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The Efficiency of Hybridization and Seed Production in *Musa* spp

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In this study, *Musa* species and cultivars with different ploidy levels and genomic constitutions were hybridized to estimate the efficiency of the hybridization process and the yield of hybrid seeds. The findings are as follows. The hybridization of 'Dwarf Cavendish' (a triploid) as the female parent with the pollen of *Musa balbisiana* (a species of wild banana) produced few hybrid seeds, and that of 'Pei Chiao' as the female parent with the wild banana yielded no hybrid seed. The hybridization between 'Monkey' and the pollen of diploid wild bananas had a seed–set rate of 8%–12% and produced 1–19 hybrid seeds. The hybridization between 'Pelipia' and the pollen of diploid wild bananas had a seed–set rate of 6%–50%. The hybridization of 'Pelipia' with *M. balbisiana* produced 1–219 hybrid seeds, 12–244 with *M. itinerans* var. *formosana* and 1–14 with *M. textilis*. The hybridization of *M. balbisiana* with *M. itinerans* var. *formosana* as the male parent (rather than as the female parent) produced seeds. Some of the hybrid seeds from the hybridization of triploid cultivars with the pollen of wild diploids were irregular or deformed in appearance. The hybridization of triploid cultivars with wild diploids as female parents produced more hybrid seeds, most of which were normal in appearance and some of them contained (a) no embryo or endosperm, (b) deformed embryos and no endosperm, or (c) deformed embryos and endosperms. Accordingly, the production of hybrid seeds does not necessarily indicate successful hybridization. The findings of this study may inform the selection of hybrid parents and banana breeding.

Key words: *Musa* spp., banana, hybridization, hybrid seed

INTRODUCTION

Bananas and plantains (*Musa* spp.) are an important crop in subtropical zones and have a worldwide production of approximately 100 million tons per year (Heslop–Harrison, 2011). Most *Musa* cultivars are sterile and produce seedless fruits through parthenocarpy. Cultivated *Musa* hybrids and species are mostly triploid; only a few of them are diploid and tetraploid. The genetics and sterility of bananas and plantains cause difficulty with developing new varieties through hybridization (Heslop–Harrison and Schwarzacher, 2007). Banana cultivars are derived from intra– and inter–specific hybridization between *M. acuminata* Colla (AA) and *M. balbisiana* Colla (BB) (Simmonds and Shepherd, 1955). The earliest edible bananas are diploids that have evolved through the development of sterility and parthenocarpy of *M. acuminata* (Valmayor *et al*., 2000). Pollefeys *et al*. (2004) collected wild seedless bananas and parthenocarpic hybrids and mutants from various sources, domesticating them to produce seedless cultivars that are diploid (AA and AB), triploid (AAA, AAB, and ABB), and tetraploid (AAAA, AAAB, AABB, and ABBB). Worldwide, there are over 1,000 domesticated *Musa* cultivars with high genetic diversity (Heslop–Harrison and Schwarzacher, 2007).

Farmers propagated, grew, and cropped parthenocarpic, high–yielding, and disease–resistant banana plants during the first several millennia of banana cropping; undertook field trials of numbers of progeny during the twentieth century in which different species were crossed and the optimum progeny were selected; and have improved crops since the twenty–first century by using genetic maps and DNA markets to identify useful variant alleles of genes, conduct recombination between desirable traits, and combine different resistance genes and accelerate their selection (Heslop–Harrison, 2011). Many innovative techniques have been developed to facilitate plant breeding—e.g., micropropagation, anther culture, in vitro selection, embryo rescue, somaclonal variation, and somatic hybridization and transformation (Karp, 1995). Breeding *Musa* through mutation compensates for the weaknesses of existing *Musa* cultivars (Heslop–Harrison and Schwarzacher, 2007); for example, chemical mutagens can be used to induce mutations in the Highgate cultivar (AAA) for tolerance to *Fusarium oxysporum* f. sp. cubense (Bhagwat and Duncan, 1998). Combining both sexual and asexual reproduction systems might be the optimal strategy to increase the diversity of vegetative reproductive crops, such as bananas, with desirable traits (Uma and Arun, 2016).

Crucial targets of *Musa* spp. breeding include resistance to pathogens, diseases, and pests, short life cycles, low plant heights, high production, parthenocarpy, and species quality (Ortiz and Swennen, 2014). The genetic resources of wild bananas are regarded as a treasury database for providing useful resistance traits that can

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be effectively applied in crop modification (Padmesh *et al.*, 2012). *M. acuminate* has many subspecies, among which hybrid cultivars are studied through cell biology; all diploid cultivars express hybridization at one or more translocations (Lanaud *et al.*, 1992). Wild *Musa* spp. and related species not only provide natural introgression, but also increase the diversity of bananas (Uma and Arun, 2016).

