before the Spanish occupation, Filipinos have already been domesticating abaca and have been utilizing its fiber, supplying abaca to the world market (Halos 2008). When Ferdinand Magellan reached the country in 1521, the Filipinos were already wearing clothes woven from abaca (FIDA 2006). This has long been known and was first recorded by Pigafetta in 1521 (Tabora 1977).

Demand for abaca, particularly in pulp form, has been increasing due to the growing apprehension regarding environmental protection and forest conservation, which

provided more opportunities for the use of natural fibers like abaca. It is expected that demand for abaca fiber, particularly by local pulp processors, will continue to expand as world demand for abaca pulp continues to grow. In spite of the high demand for abaca and its high price, local production has not kept pace with increasing need (CFC/UNIDO/FIDA 2009). Between 2009 and 2013, Philippine abaca production decreased by 1%. Ecuador, the second largest abaca-producing country, supplies 15% of the world market. However, in 2014, Ecuador announced that it would cut down its abaca production by as much as 7,000 MT by the following year (PRDP 2014). This implies that there is a need for the Philippines to double the current production of abaca fiber, while ensuring an excellent and good quality fiber.

Viruses are a major threat to abaca and banana crops in the Philippines. Viral diseases pose serious problems to germplasm movement, particularly in the exchange and trade of the vegetative parts. These diseases are systemic in nature and oftentimes, during the early phase of their development, infected plants show no signs or some symptoms. Vegetative parts which are usually the ones being transported or exchanged could be infected without being detected by an expert observer. This is one of the ways viruses are spread into new areas. To the producers of banana and abaca, however, economic loss counts as the more significant factor to consider once viruses have invaded these host plants. The cost sustaining productivity is high once the viruses have established in an area. There are traditional methods of controlling the virus, such as use of healthy planting materials, quarantine procedures, immediate rouging of infected abaca plants, and heat treatment. Although these methods were proven to be effective, cases of bunchy top disease are still rampant (Bajet and Magnaye 2002).

Even though abaca is endemic to the Philippines, very limited attention is devoted to varietal improvement of the crop. The abaca industry is still relying solely on traditional varieties for its survival in the absence of new and improved ones. This is one of the many reasons for the decline of the abaca industry. The old abaca varieties had outlived their usefulness and now become easy prey for disease devastation. As early as 1980, there was a rapid decline in abaca production not only due to the unavailability of improved varieties but also to the havoc brought about by three major virus diseases, namely, abaca bunchy top (ABT), abaca mosaic (AM) and recently, abaca bract mosaic (BM). Resistant varieties were identified from the abaca germplasm and although resistant, they are often of inferior quality. The traditional varieties, although of superior fiber quality, are highly susceptible to these diseases. Some of the improved varieties being planted by farmers are likewise susceptible to these virus diseases. Backcross breeding was done in the Institute of Plant Breeding, University of the Philippines Los Baños with an abaca cultivar Abuab (*M. textilis* Nee) and a diploid wild banana cultivar Pacol (*M. balbisiana* Colla) as parental lines. The objective was to introduce virus-resistant genes from Pacol to the hybrids and simultaneously recover good fiber quality from the recurrent parent (abaca). To date, three generations of backcrosses had already been developed (Lalusin 2010). It is substantial to develop a comprehensive varietal improvement program for abaca in the country. Adequate supply of disease-resistant planting materials, which give higher yields of superior fiber, must be achieved.

Construction of karyograms will aid in creating chromosome maps for the cultivars of *M. textilis* Nee as well as *M. balbisiana*, as this cytogenetic tool links genetic and phenotypic characteristics (Dahmer 2013). Also, this will help identify genetic problems as the cause of disorder or a disease. Through cytogenetic and molecular characterizations, banding patterns will be achieved, revealing chromosome polymorphisms. Based on Portieles et al. (2004), polymorphisms could provide markers for the identification of individual chromosome segments to assist in the monitoring of introgression of the donor genome and the mapping of the gene linkage groups at the chromosome level. Morphological characterization and karyotype analysis will help confirm correctly the stability and parental contribution of desired traits such as good fiber quality and disease resistance from generation to generation.

To date, identification of all chromosomes within the karyotype of the genus *Musa* is not possible. Cizkova et al. (2015) reported that detailed cytogenetic studies were complicated by the small size of chromosomes, their morphological similarity and lack of chromosome-specific landmarks. The difficulties with the classification of cultivars and newly generated hybrids between or among *Musa* species using morphological and cytogenetic descriptors call for characterization of *Musa* accessions using stable and reproducible characters.

This study was conducted to determine the chromosome number of progenies from the cross between *M. textilis* Nee (2n = 20) and *M. balbisiana* Colla (2n = 22). Specifically, the study evaluated the parents and backcrosses of abaca hybrids based on selected agronomic traits. The tensile strength and the karyograms were compared based on chromosome number and relative chromosome length.

MATERIALS AND METHODS

Plant Materials and Place of Study

Six varieties/breeding lines (Abuab, Inosa, Pacol; hybrids: BC_1F_1, BC_2F_1, and BC_3F_1) were used in this study. Three plants per variety/breeding line (Table 1) were used for morphological characterizations. Two-month-old field-grown suckers were randomly chosen per variety/ breeding line (a total of 12 plants) for cytogenetic characterization based on the uniformity of sizes. These were collected at the Feed and Industrial Crops (FIC) experimental area, IPB.

Table 1. List of selected abaca (*Musa textilis* Nee) parentals Abuab, Inosa, banana Pacol (*M. balbisiana* Colla) and their hybrids BC_1F_1, BC_2F_1, and BC_3F_1.

Entry No.	Plant Sample Code	Variety/ Breeding Line
1	A1	
2	A2	Abuab
3	A3	
4	I1	
5	I2	Inosa
6	I3	
7	P1	
8	P2	Pacol
9	P3	
10	BC_1-A	
11	BC_1-B	BC_1F_1
12	BC_1-C	
13	BC_2-A	
14	BC_2-B	BC_2F_1
15	BC_2-C	
16	BC_3-A	
17	BC_3-B	BC_3F_1
18	BC_3-C	

Morphological characterization was conducted at the FIC Section at the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB). Cytogenetic characterization and documentation were conducted at the Genetics and Molecular Biology Division (GMBD), Institute of Biological Sciences (IBS), University of the Philippines Los Baños (UPLB).

BC_2F_1 selections (BC_2-111, BC_2-146, and BC_2-69) from a previous study conducted by Yllano (2010) were crossed to Inosa (Fig. 1). Seventy-two hybrid plants (BC_3F_1) were produced. The 72 BC_3F_1 abaca hybrids were transplanted in the field at the IPB Experiment Station, CAFS, UPLB. Parental genotypes (Abuab and Pacol) were included as check for comparison. Abuab, instead of Inosa (another abaca cultivar), was used as representative of abaca cultivar for morphological characterization. However, both Abuab and Inosa were used for the cytogenetic characterization.

Morphological Characterization

Agronomic Characters and Fiber Quality

Agronomic characteristics of abaca hybrids and parents were assessed using parameters based on the IPB set standards as shown in Table 2.

Experimental Design and Statistical Analysis

The agronomic parameters were analyzed using analysis of variance (ANOVA) of randomized complete block design (RCBD) with three replicates per genotype serving as blocks. Pairwise treatment means comparison with respect to the parents (Abuab, Inosa, and Pacol) were done using Fisher's least significant difference (LSD) test at α = 5% and 1% probability levels (Gomez and Gomez 1984). All statistical data were generated using STAR (STAR, version 2.0, 2013).

Table 2. List of selected agronomic parameters used to quantitatively characterize abaca (*Musa textilis* Nee) parentals Abuab, banana (*M. balbisiana* Colla) parental Pacol and their hybrids BC_1F_1, BC_2F_1, and BC_3F_1.

