Analysis of Paternal Transmission of Morphological Characters in Progenies of Maternal Parents in a 4x - 2x *Musa* Polycross Mating Design

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***Abstract*- This experiment was conducted to (i) identify the progenies of paternal (pollen) parents using 20 coded morphological markers in order to establish which of these are inherited /transmitted to the progenies from their male progenitors in a 4x – 2x polycross mating design and (ii) determine using these markers, equal or unequal paternal contribution in progenies of all the maternal (seed) parents. Sixteen of the morphological characters were found to be monomorphic while four were polymorphic. The polymorphic characters were pseudostem wax (PW), pseudostem blotch (PB), presence of petiole canal (PC), and clasping of petiole margin (CPM) and these were used to screen the progenies in order to identify their male progenitors. Pseudostem wax (PW) was only present in TMB2x 5105-1, pseudostem blotch (PB) was unique to SH 3362, petiole canal (PC) and clasping of petiole margin (CPM) occurred in TMP2x 2829-62 and SH 3362, but not in TMB2x 5105-1. Chi square test showed unequal paternal contribution with respect to pseudostem wax (PW), pseudostem blotch (PB), presence of petiole canal (PC) in the progenies of the maternal parent PITA 5. It also showed unequal paternal contribution in the maternal parent PITA 3 with respect to petiole canal. Clasping of petiole margin showed equal paternal contribution in all the maternal parents.**

***Keywords:*- Plantain and banana, male progenitor, quantitative morphological descriptors, qualitative morphological descriptors, characterization, polymorphism.**

I. INTRODUCTION

Traditionally, scientists investigated successfully over the years the close relationships, diversity and other important characteristics of *Musa* spp, using several types of anatomical, agronomic and morphological features [1] [2] [3]. In their study [3] anatomical and morphological markers of leaves, stems (rhizomes) and roots of five cultivars of Indonesian bananas were employed to distinguish between the cultivars of different ploidy levels. They reported that while the morphological characters of stems and leaves of diploid (AA and BB genome) and triploid (AAA, AAB, and ABB genome) cultivars were different, the characteristics of the roots and the rhizomes were similar. The differences in leaf anatomy were based on size and number of stomatal distribution, number of subsidiary cells, number of hypodermal layers, structure and number of parenchyma palisade, size of airspace in petiole and mesophyll and the vascular bundle structure as well as size of guard cells, - triploids had longer guard cells than diploids. Much earlier, A scoring technique [1] of 15 indicative morphological characters were used to separate *Musa acuminata* from *M. balbisiana* cultivars and their hybrids. Later, more of these morpho-taxonomic / agro-morphological descriptors were developed and perfected for *Musa*, and 119 descriptors have been defined as normal for the *Musa* germplasm [4] and is now updated and published [5]. On their part, [6] used a qualitative evaluation of 74 morphological descriptors (24 plant, 25 cluster and 25 inflorescence characteristics - heart and male flowers) to characterize 20 banana clones cultivated in northern Minas Gerais, a State in eastern Brazil. The morphological descriptors included pseudostem colour, presence of anthocyanin in the pseudostem, shape of the clusters and bunches, and the persistence of remaining flowers and bracts on the rachis were used to differentiate 'Prata Anã' clones from the cultivar Prata Anã. In his research, [7] had declared that the most important qualitative morphological descriptors in *Musa* are persistence of male bud and hermaphrodite flowers, pigmentation in pseudostem, foliage, petiole and male flower, pseudostem blotching and waxiness, and leaf orientation. The most important quantitative morphological descriptors he stated however include pseudostem girth, height of tallest sucker, number of fruits and fruit sizes. These quantitative descriptors have a high heritability (> 0.8), high repeatability (> 2) and low coefficient of variation (9-15%) with the exception of the height of the tallest sucker. Many plant morphological marker traits lend themselves to easy visual recognition. These morphological markers like seed and fruit characteristics such as size, shape, colour and structure; flower and inflorescence size, shape, arrangement, position and colour; size of leaves, leaf arrangement, distribution, angle and shape [8] [9]; plant growth habit, and other important agronomic traits are some of such traits. Such morphological markers are easy to use, with no need for specialized instruments, biochemical or molecular techniques. Breeders have used such types of morphological markers successfully in the breeding programmes for various crops to identify siblings and progenies from crosses. The main disadvantages of morphological markers are the fact that they are limited in number, are influenced by age and the plant growth stages and also by a number of environmental factors