Banana is both monoecious (containing both male and female reproductive organs) and dichogamous (temporal and female organs mature before the male organs) (Fortescue and Turner, 2011). The high sterility of *Musa* diploids and triploids renders it difficult to breed plantains and bananas through hybridization; some banana cultivars have sterile pollen (Ortiz, 1995). The existence of 2*n* pollen in diploid species suggests the involvement of unilateral sexual polyploidization ($2n \times n$) in the origin of triploid *Musa* species (Ortiz, 1997b). Goigoux *et al.* (2013) assessed the pollen fertility of both diploid and doubled–diploid of Mlalis (as donor of diploid gametes) that were treated with chromosome doubling. Pollen viability and count vary between *Musa* cultivars; diploid *Musa* species produce more viable pollen than do triploid *Musa* species; and tetraploid *Musa* species have the lowest pollen viability of the three (Fortescue and Turner, 2004). Triploid female fertile clones can be crossed with male fertile, diploid wild, or cultivated accessions to improve plantains and bananas (Oselebe *et al.*, 2014). Plantains and bananas are improved by crossing heterogenomic triploid accessions (AAB and ABB) with homogenomic diploid accessions (AA and BB) (Rowe 1984). Triploid–diploid crosses between plantains and banana produce diploid, triploid, and tetraploid hybrids (Ortiz, 1997a).

Minimizing infertility barriers by crossing triploid landraces with wild diploids or tetraploids with diploids to produce sterile triploids and inter– and intra–specific hybrids can improve triploid *Musa* species with desired traits (Pillay *et al.*, 2002). Assessing the number, quality, and embryo rescue rates of hybrid seeds can enhance the efficiency of banana breeding (Ssebuliba *et al.*, 2006). In this study, *Musa* species and cultivars with different ploidy levels and genomic constitutions were hybridized to assess the efficiency of the hybridization and the yield of hybrid seeds.

MATERIALS AND METHODS

Plant materials

This study used 28 *Musa* species and cultivars (materials from four *M. acuminata* and one *M. balbisiana*, 21 intra– or interspecific hybrids of *M. acuminata* and *M. balbisiana*, one species of *M. itinerans* var. *formosana*, and one species of *M. textilis*) cultivated in a banana germplasm garden managed by the Taiwan Agricultural Research Institute (Chiayi branch) (Table 1). The garden is located at the geographic coordinates of 23°48'43"N and 120°46'80"E, with an altitude of 33 m, an average annual temperature of 23°C, and an average annual rainfall of 2000 mm.

Cross–pollination combinations

Twenty–one *Musa* species and cultivars—4 diploids (AA, AB, and BB), 15 triploids (AAA, AAB, and ABB), one species of *M. itinerans* var. *formosana*, and one species of *M. textilis*—were used as female parents. Fourteen *Musa* species and cultivars—5 diploids (AA and BB), 7 triploids (AAA, ABB, and BBB), one species of *M. itinerans* var. *formosana*, and one species of *M. textilis*—were used as male parents (or pollen sources). Cross–pollination was conducted for each tier (hand) of female inflorescences (Table 2). Cross–pollinated female inflorescences were packaged to prevent contamination.

Hybridization results and the examination of hybrid seeds

The mature fruits of *Musa* species and cultivars were harvested following cross pollination; both the number of hybrids between *Musa* species and cultivars and the seed–set rate of the hybridization were estimated. The seeds extracted from fruits were examined and the number of seeds and their development patterns were analyzed. The section profiles of hybrid seeds were observed using a stereo microscope.

RESULTS

Seed sets produced from hybridization between *Musa* species and cultivars

Table 3 presents the results of different hybrids between Musa species and cultivars. Seeds were obtained through hybridization using *M. balbisiana* (BB), 'Dwarf Cavendish' (AAA), 'Monkey' (ABB), 'Pelipia' (ABB), *M. itinerans* var. *formosana* (ABB), and *M. textilis* (ABB) as female parents. The male parents were *M. acuminata* (AA), 'Calcultair 124' (AA), *M. balbisiana* (BB), *M. itinerans* var. *formosana* (BB), *M. textilis* (BB).

