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# Classification of African plantain landraces and banana cultivars using a phenotypic distance index of quantitative descriptors

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Abstract Proper classification and establishment of relationships between and within Musa taxonomic clusters will be important tools for the genetic improvement of plantain and banana. This paper assesses the value of a phenotypic diversity index, based on 16 quantitative descriptors, for germplasm clustering and for identification of duplicates among 92 triploid plantain and banana accessions. Data were recorded during the plant and ratoon crops at Onne, a humid forest location in southeastern Nigeria. The phenotypic distance matrix was developed by calculating the average difference between each pair of accessions for all quantitative descriptors. Significant differences were observed for this phenotypic distance index between Musa taxonomic clusters. The between-cluster variance was larger (0.001779) than the within-cluster variance (0.001380). Wright's  $\phi_{\rm FS}$ , which measures the overall diversity, was 0.5663. This value suggested little gene flow among triploid taxonomic clusters via pollen, which explains the higher population differentiation exhibited by this vegetatively propagated crop with very low male fertility. The results also suggested that variation observed within each Musa taxonomic cluster arose from mutations accumulated throughout the history of cultivation of this crop. Some putative duplicates based on qualitative descriptors were not regarded as the same accession according to the phenotypic diversity index based on quantitative

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D. Vuylsteke International Institute of Tropical Agriculture, East and Southern Africa Regional Centre, P.O. Box 7878, Kampala, Uganda descriptors. Hence, gene-bank curators should assess quantitative descriptors for the identification of duplicate accessions in *Musa*.

Key words  $Musa \cdot Core \text{ collection } \cdot Duplicates \cdot Evolution \cdot Variation$ 

### Introduction

World production of plantain and banana (*Musa* spp. L.) is estimated at 85.5 million MT, of which one third is harvested in Africa south of the Sahara (http://apps.fao.org/lim500/nph-wrap.pl?Production. Crops. Primary&Domain = SUA). Africa accounts for 27.4% of the world's banana production, and 61.3% of the world's plantain production. The gross value of their annual production exceeds that of other food crops in this continent (IITA 1992).

Plantain and banana evolved from intra- and interspecific crosses of the two diploid wild species *Musa acuminata* Colla. and *M. balbisiana* Colla. in the section *Eumusa* of the genus *Musa* (Simmonds 1995). The most popular system of classification of *Musa* cultivars was developed by Simmonds and Shepherd (1955). These authors used a scoring technique to indicate the percentages of each of the two diploid *Musa* species in any cultivar. In their system, 15 diagnostic characteristics (or descriptors) were considered. The scale for each qualitative descriptor ranged from 1 (phenotype equal to *M. acuminata*) to 5 (pure *M. balbisiana*).

The most important triploid dessert bananas of the world trade market ('Cavendish' and 'Gros Michel') and the East African Highland bananas derived from intraspecific crosses of *M. acuminata* were asigned to the AAA taxonomic group following the key developed by Simmonds and Shepherd (1955). Similarly, those cultivars derived from interspecific crosses between *M. acuminata* and *M. balbisiana*, such as the triploid

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cooking bananas, were assigned to the ABB group, and the triploid plantains as well as the locally consumed triploid dessert bananas of India and Brazil to the AAB group. Later, four plantain sets were defined based on inflorescence morphology: French, French Horn, False Horn, and True Horn (Tezenas du Montcel et al. 1983). Within each plantain set, landraces were further clustered according to their total number of leaves, which is significantly associated with plant height (Swennen

Recent advances in *Musa* cross-breeding (Ortiz and Vuylsteke 1996; Vuylsteke et al. 1997) have shown the potential of hybridization and selection to develop new plantain and banana cultivars. However, further genetic gains using ploidy manipulations will depend on the proper utilization of *Musa* genetic resources (Ortiz 1997). Hence, the classification and establishment of relationships between and within *Musa* taxonomic clusters will be very important for the betterment of the genome of plantain and banana (Osuji et al. 1997).