Trait	Description
Plant height (cm)	Height of the mother plant from base to leaf whorl after a plant exhibits its flag leaf
Girth (cm)	The girth measured at the top, middle and bottom part of the plant after a plant exhibits its flag leaf
Pseudostem fresh weight (kg)	Mean fresh weight of the pseudostem before fiber extraction
Leaf sheath number	Number of sheaths per plant that can be extracted for fiber
Fiber dry weight (kg)	Total weight of fiber extracted per plant
Percent fiber recovery (%)	Computed as the ratio of total fresh weight of fiber extracted and pseudostem fresh weight 100.
Tensile strength	Computed by taking 30 sets of 10 fiber strands that are 20 cm in length and knotted at both ends from each plant. Each set was weighed and subsequently measured for its breaking load using the tensile tester. Each genotype constituted five plants as replicates which were subjected to tensile strength measurement. The tensile strength was computed using the formula: Tensile strength (kg/g-m) = Breaking load (kg)/ [weight of sample (g/20 cm) x 5]

Cytogenetic Characterization

Collection of Plant Materials and Fixation of Root Tips

Parental lines (Abuab, Pacol, and Inosa) and their hybrids (BC_1F_1, BC_2F_1, and BC_3F_1) obtained from the Feeds and

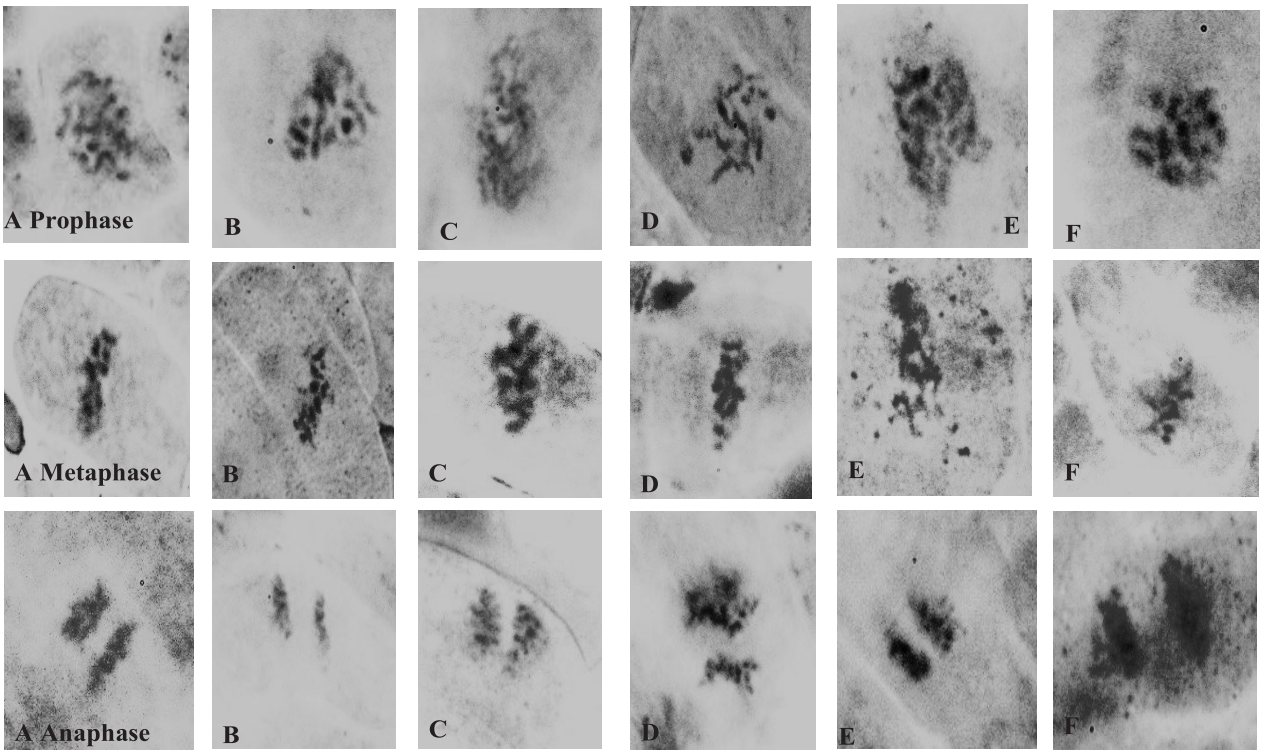

Fig. 1. Prophase, metaphase, and anaphase in cultivars of abaca (*Musa textilis* Nee): Abuab (A) and Inosa (B), adulterant cultivar of banana (*M. balbisiana* Colla): Pacol (C), and their hybrids: BC_1, BC_2, BC_3 (D, E, F) (1000x).

Industrial Crops (FIC) work area of IPB were subjected to cytogenetic analysis. A total of 12 plants, two each from the six samples, were used in this study. To determine the time when somatic cells of plant samples were actively dividing, root tips were collected every after 30 min from 10:00 a.m. to 12 noon. After the soil was washed off, the root tips were placed in a vial with cold water and kept inside the freezer at different durations of cold shock treatment (2 h, 4 h, and 6 h). Fixation of root tips was done using freshly prepared Farmer's solution (3:1 v/v 95% ethyl alcohol: glacial acetic acid) for at least 24 h and then transferred to a vial containing 70% ethanol for storage.

Slide Preparation

Root tips stored in 70% ethanol were washed with distilled water and hydrolyzed in 1 N HCl for about 6–10 min to facilitate easier maceration of root tips. After hydrolysis, root tips were rinsed again with distilled water and excess water was removed. The opaque portion of root tips that contains dividing cells was removed and placed on a clean slide. Slides were prepared using acetocarmine squash technique, wherein a drop of 2% acetocarmine stain was placed on the root tip which was then squashed with a bent needle to facilitate spreading of cells. A coverslip was placed on top of the slide. To further spread and disperse the cells, unused rubber eraser of a pencil was used to tap the top of the coverslip. Using alcohol lamp, the slides were gently heated over the flame to enhance acetocarmine staining. Afterwards, the coverslip was pressed against the slide to flatten the cells and to orient the chromosomes in one plane.

Alternate heating and pressing were done to ensure adherence of cells on the slide. Destaining was done using a drop of 45% acetic acid (HOAc) at the edge of the coverslip if the cells were overstained. Presence of dividing cells, chromosome counts, and morphology of chromosomes were visualized through a Zeiss Axioskop miscroscope and photomicrographs were taken using Cannon EOS 550D camera after optimal staining of the chromosome. Then the prepared slides were sealed with paraffin wax for temporary mounting.

Karyotype Analysis and Karyogram Construction

Different mitotic stages of cells were observed. Prometaphase cells were viewed under the microscope using Oil Immersion Objective (OIO). At least 10 well-spread prometaphase cells were considered in determining the probable chromosome number of each plant sample. Karyotypes of abaca, banana, and their hybrids (BC_1F_1, BC_2F_1, and BC_3F_1) were investigated with respect to the relative lengths of the chromosomes, arm ratio, and chromosomal count. For karyotyping, three

well-spread prometaphase cells of each plant sample were chosen and used. Photomicrographs were taken using Zeiss Axioskop Miscroscope and Cannon EOS 550D camera. Photomicrographs of prometaphases were enhanced using Adobe Light Room version 5.7. Interpretative drawings were made using tracing paper on top of printed photomicrographs. Actual chromosome lengths of each species were measured using Image J. Relative lengths of chromosomes were computed using the formula:

Relative Length (size) = Length of a particular chromosome/ Total haploid chromosome length

From the photomicrographs taken, karyograms were constructed manually and the chromosomes were arranged according to computed relative length. Relative chromosome lengths among the hybrids and parentals were compared.

Data Analysis

The average chromosome number of the six varieties/ breeding lines (parentals: Abuab, Inosa, Pacol; hybrids: BC_1F_1, BC_2F_1, and BC_3F_1) were obtained by getting the mean from the 10 prometaphase spreads counted in each variety. Relative lengths of chromosome pairs were compared among them. Finally, cytogenetic characterization data were correlated to the morphological data gathered.

RESULTS AND DISCUSSION

Morphological Characterization

Agronomic characteristics and tensile strength of abaca hybrids (BC_1F_1, BC_2F_1, and BC_3F_1) and parents (Abuab, Pacol) were assessed based on the parameters used at the Institute of Plant Breeding, such as plant height, girth, pseudostem fresh weight, leaf sheath number, fiber dry weight, percent fiber recovery, and tensile strength. Based on the ANOVA table generated (Table 3) and pairwise mean comparison (Table 4) with respect to both parents Abuab and Pacol, possible parental sources of trait contribution to the hybrids (BC_1F_1, BC_2F_1, and BC_3F_1) were traced. Parameters used for morphological characterization are quantitative traits. Quantitative genetic variation underlies susceptibility to common complex diseases in different organisms such as plants. Knowledge of the genetic basis of variation for quantitative traits is thus critical for increasing the rate of selective improvement of agriculturally important species (Mackay 2009).

Plant Height

Abuab and its hybrids for three generations have a higher average plant height, ranging from 320 cm to 361 cm respectively, compared to the average plant height of banana (Pacol) of 223 cm. Among the five genotypes, Abuab and all backcross progenies had significantly different plant height from that of Pacol. This result indicates that there was higher parental contribution from Abuab to its backcross progenies with reference to plant height. Plant height trait of the hybrids could have possibly been contributed by its Abuab (abaca cultivar) parent, since it was found to be significantly different from Pacol (banana cultivar).