Table 2 shows the seed–set rates of different hybrids between *Musa* species and cultivars. The seed–set rate was 100% for the hybridization of *M. balbisiana* (female parent) with *M. acuminate*, 20% with 'Calcultair 124', 43% with *M. balbisiana*, 75% with *M. itinerans* var. *formosana*, and 50% with *M. textilis*. The seed–set rate for the hybridization of 'Dwarf Cavendish' (female parent) was 33% with *M. balbisiana*. The seed–set rate for the hybridization of 'Monkey' (female parent) was 10% with both 'Calcultair 124' and *M. balbisiana*, 8% with *M. itinerans* var. *formosana*, and 12% with *M. textilis*. The seed–set rate for the hybridization of 'Pelipia' (female parent) was 9% with *M. balbisiana*, 6% with *M. itinerans* var. *formosana*, and 50% with *M. textilis*. The seed–set rate for the hybridization of *M. itinerans* var. *formosana* (female parent) was 100% with 'Calcultair 124', 17% with *M. itinerans* var. *formosana*, and 11% with *M. textilis*. The seed–set rate for the hybridization of *M. textilis* (female parent) was 100% with *M. balbisiana*, *M. itinerans* var. *formosana*, and *M. textilis*.

Hybrid seeds produced

Table 3 also summarizes the number of seeds pro-

Table 1. Plant materials of the genus *Musa* used in this study

No.	Accession ID [a]	Species/hybrid	Genome	Common name	Abbreviation
1	00264380	*M. acuminata*	AA w [c]	*Musa acuminata*	ACU
2	– [b]	*M. acuminata*	AA	Calcultair 124	CAL
3	00264568	*M. acuminata*	AA cv [c]	Cultivar Rose	CRO
4	00105288	*M. acuminata*	AA	Sucrier	SUC
5	00105297	*M. acuminata* × *M. balbisiana*	AB	Ney Poovan	NPO
6	00105304	*M. balbisiana*	BB w [d]	*Musa balbisiana*	BAL
7	00105411	*M. acuminate* (Triploid)	AAA	Dwarf Cavendish	DCA
8	00264479	*M. acuminate* (Triploid)	AAA	Gctcv–119	GCT
9	00105466	*M. acuminate* (Triploid)	AAA	Giant Cavendish	GCA
10	00264317	*M. acuminate* (Triploid)	AAA	Manang	MAN
11	00106025	*M. acuminate* (Triploid)	AAA	Pei Chiao	PCH
12	00264488	*M. acuminate* (Triploid)	AAA	TC1–229	TC1
13	00105813	*M. acuminate* (Triploid)	AAA	Valery	VAL
14	00264577	*M. acuminate* (Triploid)	AAA	Yangambi KM5	YAN
15	00106043	*M. acuminata* × *M. balbisiana* (Triploid)	AAB	Assam	ASS
16	00264737	*M. acuminata* × *M. balbisiana* (Triploid)	AAB	Tibok	TIB
17	00264835	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Ice Cream	ICR
18	00263892	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Klue Namwa Khau	KNK
19	00263909	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Medine	MED
20	00264844	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Monkey	MON
21	00264853	*M. balbisiana* (Triploid)	ABB	Nibah	NIB
22	00264335	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Pelipia	PEL
23	00263927	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Pisang Awak	PAW
24	00264559	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Pisang Nangka	PNA
25	00264899	*M. acuminata* × *M. balbisiana* (Triploid)	ABB	Pitogo	PIT
26	00264826	*M. balbisiana* (Triploid)	BBB	Cooking	COO
27	00105242	*M. itinerans*	unknown w [e]	Formosana	FOR
28	00105206	*M. textilis*	TT w [f]	Abaca	ABA

[a] Accession ID was based on the National Plant Genetic Resources Center of Taiwan Agricultural Research Institute

[b] No Accession ID

[c] *M. acuminate AAw* wild type, *AAcv* cultivar

[d] *M. balbisiana BBw* wild type

[e] *M. itinerans* diploid wild type

[f] *M. textilis* diploid wild type

duced from different hybrids between *Musa* species and cultivars. Hybridizing 'Dwarf Cavendish' as the female parent with the pollen of *M. balbisiana* produced one hybrid seed. Two of the hybrids between 'Monkey' (female parent) and 'Calcultair 124' produced 4 and 7 hybrid seeds. The hybridization of 'Monkey' (female parent) produced 1–7 hybrid seeds with *M. balbisiana*, 2–19 hybrid seeds with *M. itinerans* var. *formosana*, and one hybrid seed with *M. textilis*. The hybridization of 'Pelipia' (female parent) produced 1–219 hybrid seeds with *M. balbisiana*, 12–217 hybrid seeds with M. *itinerans* var. *formosana*, and 1–14 hybrid seeds with *M. textilis*. Two of the hybrids between *M. balbisiana* (female parent) with *M. acuminata* produced 68 and 147 hybrid seeds. The hybridization of *M. balbisiana* (female parent) produced 182 hybrid seeds with 'Calcultair 124', 21–303 hybrid seeds with *M. balbisiana*, 3–3297 hybrid seeds with *M. itinerans* var. *formosana*, and