Duplicate accessions should be identified within a collection and between gene banks to avoid a waste of capacity. Qualitative morphological descriptors and landrace names have been used to determine whether or not 2 plantain landraces were duplicates (Swennen 1990). The humid lowlands of West and Central Africa are regarded as the secondary center of plantain diversification (De Langhe 1961, 1964). African plantain landraces were originally collected in Nigeria, Ghana, Ivory Coast, Cameroon, Congo, Gabon, and Burundi (Vuylsteke et al. 1997). However, based on Swennen's investigation, only 113 plantain landraces were described as unique.

Although quantitative descriptors may be affected by the environment, these descriptors are often used for studying similar adaptation patterns. They are also useful characteristics to define potential divergent heterotic crop gene pools for further hybrid production. Hence, the objective of this paper was to assess the value of a phenotypic diversity index based on quantitative descriptors for germplasm clustering and for the identification of duplicates in *Musa*.

#### Materials and methods

et al. 1995).

Ninety-two plantain and banana triploid cultivars (Fig. 1) were grown in the *Musa* field gene bank of the International Institute of Tropical Agriculture (IITA) at Onne ( $4^{\circ}51'$  N,  $7^{\circ}3'$  E and 10 m above sea level) in southeastern Nigeria (for site characterization see Ortiz et al. 1997). Row spacing between cultivars was 3 m, and plants of the same cultivar were separated by a space of 2 m. Data were recorded for each cultivar of the plant and ratoon crops on five plants per plot; i.e., one plant was considered as the basic experimental unit. Bunches were harvested when green fruit from the oldest hands started yellowing. The descriptors evaluated at flowering, were date, plant height (cm) and girth (cm), height of tallest sucker (cm), and the leaf length/width ratio, which was measured in the seventh standing leaf counted from the youngest (i.e., the highest) unrolled leaf downwards. At harvest the descriptors recorded were

A phenotypic distance matrix was created by calculating the difference between each pair of accessions for each quantitative descriptor. The distance index was calculated by averaging all the differences in the phenotypic value for each descriptor divided by the respective range (Gower 1985). The analysis of variance for the phenotypic distance index based on quantitative morphological descriptors considered the variation between and within Musa taxonomic clusters. The phenotypic distances were treated as deviations from a taxonomic cluster position mean, and the analysis considered the squared deviations as variances (Johns et al. 1997). Thus, the total sum of squares was partitioned into between and within taxonomic clusters. Variance components were calculated for each of these sources of variation, i.e., between taxonomic clusters ( $\sigma_c^2$ ), and within taxonomic clusters ( $\sigma_{\rm W}^2$ ). The ratio  $\phi_{\rm FS}$ , which measures the degree of population divergence (Wright 1951, 1965), was calculated by dividing the between-cluster mean square ( $\sigma_{\rm C}^2$ ) and the total-mean square  $(\sigma_{\rm W}^2 + \sigma_{\rm C}^2)$ . Wright  $\phi_{\rm FS}$  represents the correlation between random genetic accessions within a taxonomic cluster relative to random accessions from the population (i.e., triploid Musa species) at large. A value close to 1 (maximum) indicates greater partitioning of the population into taxonomic clusters.

The phenotypic distance index of the the putative duplicate landraces reported by Swennen (1990) was compared with the lowest value calculated for 2 known distinct accessions in the same taxonomic cluster. It was expected that two putative duplicates should have a distance index of zero or at least lower than the lowest distance index between 2 known distinct landraces. Average linkage cluster analysis was performed on the phenotypic diversity matrix to study the pattern of variation and relationship between *Musa* accessions according to their known taxonomic designation and geographical origin. All statistical analyses were calculated with SAS (Anonymous 1990).