Girth

The girth parameter was measured at the top, middle and bottom parts of the plant after a plant exhibited its flag leaf. For the girth measured at the top of the plants, the lowest measurement was in BC_2F_1 (24 cm), while Pacol had the highest measurement of 38.2 cm (Table 4). However, no significant differences were identified among the five genotypes for this trait. As one of the traits that had the lowest R^2 value (0.40), variation is explained more by random error rather than the treatment (genotype assignment).

Table 3. Analysis of variance using RCBD of agronomic traits and tensile strength of abaca (*Musa textilis* Nee) parental Abuab, banana (*M. balbisiana* Colla) parental Pacol, and backcross hybrids BC_1F_1, BC_2F_1, and BC_3F_1

S.V.	DF	Mean Square								
		Plant Height (cm)	Girth (cm)			Pseudostem Fresh Weight (kg)	Leaf Sheath No.	Fiber Dry Weight (kg)	Percent Fiber Recovery	Tensile Strength
			Top	Middle	Bottom					
Replicates	2	465.27	6	6.54	8.79	2.87	14.47	0.0002	0.0016	2.99
Genotype	4	10094.57**	25.01	53.06*	101.37*	105.4*	22.17	0.0076**	0.31**	42.26*
Error	8	983.02	17.47	8.17	15.87	18.51	17.57	0.0005	0.019	8.48
Total	14									
C.V. %		9.63	13.54	7.65	8.07	23.71	21.62	27.53	27.56	13.38

*Significant at 0.05 probability level, **Significant at 0.01 probability level
Genotypes: Abuab (*Musa textilis* Nee), Pacol (*M. balbisiana* Colla), BC_1F_1, BC_2F_1, and BC_3F_1

Table 4. Parameters of agronomic traits and their pairwise comparison in abaca hybrids BC_1F_1, BC_2F_1, and BC_3F_1, with respect to both parents Abuab (*Musa textilis* Nee) and Pacol (*M. balbisiana* Colla).

Parameter	Plant Height (cm)	Girth (cm)			Pseudostem Fresh Weight (kg)	Leaf Sheath No.	Fiber Dry Weight (kg)	Percent Fiber Recovery	Tensile Strength (kg/g-m)
		Top	Middle	Bottom					
N	15	15	15	15.49.36	15	15	15	15	15
Mean	325.73	30.87	37.34	48	18.14	19.33	0.08	0.50	21.77
Median	338	30	36	6.27	18	18	0.07	0.44	21.83
Std. dev.	59.27	4.24	4.56	38	6.41	4.29	0.05	0.32	4.17
Minimum	194	24	31	63.2	10	13	0.005	0.02	15.82
Maximum	390	38.2	46.2	57.07	31	27	0.17	1.13	29.97
Abuab	361[b]		42.43	53.73	28		0.036[b]	0.12[b]	27.21[b]
Pacol	223		40.93	45.33[ab]	18.67[a]		0.088[a]	0.5[a]	20.88[a]
BC_1F_1	342.67[b]		35.33[ab]	43.33[ab]	13.97[a]		0.043[b]	0.31	18.83[a]
BC_2F_1	358[b]		32.23[ab]		13.25[a]		0.079[a]	0.60[a]	18.15[a]

[a]Significantly different from Abuab
[b]Significantly different from Pacol

Girth measured at the middle part ranged from 31 cm (BC_2) to 46.2 cm (Abuab). There were significant differences found among the genotypes for this trait. Girth measured at the bottom part ranged from 38 cm to 63.2 cm with BC_2 having the lowest and Abuab having the highest measurement. Significant differences were also found among genotypes for this trait. For the trait girth width at the middle and bottom parts, all backcrosses of three generations were found significantly different with respect to Abuab. However, among the hybrids, BC_1F_1 and BC_2F_1 were significantly different with respect to both parentals Abuab and Pacol. BC_3F_1 hybrids were found to be significantly different only from its parent Abuab. BC_3F_1 could have possibly inherited the trait girth width at the middle and bottom of Pacol, while the other two hybrids (BC_1F_1 and BC_2F_1) may have exhibited an independent girth trait, but has lower size compared to the parentals.

Pseudostem Fresh Weight

The pseudostem was measured just before fiber extraction. The fresh weight of each pseudostem of the plant samples ranged from 10 kg (BC_2) to 31 kg (Abuab). All the backcross progenies were significantly different from Abuab for this trait. Pacol is more likely similar to the abaca hybrids, which may indicate its high parental contribution of the said trait. Pseudostem fresh weight trait of all the hybrids could have possibly been contributed by its parental Pacol.

Leaf Sheath Number

The leaf sheath number ranged from 13 sheaths (Pacol) to 27 sheaths (BC_2). Among the five genotypes, no significant differences were detected for this trait. It was one of the agronomic parameters that had the lowest R^2 (0.34). Transmission of genes from either of the parents for the mentioned trait did not significantly affect the performance of the backcross hybrids.

Fiber Dry Weight

The dry weight of the fiber extracted from the plant sampled ranged from 0.0052 kg (Pacol) to 0.1697 kg (BC_3F_1). Highly significant differences among the mean fiber dry weight of the samples were identified with respect to Pacol (0.088 kg) and Abuab (0.036 kg), a characteristic which is therefore supporting the speciation of these two Musa species. BC_1F_1 hybrids (0.043 kg) were significantly different from Pacol, while BC_2F_1 (0.079 kg) was significantly different from Abuab. BC_3F_1 hybrids (0.16 kg), on the other hand, were significantly different from both parents, having the least amount of fiber weight among the samples. For the fiber dry weight trait, BC_1F_1 could have inherited it from its parental Abuab, while BC_2F_1 could have inherited the trait from its parental Pacol. However, BC_3F_1 exhibited an independent fiber dry weight trait but its value is lower compared to both parentals.

Percent Fiber Recovery

The percentage of fiber recovered from both samples ranged from 0.02% (Pacol) to 1.13% (BC_3). Pacol (0.5%) and Abuab (0.12%) were highly significantly different from each other. BC_1F_1 hybrids (0.31%) were not significantly different from either of the two parents. BC_2F_1 hybrids (0.60 %) were significantly different from Abuab. BC_3F_1 hybrids (0.98%) were significantly different from both Abuab and Pacol. BC_1F_1 showed an average trait of the parentals' fiber recovery. BC_2F_1 has more likely inherited the said trait from Pacol, while BC_3F_1 again exhibited an independent fiber recovery trait, but this time is of higher value compared to its parentals. This result showed that through generations of backcrossing,

percent fiber recovery increases and improves.

Tensile Strength

The computed tensile strength of the fiber ranged from 15.82 kg/g-m (BC_2) to 29.97 kg/g-m (Abuab). Highly significant differences for tensile strength were found between Pacol (20.88 kg/g-m) and Abuab (27.21 kg/g-m). BC_1F_1 (18.83 kg/g-m) and BC_2F_1 (18.15 kg/g-m) were both significantly different from Abuab, while BC_3F_1(23.81 kg/g-m) was not significantly different from either one or both of the parents. For the trait tensile strength, BC_1F_1 and BC_2F_1 could have inherited it from the parent Pacol, while BC_3F_1 exhibited an average of the parentals' tensile strength trait. As expected, significant differences were found among the traits fiber quality, percent fiber recovery, and tensile strength in Pacol and Abuab. Abuab, compared to Pacol, is known for its good fiber quality.

Based on the results gathered, the plant height trait of the hybrids could have possibly been contributed by its Abuab (abaca cultivar) parent, since it was found to be significantly different from Pacol (banana cultivar). However, BC_3F_1 could have possibly inherited the trait girth (middle and bottom) from Pacol. Pseudostem fresh weight trait of all the hybrids could have possibly been contributed by its parental Pacol. For the fresh dry weight trait, BC_1F_1 could have inherited it from its parental Abuab, while BC_2F_1 could have inherited the trait from its parental Pacol. BC_1F_1 showed an average trait of the parentals' fiber recovery.

For the tensile strength trait, BC_1F_1 and BC_2F_1 could have inherited it from the parent Pacol, while BC_3F_1 exhibited an average of the parentals' tensile strength trait. Among the agronomic traits evaluated, two agronomic traits, namely, leaf sheath number and girth measured at the top, did not have any significant differences among the five genotypes, and with respect to either one of the parents. This result could mean that these two agronomic traits are conserved among *Musa* species. However, as confirmed by the remaining agronomic traits and fiber quality assessment, the recurrent parent genome (Abuab) was continuously recovered until the third generation of backcross. Although the observed possible contribution of parents to hybrids had been traced, it still needs to be validated by subjecting a larger population size of plant samples to morphological characterization, karyotype analysis, as well as molecular characterization.