such as climate and plant nutrition [10] [6]. According to [11] a number of these morphological characteristics are also prone to mutations, including plant stature, bunch and fruit shapes and fruit pulp stringency. Because morphological markers are phenotypic traits, their expression in hybrids is highly dependent on the environment. Since ancient times, man has successfully used various morphological markers to investigate observed variations in order to utilize them for breeding. Today genetic characterization using molecular markers and DNA fingerprinting is a rapid and accurate technique for hybrid identification. In their review article on genetic, morphological, and chemical patterns of plant hybridization, [12] asked a pertinent question which is; why then should researchers continue using other markers such as morphological, chemical profiles and chromosome number in hybridization related studies? They pointed out that one reason is that it has been found that these additional markers may unveil hybrid individuals that go undetected when using problematic DNA markers such as RAPD or AFLP as pointed out [13]. Thus, although DNA fingerprinting is the most reliable tool for hybrid identification, the use of additional markers will continue to be employed as they provide insights of the ecological performance of hybrids. Morphological marker characterization should therefore be complementary to DNA molecular markers. In their study [14] characterized 27 cultivars (*M. acuminata* (AA genome), and *M. balbisiana* (BB genome), polyploids and interspecific hybrids using morphological traits and simple sequence repeats (SSRs). It was reported [15] that overall, the data obtained from 36 cultivars using molecular markers were in accordance with the initial classification based on morphological characters except in two cultivars. This is indicative of the informative trait and reliability of the morphological-based genome classification system in most cases as emphasized [16]. Our study seeks to identify progenies of paternal (pollen) parents using 20 coded morphological markers in order to establish which of these are inherited /transmitted to the progenies from their male progenitors. In addition, we seek to determine whether these traits are transmitted in equal measure by the paternal parents in the progenies of all the maternal (seed) parents. Maternal and (or) paternal inheritance of a character has been identified in many plant species [17] [18] [19] [20] [21] [22]. Previous studies revealed that the mitochondrial DNA in cucumber is paternally inherited [23] [24] [25] while the chloroplast DNA might be maternally inherited [26].

This study therefore describes the use of 20 morphological markers to identify the relationship between three diploid paternal (pollen) parents and their progenies from four different tetraploid maternal (seed) parents in a 4x – 2x polycross mating design.

II. MATERIALS AND METHODS

This study was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4°51'N, 7° 03'E, 10m above sea level), in Rivers State, south-eastern Nigeria. The rainfall pattern is monomodal, distributed over a 10month period from February through December, with an annual average of 2400mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July and September. The mean annual minimum and maximum temperatures are 25^0C and 27^0C, respectively, while solar radiation / sunshine lasts an average of 4hours daily [27]. The soil is derived from coastal sediments of the Niger Delta, freely drained and acidic (pH 4.3), and made up of mainly Kaolinite. Onne soils are also high in phosphorus 60mg kg^{-1}, manganese 0.2mmol kg^{-1}, but low in nitrogen [28], [29].

.Parental selection, hybridization and seedling evaluation

The following four tetraploid (4x) maternal (seed) parents – PITA 5; PITA 7; PITA 3; and PITA 14 and three diploid paternal (pollen) parents - TMP2x 2829-62; TMB2x 5105-1; and SH 3362 were selected from IITA's collection. Pedigree information on the parental hybrids is provided (Table 1). Both maternal and paternal selections were established in 2 polycross mating blocks. The seed parents were replicated at 12 plants per clone, and each crossing block had 31 plants of each of the three male parents in order to generate synthetic hybrids from the polycross blocks. Details of the polycross blocks and arrangement of male and female parents have been described elsewhere [30]. The experiment was located 200m in the south and 270m in the east away from any other plantain and banana fields; a distance which is more than the pollen dispersal distance or isolation distance of plantain and banana. This was done in order to exclude invasion of foreign pollen.