Table 2. Number of hybrid seed sets from different hybrids between *Musa* species and cultivars

♀\♂	ACU	CAL	CRO	SUC	BAL	GCA	MAN	YAN	MED	NIB	PAW	COO	FOR	ABA
CRO	–[a]	1 (0)	–	–	1 (0)	–	–	–	–	–	–	–	1 (0)	2 (0)
SUC	3[b] (0)[c]	5 (0)	–	–	28 (0)	–	–	–	–	–	–	–	24 (0)	4 (0)
NPO	4 (0)	6 (0)	1 (0)	1 (0)	14 (0)	1 (0)	1 (0)	–	–	–	–	–	16 (0)	9 (0)
BAL	2 (100)	5 (20)	1 (0)	–	7 (43)	–	1 (0)	–	–	–	1 (0)	2 (0)	4 (75)	10 (50)
DCA	–	2 (0)	–	–	3 (33)	–	–	–	–	–	–	–	2 (0)	2 (0)
GCA	–	–	–	–	12 (0)	–	–	–	–	–	–	–	13 (0)	–
GCT	–	1 (0)	–	–	2 (0)	–	–	–	–	–	–	–	1 (0)	1 (0)
PCH	14 (0)	13 (0)	4 (0)	1 (0)	60 (0)	6 (0)	–	–	–	3 (0)	–	1 (0)	50 (0)	9 (0)
TC1	–	1 (0)	–	–	2 (0)	–	–	–	–	–	–	–	1 (0)	2 (0)
VAL	2 (0)	–	1 (0)	–	–	–	2 (0)	–	–	–	–	–	–	1 (0)
ASS	–	4 (0)	–	–	2 (0)	–	–	–	–	–	–	–	–	–
TIB	2 (0)	2 (0)	–	–	19 (0)	1 (0)	–	–	–	1 (0)	–	–	19 (0)	3 (0)
ICR	–	1 (0)	–	–	1 (0)	–	–	–	–	–	–	–	2 (0)	3 (0)
KNK	2 (0)	2 (0)	–	–	2 (0)	–	–	–	–	–	–	–	–	4 (0)
MED	1 (0)	1 (0)	1 (0)	–	2 (0)	–	–	–	–	–	–	–	2 (0)	1 (0)
MON	17 (0)	21 (10)	–	–	41 (10)	6 (0)	–	1 (0)	–	1 (0)	–	–	48 (8)	25 (12)
PEL	7 (0)	10 (0)	–	–	65 (9)	4 (0)	–	–	1 (0)	1 (0)	–	–	62 (6)	12 (50)
PIT	–	2 (0)	–	–	–	–	–	–	–	–	–	–	2 (0)	2 (0)
PNA	–	1 (0)	–	–	1 (0)	–	–	–	–	–	–	–	1 (0)	2 (0)
FOR	–	1 (100)	1 (0)	1 (0)	7 (0)	–	–	–	–	–	–	3 (0)	6 (17)	9 (11)
ABA	–	1 (0)	–	–	2 (100)	–	–	–	–	–	–	–	2 (100)	2 (100)

[a] No hybridization
[b] Number of tiers of inflorescence of hybridized female flowers
[c] Percentage of seed sets from hybridization

Table 3. Number of seeds from hybridization between *Musa* species and cultivars

♀\♂	ACU	CAL	BAL	FOR	ABA
BAL	2[a] (147, 68)[b]	1 (182)	3 (303, 21, 30)	3 (5, 3297, 3)	5 (863, 6, 306, 175, 29)
DCA	–[c]	0	1 (1)	0	0
MON	0	2 (4, 7)	4 (1, 1, 1, 7)	4 (4, 19, 2, 12)	3 (1, 1, 1)
PEL	0	0	6 (38, 1, 219, 118, 6, 7)	4 (25, 217, 244, 12)	6 (2, 3, 6, 3, 1, 14)
FOR	–	1 (853)	0	1 (506)	1 (773)
ABA	–	0	2 (275, 3)	2 (278, 27)	2 (69, 83)