Generally, it was observed that the hybrids/progenies performed better than their parents as far as fiber dry weight and percent fiber recovery were concerned, thus the goal of the abaca development program of IPB was realized. Overall, BC_3F_1 progenies are very promising for having the highest fiber dry weight and percent fiber recovery with no significant difference from its abaca parent in terms of tensile strength. Therefore, future abaca backcross breeding for two or more generations could possibly exceed the overall performance of the existing abaca varieties to date.

Karyotype Analysis

In the breeding program at IPB, BC_1 with phenotypic characteristics like banana were discarded. BC_1 with abaca-like phenotypic characteristics were chosen instead for backcross breeding and were expected to have a diploid chromosome number of 2n = 20 compared to BC_1 with banana-like features (2n = 22). To confirm this, BC_1 with abaca-like feature was subjected to cytogenetic characterization.

Optimized Collection and Fixation of Root Tips

The most favorable time of collection was from 10:00 a.m. to 10:30 a.m. Root tips of about 1–4 cm were harvested between this time frame due to the high number of dividing cells observed compared to other time slots tried (10:30 a.m.–11:00 a.m.; 11:00 a.m.–11:30 a.m.; 11:30 a.m.–12:00 noon). After the soil has been washed off, the root tips were placed in a vial with cold water and kept inside the freezer at different durations of cold shock treatment (2 h, 4 h, and 6 h). The first batches of root samples were pre-treated on a 6-h cold shock, following the protocol of Africa (1992). It was observed then that chromosomes of the sample roots shortened so much. It is important to note that longer pretreatment will shorten the chromosome length considerably (Sharma and Sharma 1965). On the other hand, 4-h cold shock provided comparably longer and visible chromosomes, but low frequency of prometaphase cells was observed. Finally, a 2-h cold shock pretreatment resulted in considerably larger chromosomes, and higher number of well-spread prometaphase cells that helped in the construction of karyograms.

Some thick root tips were cut at the ends into lengthwise or quarters so that the fixative would be well-absorbed by the cells. Farmer's solution is the most widely used fixative as described by Sharma and Sharma (1965). Moreover, easier maceration of root tips was observed if roots were stored in freshly prepared Farmer's solution for a minimum of 48 h instead of subjecting them to 24-h fixation.

Stages of Mitosis

All stages of mitosis except telophase were observed in the slides prepared for prometaphase cells of Abuab, Inosa, Pacol, BC_1, BC_2, and BC_3. Representative photomicrographs showed the normal chromosome

behavior during mitosis (Fig. 1).

Prophase. In this stage, chromosomes are visible and are starting to coil up. The chromatids, which are the two longitudinal halves of the chromosome, become thicker, straighter and smoother as prophase progresses from early to late stage. At the end of this stage, the nucleolus disappears through degradation (Singh 2003).

Metaphase. Chromosomes can be seen aligned at the metaphase plate. The chromosomes are maximally contracted and positioned halfway between the poles on the spindle equator (Pines and Rieder 2001).

Anaphase. Two compact groups of chromosomes could be seen separated and positioned near each opposite pole. The sister chromatids separate through an independent force pushing the kinetochore fiber forward. The spindle fiber attached to the kinetochores drags the two sister chromatids toward the opposite poles. At the end of this stage, the spindle fiber disappears. Chromosome number of the organisms remain the same since the compact groups of chromosomes that migrated at opposite poles were formerly sister chromatids (Singh 2003).

Telophase. No telophase stage was observed among the slides prepared. At this end stage of mitosis, the spindle breaks down as adjacent chromosomes fuse to form the two daughter nuclei, and a microtubule-based mid-body assembles near the original spindle equator and participates in cytokinesis (Pines and Rieder 2001).

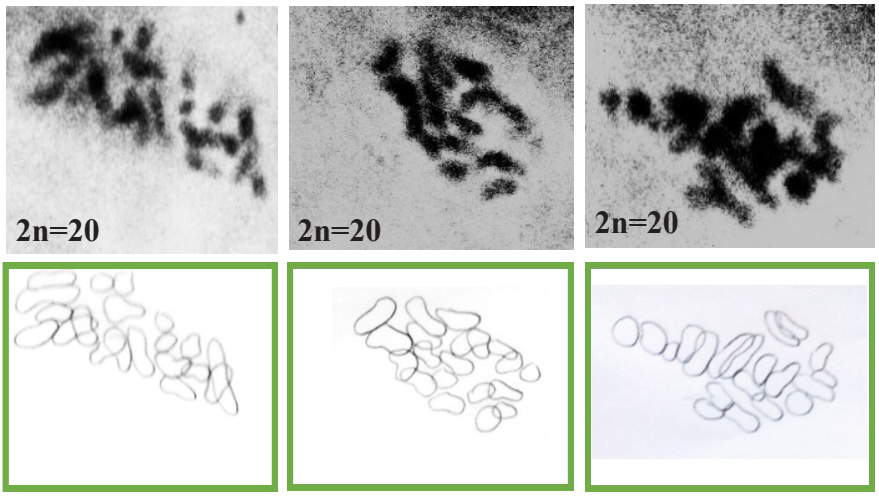

Fig. 2. Representative cells at prometaphase in abaca (*Musa textilis* Nee) cv. Abuab and their interpretative drawings (1000x).

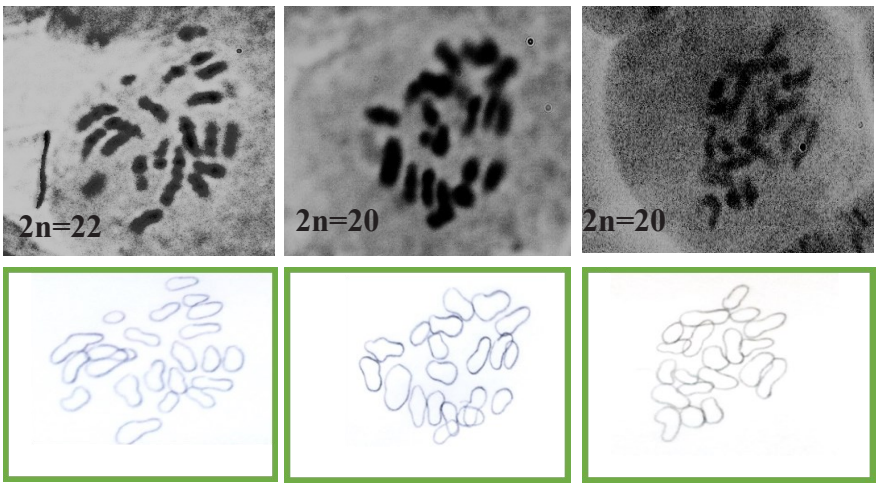

Fig. 3. Representative cells at prometaphase in abaca (*Musa textilis* Nee) cv. Inosa and their interpretative drawings (1000x).

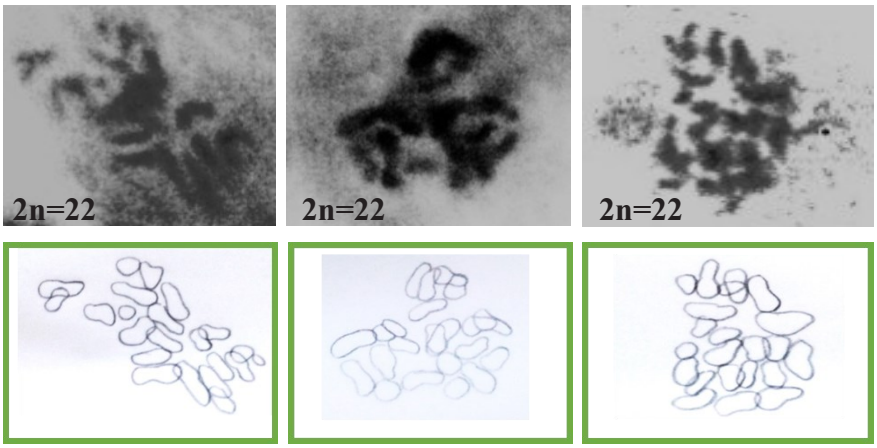

Fig. 4. Representative cells at prometaphase in banana (*Musa balbisiana* Colla) cv. Pacol and their interpretative drawings (1000x).