Table 1. Diploid and Tetraploid bananas and plantains used in the experiment Nigeria

Maternal Parents (4x)	**Source**	**Pedigree**	**Agronomic characteristics**
PITA 5	IITA	Bobby Tannap x Pisang Lilin	Stable high yield and bunch weight, regulated suckering and Black Sigatoka Resistance (BSR)
PITA 3	IITA	Obino l'ewai x Calcutta 4	High yielding, big fruits, good pulp quality and colour. BSR
PITA 14	IITA	Mbi Egome 1 x Calcutta 4	High yielding, short cycling, BSR, and field resistance to Banana streak virus (BSV)
PITA 7	IITA	Obino l'ewai x Pisang Lilin	Stable high yield and bunch weight, regulated suckering and moderately resistant to black Sigatoka disease
Paternal Parents (2x)	**Source**	**Pedigree**	**Agronomic characteristics**
TMP2x 2829-62	IITA	Bobby Tannap x Calcutta 4	Male and female fertile, good bunch, fruit parthenocarpy and BSR
TMB2x 5105-1	IITA	Pisang Lilin x Calcutta 4	Male fertile, fruit parthenocarpy and BSR
SH 3362	FHIA Honduras	SH 3217 x SH 3142	Good bunch size, BSR and resistant to race 4 of Fusarium wilt

Source: [31] [32] IITA. Prefix PITA (hybrids derived from plantain landraces), Serial cross numbers with / TMP stand for 'tropical *Musa*

plantain' (plantain-derived) & TMB for 'tropical *Musa* banana (banana-derived hybrid). Accessions 'Bobby Tannap' and Obino l'ewai- plantain landraces are triploid AAB plantains from west Africa that are susceptible to black Sigatoka disease; SH 3362 is a diploid banana from the Fundación Hondureña de Investigaçion Agricŏla (FHIA) in Honduras. 'Calcutta 4'- wild banana, 'Pisang lilin- dessert banana (AA) from southeast Asia that are resistant are diploid *Musa acuminate*; Mbi Egome is a medium French plantain cultivar

Assessment of paternal transmission of characters to progenies using morphological markers

At maturity, fruits were harvested, bunches ripened and the seeds extracted from each of the four tetraploid maternal parents (PITA 5; PITA 7; PITA 3; and PITA 14). Hard botanical seeds obtained were established in soil in the nursery. The resulting seedlings were transplanted to perforated nursery bags containing a mixture of topsoil and poultry manure in 7:1 ratio. Forty-three healthy seedlings of 80 nursery seedlings obtained from the four female parents were established in the field. At eight months after field establishment, progenies were evaluated based on standard descriptors of 20 morphological characters of the male parents. These characters differentiated the pseudostem, leaves, petiole, stalk of the leaf sheath and blade, and suckers produced. Data were generated by giving appropriate codes that described the phenotypes (Table 2). Chi-square test was performed to compare the contribution of each male parent to progenies of each female parent.

Table 2. Morphological characters and codes used for phenotypic tagging of the paternal parents and 43 resulting progenies of the maternal parents in a polycross mating design at IITA High Rainfall Station Onne, Rivers State, Nigeria.

S/N	**Morphological Characters**	**Codes**
1	Pseudostem colour	1=yellow; 2=green-yellow; 3=green; 4=green-red; 5=red; 6=other
2	Red colour on pseudostem	0=none. 3=slight. 5=moderate. 7=extensive
3	Pseudostem blotching	0=none. 3=slight. 5=moderate. 7=extensive
4	Colour of pseudostem blotch	1=brown. 2=black 3=other
5	Pseudostem/ foliage pigment	0=none. 3=slight. 5=moderate. 7=extensive
6	Pseudostem waxiness	0=none. 3=slight. 5=moderate. 7=heavy
7	Leaf orientation	1=erect. 2=intermediate. 3=dropping
8	Leaf colour	1=green. 2=green with red
9	Leaf margin	1=smooth. 2=crenated
10	Laminar shape	1=inhibited. 2=intermediate.
11	Shape of laminar base	1=cuneate. 2=rounded. 3=auriculate.
12	Petiole margin	1=spreading. 3=erect. 5=enrolled
13	Petiole margin clasping	0=absent. 1=present.
14	Colour of petiole margin	1=green. 2=other.
15	Colour of petiole margin medium section	1=green. 2=other
16	Colour of petiole margin dorsal section	1=green. 2=other
17	Dried petiole margin	0=absent. 1=present.
18	Petiole canal	1=spreading. 3=erect. 5=enrolled.
19	Suckering	1=inhibited. 2=intermediate. 3=1 or 2 suckers not inhibited. 4=no sucker inhibited,
20	Sucker orientation	3=erect. 5=intermediate. 7=divergent