[a] Number of hands in hybrid seed sets
[b] Number of seeds/hands
[c] No hybridization

6–863 hybrid seeds with *M. textilis*. The hybridization of *M. itinerans* var. *formosana* (female parent) produced 853 hybrid seeds with 'Calcultair 124', 506 hybrid seeds with *M. itinerans* var. *formosana*, and 773 hybrid seeds with *M. textilis*. Two of the hybrids between *M. textilis* (female parent) with *M. balbisiana* respectively produced 3 and 273 hybrid seeds. Two of the hybrids between *M. textilis* (female parent) with *M. itinerans* var. *formosana* produced 27 and 278 hybrid seeds. Two of the hybrids between *M. textilis* (female parent) with *M. textilis* produced 69 and 83 hybrid seeds.

Appearance of hybrid seeds

The analysis of the appearances of hybrid seeds from crosses between *Musa* species and cultivars revealed the following findings. Hybrid seeds from the hybridization of 'Dwarf Cavendish' (female parent) with *M. balbisiana* were normal in appearance (Fig. 1a). Those from the hybridization of 'Monkey' (female parent) with *M. balbisiana* were abnormal and spindle–shaped in appearance (Fig. 1b). Those from the hybridization of 'Pelipia' (female parent) with *M. textilis* had a normal oval appearance (Fig. 1c). Those from the hybridization of *M. balbisiana* (female parent) with the pollen of 'Calcultair 124', *M. acuminata*, and *M. textilis* respectively appeared elliptical (Fig. 1d), oval or deformed and shriveled (Fig. 1e–g), and as a normal oval (Fig. 1h). Those from the hybridization of *M. textilis* (female parent) with *M. balbisiana* and *M. itinerans* var. *formosana* had a normal oval appearance (Fig. 1i and 1j). Seeds from the self–pollination of 'Pelipia' contained embryos and endosperms (Fig 1k–l). The self–

pollination of 'Calcultair 124' produced normal oval seeds with embryos and endosperms and abnormal flat seeds with degenerated embryos and without endosperms (Fig. 1m–p). The self–pollination of *M. balbisiana* produced normal oval seeds with embryos and endosperms (Fig. 1q–r). The self–pollination of *M. itinerans* var. *formosana* produced normal seeds with a flat oval appearance, embryos and endosperms, and immature seeds colored in translucent white (Fig. 1s–u). The self–pollination of *M. textilis* produced normal oval seeds with embryos and endosperms and abnormal seeds with a normal oval appearance and without embryos or endosperms (Fig. 1v–x).

The analysis of the sectional profiles of hybrid seeds from crosses between *Musa* species and cultivars yielded the following findings. Hybrid seeds from the hybridization of *M. balbisiana* (female parent) with 'Calcultair 124' contained either shriveled endosperms or no embryo and endosperm (Fig. 2a–c). Those from the hybridization of *M. balbisiana* with *M. acuminata* contained normal embryos and endosperms, deformed embryos and endosperms, or no embryo or endosperm