Karyogram Construction and Karyotype Analysis

Figures 2–7 show photomicrographs of prometaphase cells with their respective interpretative drawings which were used to make representative karyograms (Fig. 12–14) for the six breeding lines: Abuab, Inosa, Pacol, BC_1,

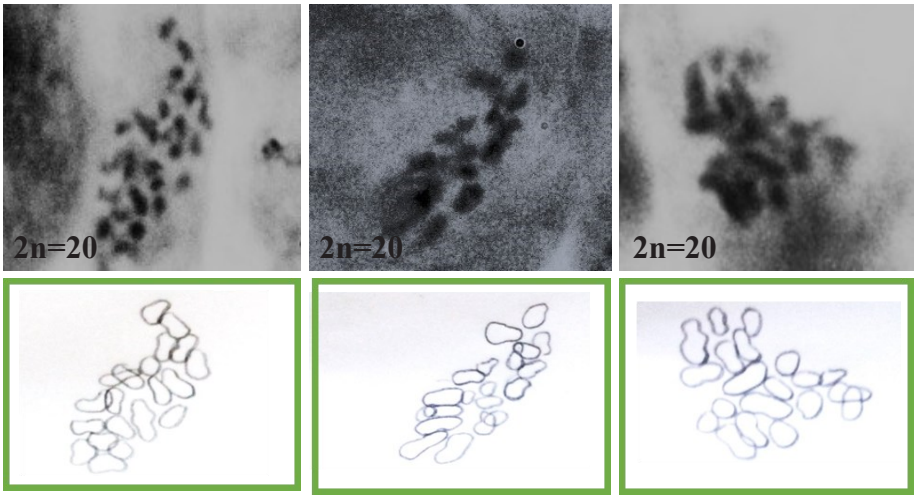

Fig. 5. Representative cells at prometaphase in BC_1F_1 and their interpretative drawings (1000x).

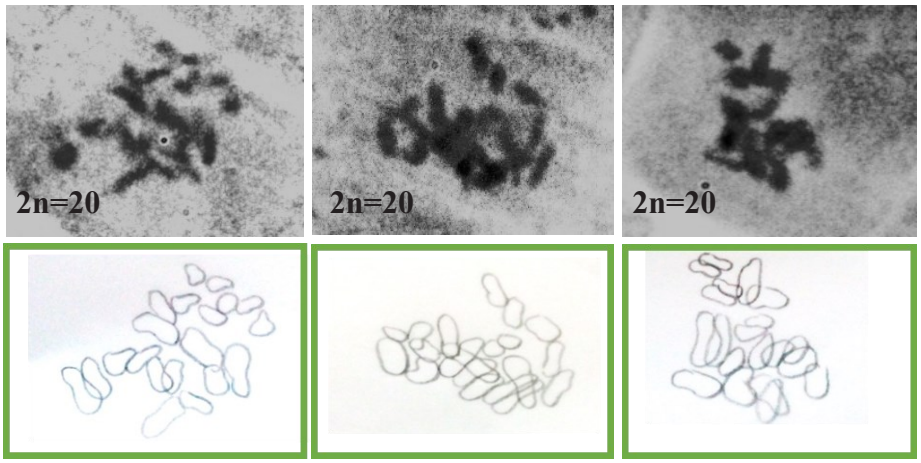

Fig. 6. Representative cells at prometaphase in BC_2F_1 and their interpretative drawings (1000x).

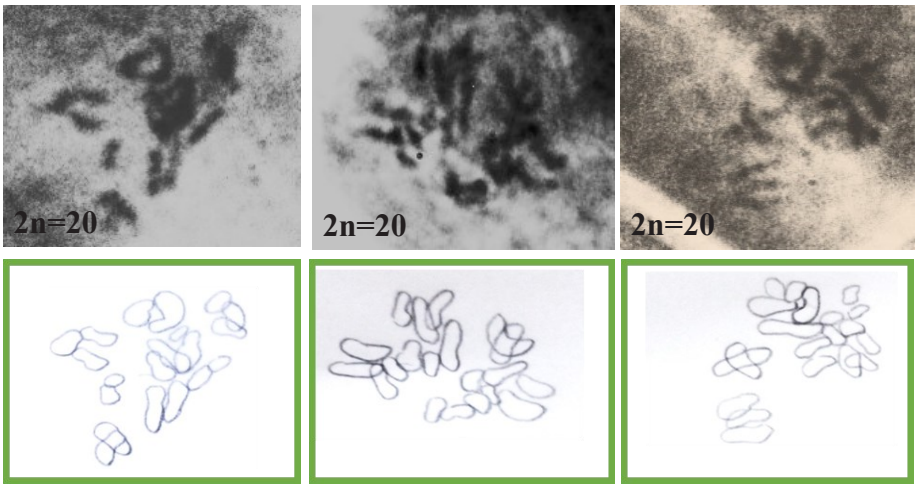

Fig. 7. Representative cells at prometaphase in BC_3F_1 and their interpretative drawings (1000x).

BC_2, and BC_3. From the photomicrographs taken, karyograms were constructed manually and the chromosomes were arranged according to their relative sizes.

Comparative Karyotype Analysis

Figure 8–14 show representative prometaphase cells, interpretative drawings, and karyograms of Abuab, Pacol, Inosa, BC_1F_1, BC_2F_1, and BC_3F_1. Chromosomes of the best prometaphase cell observed were counted to determine the probable chromosome number of each breeding line. The expected basic chromosome number for Pacol is n = x = 11 since this belongs to the section Eumusa, whereas that of Australimusa to which abaca (Abuab and Inosa) belongs is n = 10. Backcross hybrids were expected to have the same chromosome number as abaca, n = 10, since all BC_1F_1 hybrids with banana-like phenotype (2n = 21) were discarded in the breeding program. Karyograms and chromosomal count of progenies confirmed the success of abaca-like phenotype selection in the BC_1F_1 generation.

The chromosomal counts of the six breeding lines are shown in Table 5. Abuab, a cultivar of *M. textilis* Nee, and the rest of the abaca hybrids (BC_1, BC_2 and BC_3), as expected, had an observed diploid chromosome number of 2n = 20. Inosa, another cultivar of *M. textilis* Nee, had either 20 or 22. Inosa has been reported to have chromosome numbers varying from 2n = 17 to 2n = 23 (Javier and Oracion 1988). Moreover, it has been reported that abaca varieties and hybrids possess a high degree of somatic mosaicism or variability due to aneuploidy, with chromosome counts ranging from 2n = 12 to 2n = 20 (Moreno 2003). This could possibly explain

Table 5. Diploid chromosome number of breeding line parents Abuab and Inosa (*Musa textilis* Nee), and Pacol (*M. balbisiana* Colla) and their hybrids (BC_1F_1, BC_2F_1, and BC_3F_1).

	Musa Breeding Lines					
Cell	Abuab	Inosa	Pacol	BC_1F_1	BC_2F_1	BC_3F_1
1	20	22	22	20	20	20
2	20	20	22	20	20	20
3	20	20	22	20	20	20
4	20	20	22	20	20	20
5	20	20	22	20	20	20
6	20	20	22	20	20	20
7	20	22	22	20	20	20
8	20	20	22	20	20	20
9	20	20	22	20	20	20
10	20	20	22	20	20	20
Mean	20	20.4	22	20	20	20

why Inosa, an abaca cultivar, can oftentimes be observed with a chromosome number of n = 11, similar to that of Pacol, a banana cultivar.

Abaca varieties Abuab and Inosa have long been found to be cross compatible with several Musa species including *M. balbisiana* (banana variety: Pacol) (Brewbaker et al. 1956). The indigenous Musa species (*M. acuminata*, *M. balbisiana* and *M. textilis*) overlap and natural hybrids among these species exist. *M. balbisiana* cv. Pacol produces low quality fiber that has been used as an adulterant to abaca. Natural hybrids of Pacol and abaca exist in the Bicol region and are known as Canton and Minay (Valmayor et al. 1956). The natural hybrid between the diploid banana and abaca called Minay/ Minary/Minray has 2n = 21 (Tabora and Carlos 1978) whereas Canton has 2n = 20 (Valmayor et al. 1956).

Relative Chromosome Length

The average relative lengths of the chromosomes of the six breeding lines are compared in Table 6. Chromosomes of Pacol were comparably longer than those of Abuab. However, chromosome pairs 1, 2, 7, 8, 9, and 10 of Inosa (another *M. textilis* Nee cultivar) are comparably longer than those of Pacol. Relative lengths of chromosome pairs 1 to 4 of backcross hybrids were found to be increasing through generations. On the other hand, chromosome pairs 5 to 9 were found to be decreasing through generations. Moreover, the chromosome pairs of the hybrids were relatively longer compared to both parentals. There is no statistical analysis to show if the differences in relative lengths observed were significant. Chromosome pair 1 of both Inosa (abaca cultivar) and Pacol (banana cultivar) was found to have the same size (0.140). As evidenced by the similarity of their chromosome 1 and based on the closeness of the remaining chromosome measurements of *M. textilis* Nee cultivars (Abuab and Inosa) and *M. balbisiana* Colla cultivar (Pacol), they are indeed closely related species. Closely related species have more similar chromosomes than those of more distantly related ones (Sharma and Sen 2002).