III. RESULTS AND DISCUSSION

Paternal transmission and contribution to progenies using morphological markers

Morphological variation among male parents was used to confirm possible paternal transmission to progenies. Of the twenty morphological characters scored, sixteen characters were monomorphic while four were polymorphic and served as identifiable markers among the male parents (Table 3). These were pseudostem wax (PW), pseudostem blotch (PB), presence of petiole canal (PC), and clasping of petiole margin (CPM) and were therefore used to screen the progenies in order to identify their male progenitors. Hence, PW was only present in TMB2x 5105-1, PB was unique to SH 3362, PC and CPM occurred in TMP2x 2829-62 and SH 3362, but not in TMB2x 5105-1. This means that the polymorphic character identifying progenies of TMB2x 5105-1 was extensive wax on the pseudostem (PW), whereas extensive blotch on pseudostem (PB) was peculiar to progenies of SH 3362. The presence of petiole canal (PC) and clasping of the petiole margin (CPM), differentiated progenies of TMP2x 2829-62 and SH 3362 from progenies of TMB2x 5105-1. These four morphological differences among the male parents were used to establish their contribution to 43 of the progenies. Chi-square analysis performed on informative progenies showed significant differences ($P > 0.05$) in pseudostem wax (PW) and pseudostem blotch (PB) and petiole canal for progenies of the maternal parent PITA 5 (Table 3).

Table 3: Estimates of paternal contribution to progenies of different maternal /seed parents from the 4x – 2x *Musa* polycross mating design using four polymorphic morphological markers at IITA High Rainfall Station, Onne, Rivers State, Nigeria.

Morphological markers	Maternal parents	Total no. of progenies	No. of informative individuals	Paternal contribution			
				TMP2x 2829-62	TMB2x 5105-1	SH 3362	X^2
Pseudostem waxiness	PITA 5	19	13	5.373	6.004	5.373	**11.377****
	PITA 3	8	5	1.760	1.260	1.760	**3.021**
	PITA 7	11	7	1.280	0.917	2.735	**0.817**
	PITA 14	5	0	0.817	0.817	0.817	**1.633**
Pseudostem blotching	PITA 5	19	3	5.373	1.268	5.373	**6.640***
	PITA 3	8	2	1.760	0.010	1.760	**1.771**
	PITA 7	11	2	2.735	0.371	2.735	**3.106**
	PITA 14	5	1	0.817	0.017	0.817	**0.833**
Clasping petiole margin	PITA 5	19	19	5.373	0.510	0.004	**5.883**
	PITA 3	8	8	1.760	0.189	0.510	**1.950**
	PITA 7	11	11	2.735	0.417	0.189	**3.152**
	PITA 14	5	5	0.817	0.417	0.117	**0.833**
Petiole canal	PITA 5	19	11	5.373	5.373	2.741	**13.487****
	PITA 3	8	7	1.760	1.760	5.510	**9.031***
	PITA 7	11	3	2.735	2.735	0.008	**5.477**
	PITA 14	5	2	0.817	0.817	0.017	**1.650**

** Chi-square test, significant at 0.05; ** significant at 0.01 probability level.*

This Chi square test tells us that the null hypothesis, that paternal contribution to progenies of maternal parents was equal, should be rejected with respect to these 3 morphological characters in the maternal parent PITA 5 and that the alternative hypothesis, that paternal contribution to progenies was not equal should be accepted. It would appear therefore that all 3 paternal parents having these distinct characters had unequal contribution with the maternal parent PITA 5. Such significant differences suggest the unequal transmission of these morphological characters (Pseudostem waxiness, Pseudostem blotching and Petiole canal) from the paternal parents to the progenies of PITA 5- the maternal parent. On the contrary, Pseudostem waxiness, and Pseudostem blotching showed equal contribution in the other 3 maternal parents PITA 3, PITA 7 and PITA 14. Moreover, the morphological character Petiole canal also showed unequal transmission from the paternal parents in progenies of the maternal parent PITA 3. In contrast, paternal transmission of the character, Clasping of the petiole margin (CPM) did not differ significantly among the progenies of all the maternal parents suggesting that paternal contribution of this character in progenies was equal for all male progenitors.

IV. CONCLUSION

This study found unequal contribution of paternal parents to progenies of some maternal parents with respect to three morphological characters - pseudostem waxiness, pseudostem blotching and petiole canal, whereas clasping of the petiole margin showed equal contribution in progenies of all the maternal parents.

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