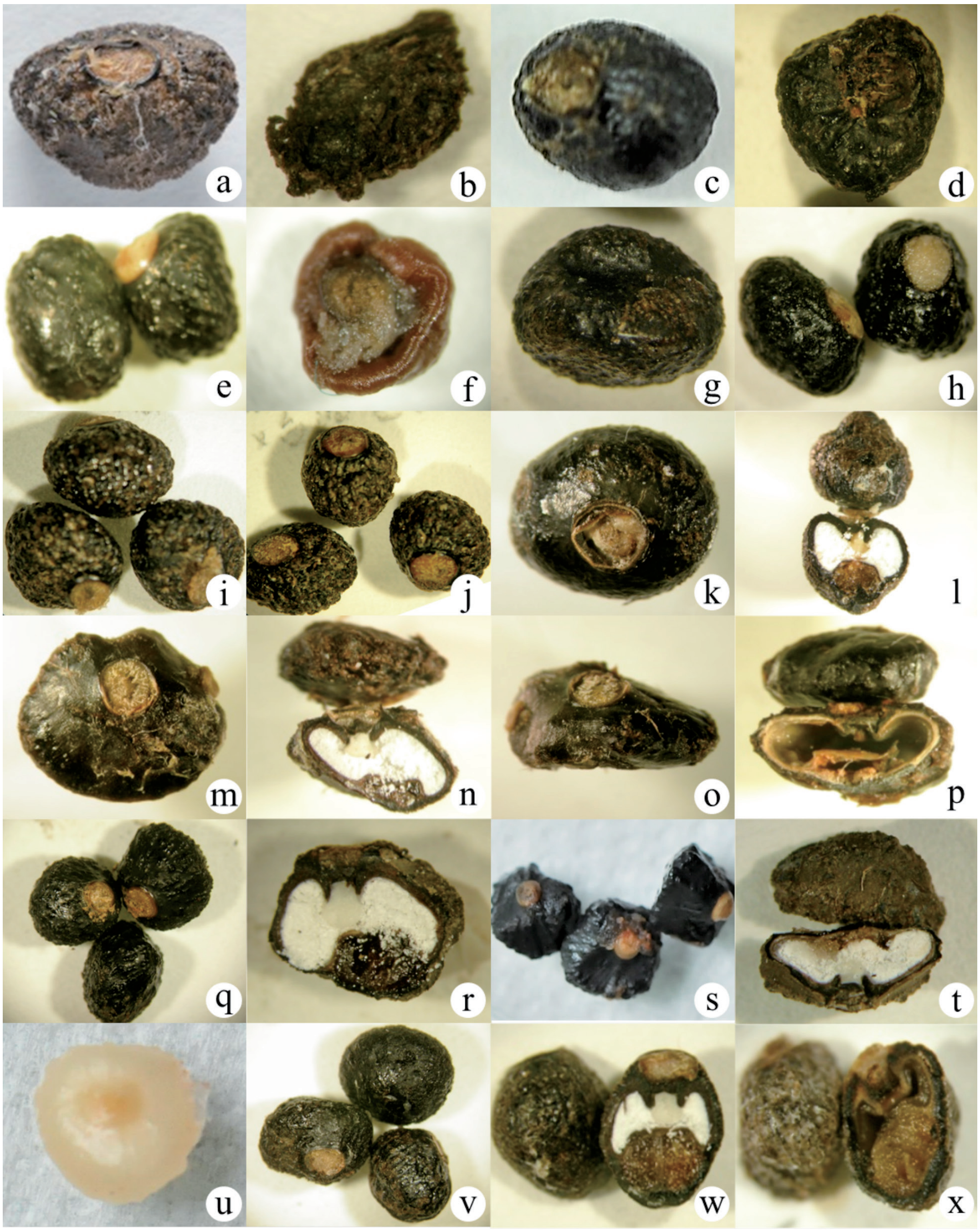

Fig. 1. Hybrid seeds of *Musa* spp. a. DCA x BAL; b. MON x BAL; c. PEL x ABA; d. BAL x CAL; e.,f. BAL x SUC; g. BAL x ACU; h. BAL x ABA; i. ABA x BAL; j. ABA x FOR; k.,l. PEL x PEL; m.–p. CAL x CAL; q.,r. BAL x BAL; s.–u. FOR x FOR ; v.–x. ABA x ABA.
(ABA: 'Abaca'; ACU: *M. acuminata*; BAL: *M. balbisiana*; CAL: 'Calcultair 124'; DCA: 'Dwarf Cavendish'; FOR: 'Formosana'; MON: 'Monkey'; PEL: 'Pelipita'; SUC: 'Sucrier')