Chromosome pair 3 of Inosa and BC_1F_1 was found to be of the same size (0.114). Abuab and Inosa, both abaca cultivars, have the same length for chromosome pair 4 (0.109). Inosa and BC_1F_1 have the same size of chromosome pair 5 (0.101). Indeed both Abuab and Inosa are cultivars of *M. textilis* Nee, as evidenced not only by their chromosomal count but also by the similarity of their chromosomes.

Table 7 shows a comparison of relative chromosome lengths of BC_1F_1 and its parents Pacol and Abuab. Chromosomes 1 and 9 had the average length of its parental chromosomes. Chromosomes 2, 5, 7, and 8 had relatively longer chromosomes compared to both parents, while the remaining chromosomes (3, 4, 6, and 10) were relatively shorter. Chromosomes 1, 4, 5 and 9 of BC_2F_1 had the average length of its parents' chromosomes. Chromosomes 2, 3 and 5 had relatively longer chromosomes compared to both parents, while chromosomes 6, 7, 8, and 10 were relatively shorter. It also shows the comparison of relative chromosome lengths of BC_3F_1 and its parents (Pacol and Abuab). Chromosomes 1, 9, and 10 of BC_3F_1 had the average length of its parents' chromosomes. Chromosomes 2, 3, 4 and 5 had relatively longer chromosomes compared to both parents, while the remaining chromosomes (6, 7,

Table 6. Average relative lengths of six breeding lines, Abuab and Inosa (*Musa textilis* Nee), Pacol (*M. balbisiana* Colla), and their hybrids BC_1F_1, BC_2F_1, and BC_3F_1.

Chromosome Pair	*Musa* Breeding Lines					
	Abuab	Inosa	Pacol	BC_1F_1	BC_2F_1	BC_3F_1
1	0.161	0.14	0.14	0.148	0.15	0.158
2	0.128	0.124	0.123	0.132	0.133	0.134
3	0.117	0.114	0.118	0.114	0.125	0.122
4	0.109	0.109	0.111	0.106	0.11	0.112
5	0.1	0.101	0.103	0.104	0.101	0.105
6	0.095	0.094	0.096	0.093	0.093	0.091
7	0.089	0.088	0.087	0.09	0.085	0.08
8	0.078	0.083	0.082	0.084	0.077	0.072
9	0.066	0.078	0.075	0.073	0.07	0.066
10	0.056	0.071	0.064	0.055	0.055	0.059
11			0.052			

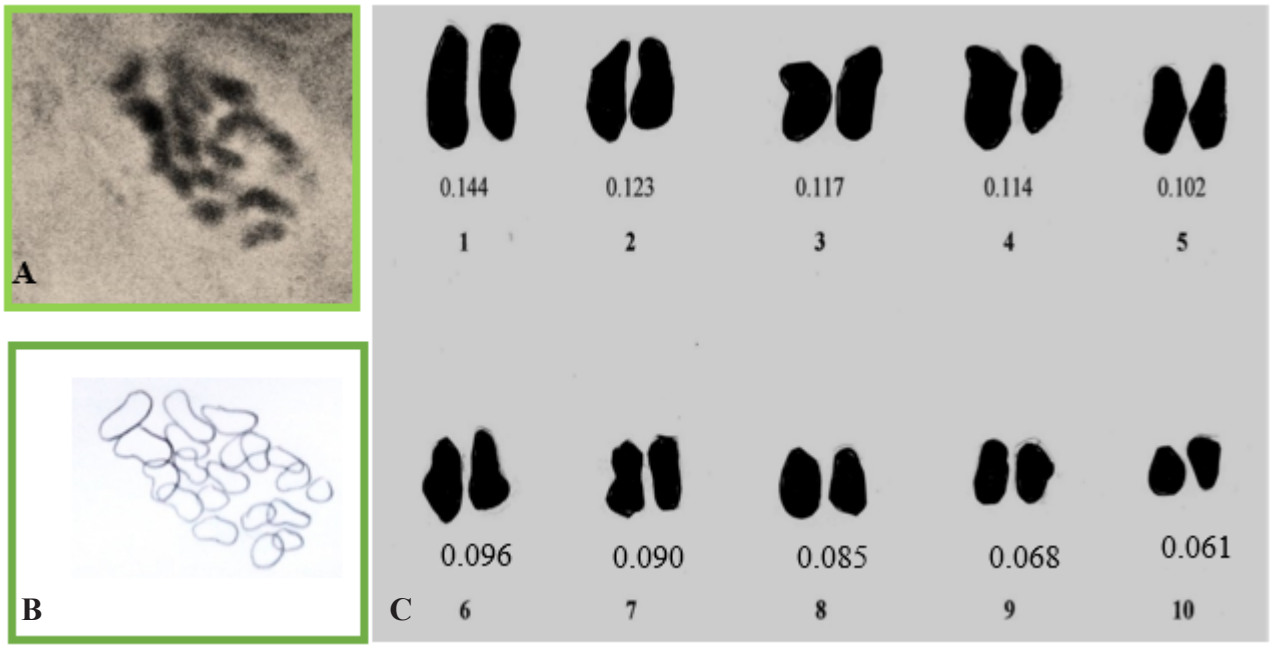

Fig. 8. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of abaca (*Musa textilis* Nee) cv. Abuab, 2n = 20 (1000x).

and 8) were relatively shorter.

Although minimal, variations in chromosomal size are apparent among the six breeding lines/varieties. These may be influenced by either differences in amount of gene products or proteins produced by the individuals, or by duplication of genes, which can influence their interactions and the rate of synthesis of individual proteins. The total mass or size of chromosomes in a nucleus has been found to be closely related to its DNA content (Sharma and Sen 2002).

Comparative karyotype analysis of related species has been used in many cases to describe patterns and directions of chromosomal evolution within a group and to infer the evolutionary role of karyotype (Badr et al. 2009). Each organism has a defined standard karyotype, which is a point of reference for mutation studies (Mellors 1955). Karyotype is the exact haploid chromosome set of an organism, while karyogram is the physical measurement of the chromosomes from the photomicrograph where chromosomes are arranged in descending order (longest to highest in terms of chromosome length) (Iijima and Fukui 1991). Chromosomal characteristics such as number, size, and morphology have been of considerable value in understanding interrelationships and delimitation of taxa (Naruhashi and Iwatsubo 1991). Comparisons of chromosomes among cultivars on the basis of their relative lengths were previously done by Hasegawa (1932) on four *Disporum* varieties. Detailed computation for relative chromosome lengths and their average were presented and compared among the six breeding lines.

Table 7. Comparison of BC_1F_1, BC_2F_1, and BC_3F_1 chromosome relative lengths with respect to abaca (*Musa textilis* Nee) parentals: Abuab and Inosa, and banana (*M. balbisiana* Colla) parental Pacol.

Chromosome Pair	Abuab	Inosa	Pacol	BC_1F_1	BC_2F_1	BC_3F_1
1	0.161	0.14	0.14	0.148	0.15	0.158
2	0.128	0.124	0.123	0.132	0.133	0.134
3	0.117	0.114	0.118	0.114	0.125	0.122
4	0.109	0.109	0.111	0.106	0.11	0.112
5	0.1	0.101	0.103	0.104	0.101	0.105
6	0.095	0.094	0.096	0.093	0.093	0.091
7	0.089	0.088	0.087	0.09	0.085	0.08
8	0.078	0.083	0.082	0.084	0.077	0.072
9	0.066	0.078	0.075	0.073	0.07	0.066
10	0.056	0.071	0.064	0.055	0.055	0.059
11			0.052			

SUMMARY AND CONCLUSION

The abaca industry is still relying solely on traditional varieties for its survival in the absence of new and improved ones. This is one of the many stated reasons for the decline in the abaca industry. Therefore, basic information generated by morphological and cytogenetic characterizations is important for abaca breeders. In general, this study sought to determine if the progenies from the cross