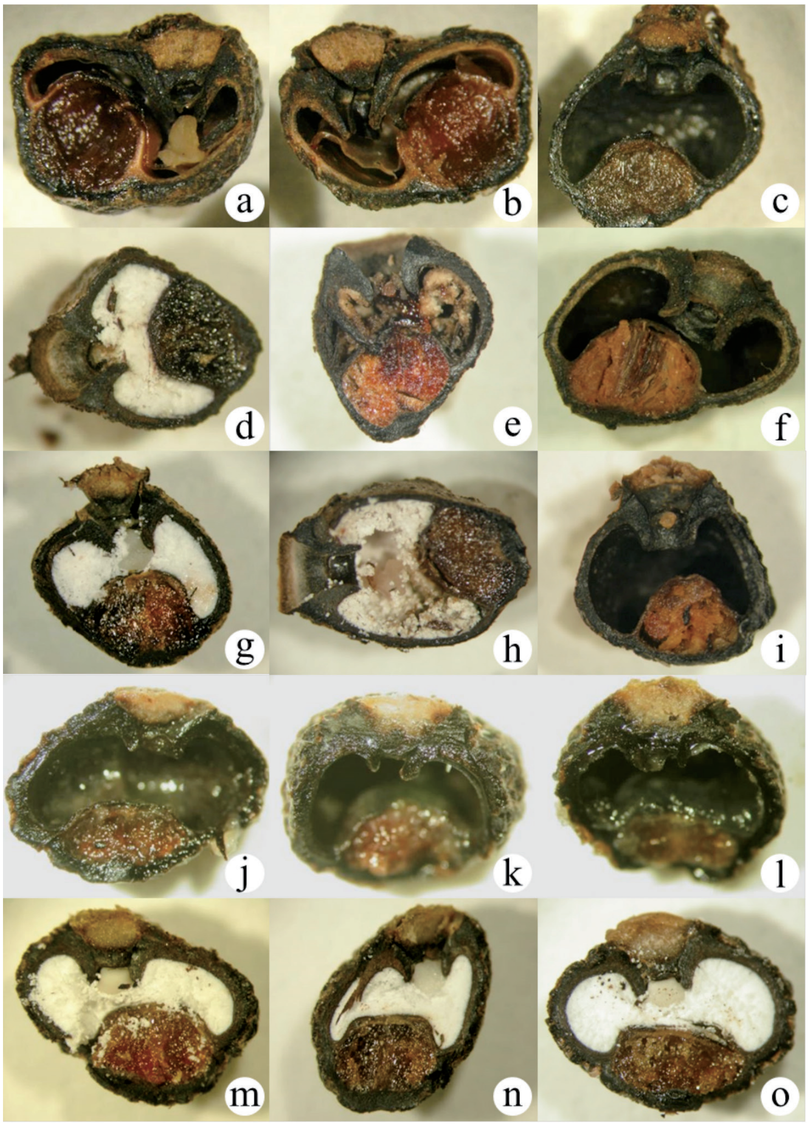

Fig. 2. Sectional profile of hybrid seeds of *Musa* spp. a.–c. BAL x CAL; d.–f. BAL x ACU; g. BAL x ABA; h.,i. BAL x FOR; j.–l. ABA x FOR; m.–o. ABA x BAL. (ABA: 'Abaca'; ACU: *M. acuminata*; BAL: *M. balbisiana*; CAL: 'Calcultair 124'; FOR: 'Formosana')

(Fig. 2d–f). Those from the hybridization of *M. balbisiana* with *M. textilis* contained embryos and endosperms (Fig. 2g). Those from the hybridization of *M. balbisiana* with *M. itinerans* var. *formosana* contained either embryos and underdeveloped endosperms or no embryo or endosperm (Fig. 2h–i). Those from the hybridization of *M. textilis* (female parent) with *M. itinerans* var. *formosana* contained no embryo or endosperm and merely translucent matters (Fig. 2j–l). Those from the hybridization of *M. textilis* with *M. balbisiana* contained embryos and endosperms (Fig. 2m–o).

DISCUSSION

The hybridization of *Musa* species and cultivars suggested that 'Dwarf Cavendish' (AAA), 'Monkey' (ABB), and 'Pelipia' (ABB) are female fertile clones that can be crossed with the wild species of *M. balbisiana* (BB), yielding seed–set rates of 33%, 10%, and 9%, respectively. 'Dwarf Cavendish' had a low seed–set rate because it produced only one hybrid seed. Moreover, 'Giant Cavendish' and 'Pei Chiao' (the main cultivar indigenous to Taiwan) (Ko *et al.*, 2009), both of which are Cavendish bananas, produced no hybrid seed (Tables 2 and 3). Edible bananas are parthenocarpic and typically seedless clones that are diploid, triploid or tetraploid; Cavendish clones (AAA) and AAB and ABB plantain groups have polyploidy, low fertility, and infertility—characteristics that limit the success of breeding programs (Shepherd, 1987).

Several triploid plantain and banana cultivars produced seeds after hand pollination with diploid parents. ABB cooking bananas had the highest rate of seed production, followed by AAB French plantains (Ortiz and Vuylsteke, 1995). The hybridization of 'Monkey' with 'Calcultair 124', *M. balbisiana*, *M. itinerans* var. *for-*

mosana, and *M. textilis* had seed–set rates 10% (4–7 seeds), 10% (1–7), 8% (2–19 seeds), and 12% (one seed), respectively. The hybridization of ‘Pelipia’ with *M. balbisiana* had a seed–set rate of 9% (1–219 seeds), the hybridization of ‘Pelipia’ with *M. itinerans* var. *formosana* had a seed–set rate of 6% (12–244 seeds), whereas the hybridization of ‘Pelipia’ with *M. textilis* had a seed–set rate of 50% (1–14 seeds) (Tables 2 and 3). Triploid female clones can be crossed with male fertile, diploid wild, or cultivated accessions to improve plantains and bananas (Oselebe *et al*., 2014). Most edible bananas can be experimentally induced to yield seeds occasionally; breeding methods are assessed according to crosses between diploids, tetraploids and diploids, or triploids and diploids (Shepherd, 1987).