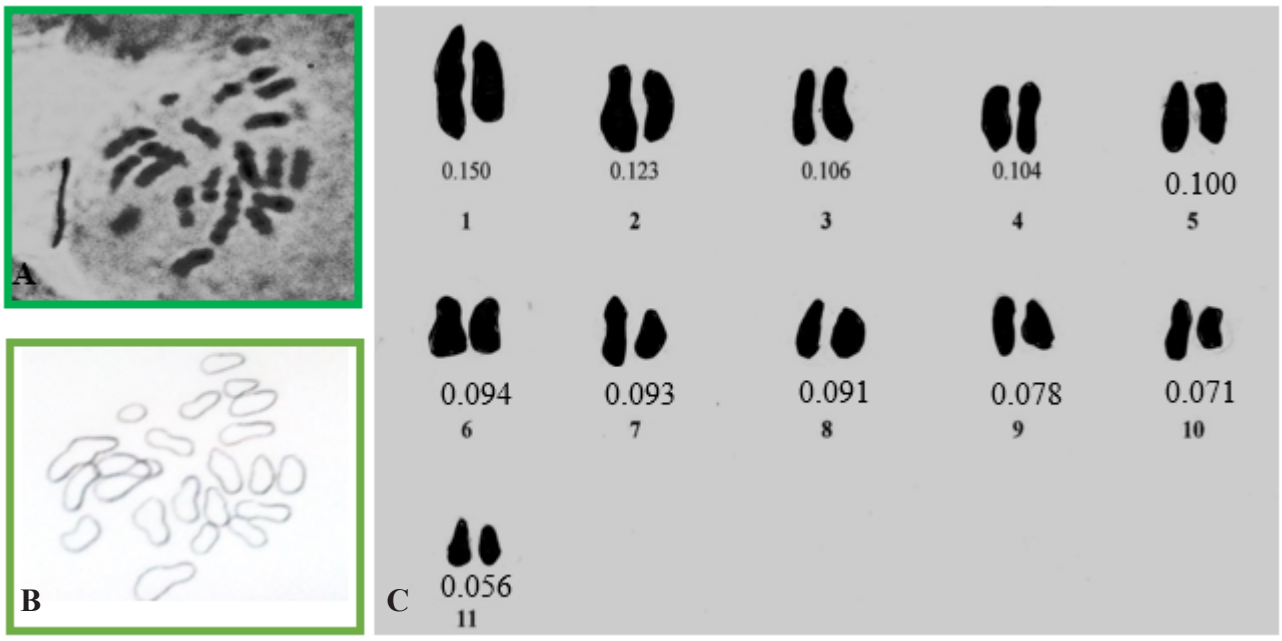

Fig. 9. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of abaca (*Musa textilis* Nee) cv. Inosa, 2n = 22 (1000x).

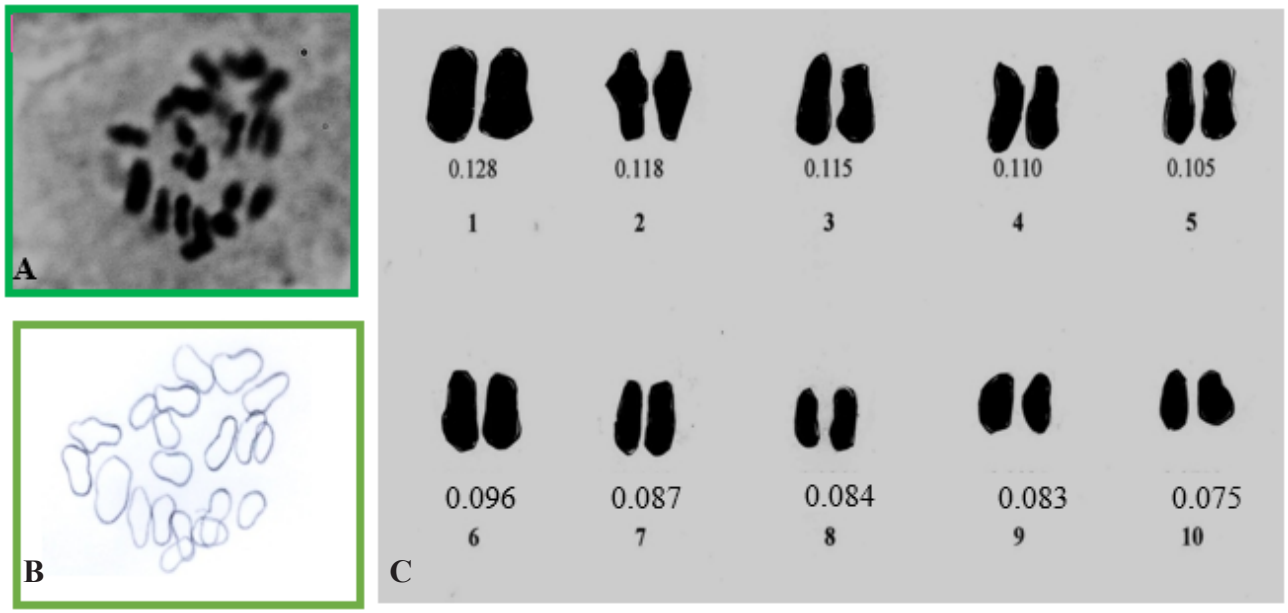

Fig. 10. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of abaca (*Musa textilis* Nee) cv. Inosa, 2n = 20 (1000x).

between *M. textilis* Nee (2n = 20) and *M. balbisiana* (2n = 22) produced at IPB were true hybrids. Parents (abaca cultivars: Abuab and Inosa; banana cultivar: Pacol) and their backcross hybrids were evaluated based on selected agronomic traits and their tensile strength. Moreover, probable diploid chromosome number using well-spread prometaphase cells and chromosome lengths were determined by constructing karyograms. This study reports the successful optimization of cytogenetic techniques such as pre-treatment, fixation, and slide preparations.

Agronomic traits of parentals (abaca and banana) and backcross hybrids were assessed using ANOVA of RCBD. Pairwise analysis was also done to statistically compare the traits of hybrids to their parentals, Abuab and Pacol. Significant differences were identified for fiber quality, percent fiber recovery, and tensile strength in

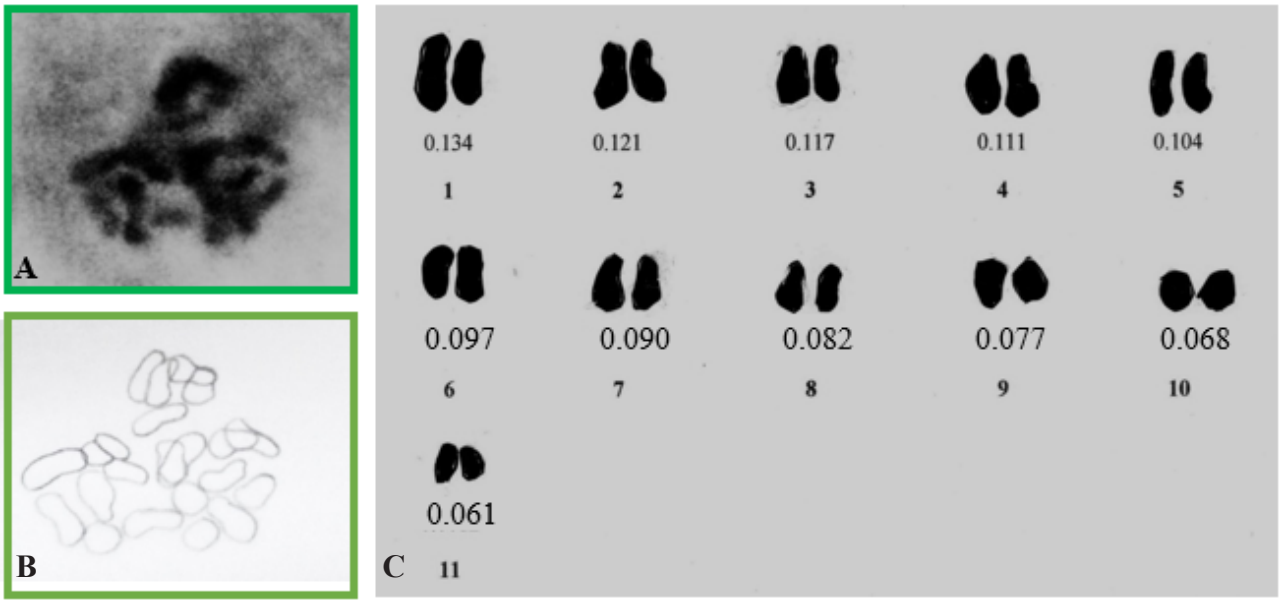

Fig. 11. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of banana (*Musa balbisiana* Colla) cv. Pacol, 2n = 22 (1000x).

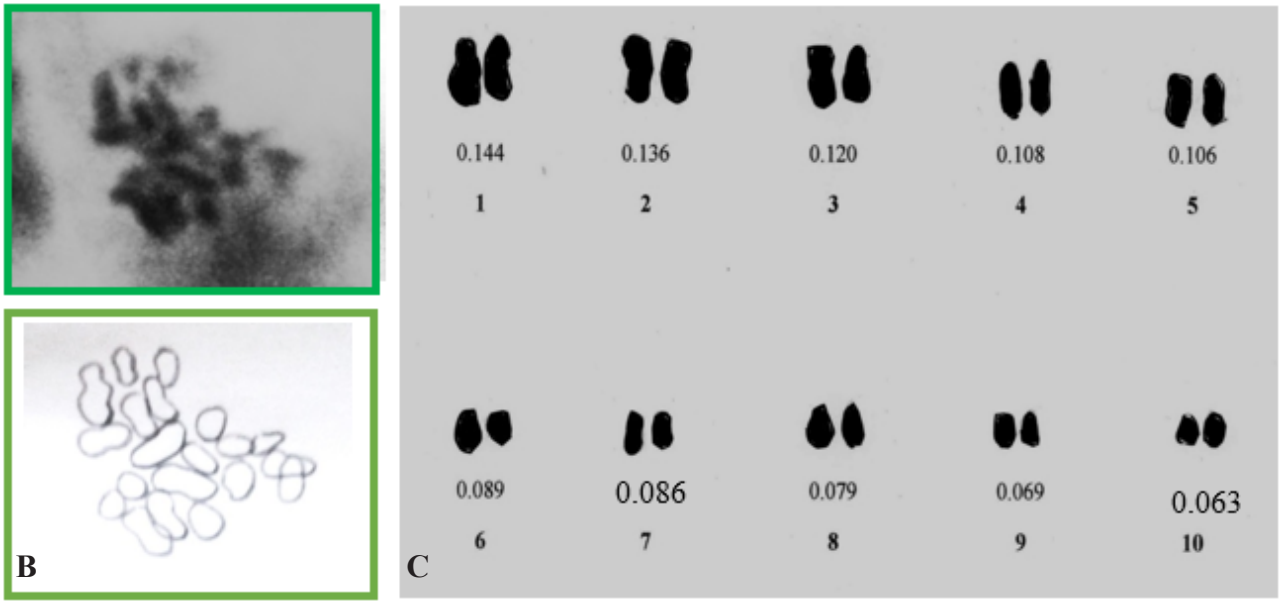

Fig. 12. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of BC_1F_1 (*Musa textilis* Nee hybrid), 2n = 20 (1000x).

Pacol and Abuab. This result was expected since these parents are two separate species of *Musa*. Significant differences with respect to either one or both parents were identified among the abaca hybrids for the same three fiber traits, except for two agronomic traits (leaf sheath number and girth measured at the top). As confirmed by the agronomic trait and fiber quality assessment, the recurrent parent genome (*M. textilis* Nee) was continuously recovered until the third generation of backcross.

The most favorable time for collecting root tips of about 1–4 cm was from 10:00 a.m. to 10:30 a.m. High number of dividing cells was observed compared to other time slots tried. After the soil has been washed off, root tips were placed in a vial with cold water and kept inside the freezer for a 2-h cold shock treatment which resulted in comparably larger chromosomes, and higher number of well-spread prometaphase cells that helped in the construction of karyograms. Abuab and the rest of the abaca hybrids (BC1, BC2 and BC3) have a diploid chromosome number of 2n = 20. Inosa has either 2n = 20

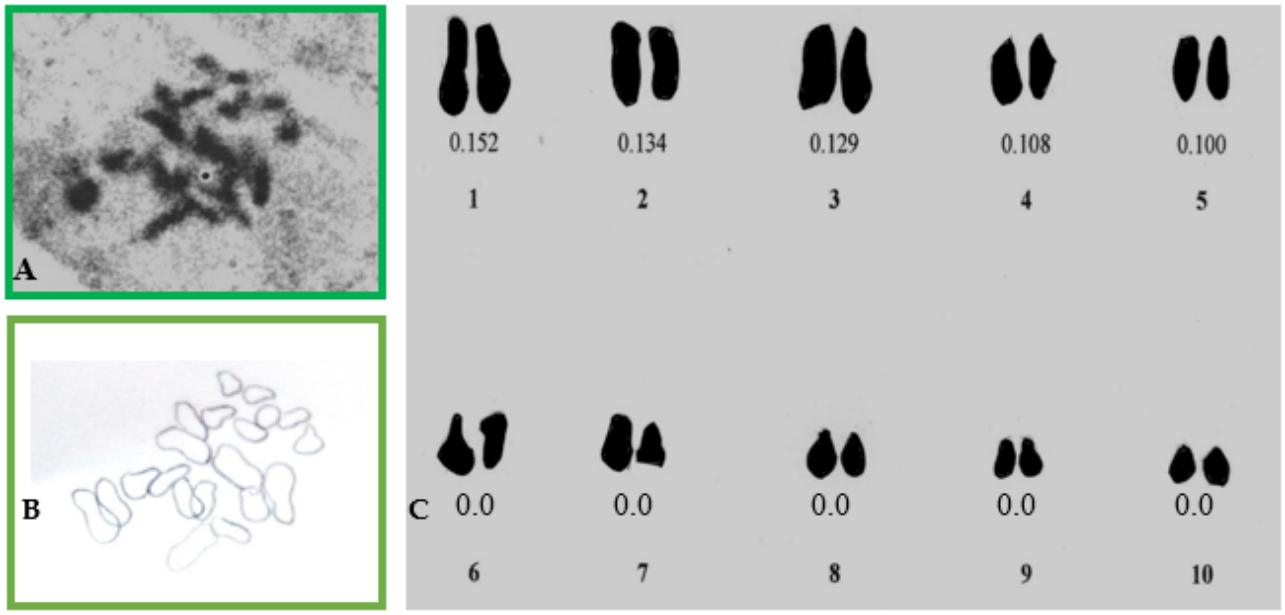

Fig. 13. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of BC_2F_1 (*Musa textilis* Nee hybrid), 2n = 20 (1000x).

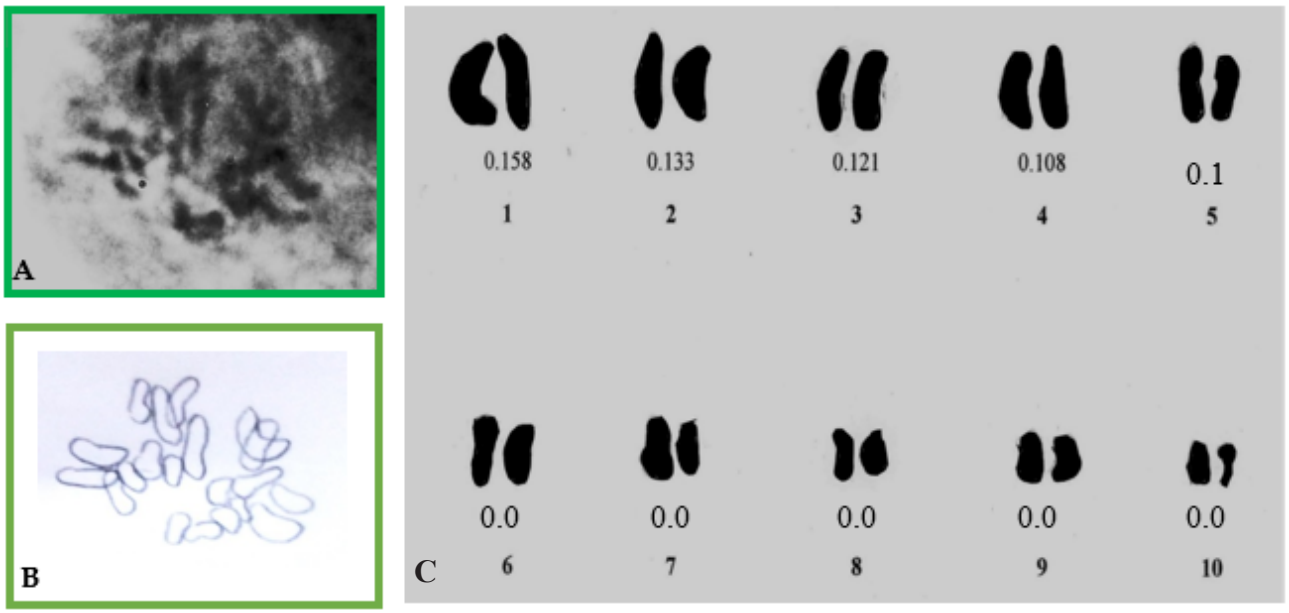

Fig. 14. Representative prometaphase cell (A), interpretative drawing (B), and karyogram (C) showing average chromosome relative lengths of BC_3F_1 (*Musa textilis* Nee hybrid), 2n = 20 (1000x).

or 2n = 22; earlier, it has been reported to have chromosome numbers varying from 2n = 17 to 2n = 23. Pacol, a cultivar of *M. balbisiana* Colla, has a chromosome number of 2n = 22. Chromosomal count of backcross progenies implies that they are true hybrids of abaca and banana.

Previously, it has been reported that detailed cytogenetic studies were complicated by the small size of chromosomes, their morphological similarity and lack of chromosome-specific landmarks. This study was able to overcome reported difficulties by successfully selecting stable agronomical parameters and optimizing cytogenetic techniques for karyotype analysis to aid in the classification of *Musa* cultivars.

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