The hybridization of the wild species *M. balbisiana* (BB) with *M. acuminata*, ‘Calcultair 124’, *M. itinerans* var. *formosana*, and *M. textilis* had seed–set rates of 100% (68–147 seeds), 20% (182 seeds), 75% (3–3297 seeds), and 50% (6–83 seeds), respectively. The hybridization of *M. itinerans* var. *formosana* with ‘Calcultair 124’ and *M. textilis* had seed–set rates of 100% (853 seeds) and 11% (773 seeds), respectively. Hybrids with the wild species *M. balbisiana* produced no seed. Notably, the hybridization of *M. textilis* with *M. balbisiana*, ‘Calcultair 124’, *M. itinerans* var. *formosana* had 100% seed–set rates and produced seeds (Tables 2 and 3). Seasonal variation in 2*n* pollen production correlates strongly with solar radiation, temperature, total pan evaporation, rainfall, and minimum relative humidity (Ortiz *et al*., 1998).

Between different sections of bananas, seeds vary slightly in appearance but are highly similar in structure and anatomy, and the outer integument of a *Musa* seed covers the micropylar and chalazal mass and the inner integument of the seed encompasses embryos and endosperms (Graven *et al*., 1996). In this study, slight differences were observed in seed appearance between the hybrids of the pollen of wild diploids with ‘Dwarf Cavendish’ (AAA), ‘Monkey’ (ABB), and ‘Pelipia’ (ABB); some hybrid seeds had a normal oval appearance, whereas others had an irregular or deformed and shriveled appearance (Fig. 1). Regarding the anatomical structure of hybrid seeds, some contained fully developed embryos and endosperms, some contained deformed embryos and deformed or shriveled endosperms, and some had a normal appearance but contained no embryo or endosperm (Fig. 2). Regarding the anatomical structure of hybrid seeds from the hybridization of *M. balbisiana* (BB) as the female parent, some contained fully developed embryos and endosperms, some contained embryos but no endosperm or underdeveloped endosperms, and some others had a normal appearance and contained either deformed embryos or no embryo or endosperm (Fig. 2). Regarding the anatomical structure of seeds from the hybridization of *M. textilis* (female parent) with *M. balbisiana*, the seeds contained normal embryos and endosperms. However, the hybridization of *M. textilis* with *M. itinerans* var. *formosana* produced seeds without embryos or endosperms (Fig. 2). Ssebuliba *et al.* (2006) studied hybrids from East African highland bananas, showing that although 72% of the seeds had a normal external appearance characterized by hard black integuments, only 59% contained embryos, of which 9% germinated. Their finding suggested that hard-seededness alone does not indicate the presence of an embryo and should not be used as a measure of seed fertility in East African highland bananas.

Interspecific and interploidy crosses are essential to producing *Musa* hybrids, with most of their hybrid seeds being immature and having low viability and germination rates under natural conditions (Ortiz and Vuylsteke, 1995). Therefore, the survival rates of hybrid seeds must be improved through micropropagation (Vuylsteke *et al*., 1990). Major factors in the success of breeding and hybridization of bananas include ploidy, genomic constitution, and male and/or female sterility. In the present study, the hybridization of *M. balbisiana* with *M. itinerans* var. *formosana* as the male parent, rather than the female parent, produced seeds. The findings of this study are expected to inform the utilization of banana germplasm and the improvement and breeding of banana.

AUTHOR CONTRIBUTION

1. Shu–Fen CHANG, proved the hybrid seed structure between *Musa* species or cultivars is not necessarily normal, and the parental selection affects the efficiency of hybridization.
2. Yung–Fu YEN, designed observational study of the appearance and sectional profile morphology of banana hybrid seeds, and offered suggestion for research.
3. Ikuo MIYAJIMA, offered advices on international tropical horticultural crops research for crossbreeding research, and revised paper and inspected final data.
4. Kuang–Liang HUANG, organized the research protocol through hybridization experiments of banana and managed lab process.

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