### Results

The phenotypic distance index, based on quantitative morphological descriptors, ranged from 0.0167 (between the medium French plantains 'Ntanga 4' and 'Obibut Ntanga 2') to 0.5198 (between the medium False Horn plantain 'Kiogo' and the cooking banana 'Nzizi') (Table 1). Within each taxonomic cluster, the lowest values of the phenotypic distance index were calculated between 2 accessions belonging to the same known taxonomic cluster, except for the giant False Horn plantains and the heterogeneous AAB bananas derived from interspecific crosses between M. acuminata and M. balbisiana. The AAB banana 'Popoulou', derived from this interspecific cross, showed on average the lowest phenotypic distance index with the plantain landraces, whereas the banana 'Pome' from the same cluster had on average the highest phenotypic distance index with the plantains. In contrast, 'Pome', which was the interspecific banana cultivar with the highest phenotypic distance index to 'Popoulou', had the lowest phenotypic distance index with the other bananas. The narrowest range of variation for the phenotypic distance index was calculated Fig. 1 Dendrogram of clusters resulting from the phenotypic distance index for *Musa* triploid accessions. Scale based on a normalized average distance between clusters. The putative genome, the group name, utilization, and donor country of these 92 plantain and banana cultivars have been reported elsewhere (Osuji et al. 1997; PBIP 1997)



for the medium French Horn plantains (Table 1), while the widest range was observed in the AAA dessert bananas. The unstable plantain 'Bise Egome 2' showed the closest phenotypic resemblance to the medium French Horn plantains.

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There were significant differences (P < 0.001) for the phenotypic distance index based on quantitative morphological descriptors between *Musa* taxonomic clusters (Table 2). The between-cluster variance ( $\sigma_{\rm C}^2$ ) was larger (0.001779) than the within-cluster variance ( $\sigma_{\rm W}^2 = 0.001380$ ). Thus, Wright's  $\phi_{\rm FS}$  was 0.5663, which

indicated the relative degree of divergence for quantitative variation between the triploid *Musa* taxonomic clusters.

The phenotypic distance index based on quantitative descriptors between 2 cultivars supports early findings that the dessert bananas 'Red' and '74.10' (collected in Nigeria) were duplicates (Table 3). This distance index was the smallest between the following putative plantain duplicates collected under different names: medium French plantains '76.15' and 'Mbi Egome 3' (both collected in Nigeria), the dwarf mutants of giant

(above diagonal) and with taxonomic cluster, except	in (in itali for 2 clust	ics in diago ters with c	onal) <i>Muse</i> only 1 culti	ı taxonom ivar	iic clusters	s. Mean pl	nenotypic	distance i	ndex with	in clusters	indicated	below the	t name of	respective
Taxonomic cluster	1	2	3	4	5	9	7	8	6	10	11	12	13	14
Plantains 1. Small French	0.0000	0.0324	0.2098	0.1058	0.1133	0.1921	0.1561	0.1506	0.2232	0.1849	0.1136	0.1291	0.1822	0.1055
2. Medium French		0.1249 0.0167	0.3606 0.1730 0.2084	0.1179	0.1245 0.0713 0.1458	0.2436 0.1593 0.2515	0.1824 0.0997	0.2797 0.1029 0.0200	0.2836 0.1861 0.2158	0.2923 0.1450 0.2180	0.0880	0.1100	0.3289 0.1380	0.2416 0.0826
3. Giant French		1401.0	0.0634	0.2931	0.2160	0.1570	0.2869	0.2373	0.1838	0.2257	0.2414	0.1926 0.1926	0.1984	0.2593
(0.1249) 4. Small French Horn			0.2171	0.4346 0.0000	0.3642 0.1138	0.3067 0.1843	0.4391 0.0566	0.0955	0.1697	0.2008	0.3617 0.1216	0.4491 0.2018	0.4881 0.2747	0.4880 0.1519
5. Medium French Horn					0.1145	0.2334 0.1023	0.1130	0.2224 0.0619	0.2333 $0.1212$	0.1253	0.0601	0.3330 0.2028	0.2065	0.313/0.1608
(0.0297) 6. Giant French Horn						0.1429 0.0395	$0.1379 \\ 0.1725$	0.2167 0.1139	0.2248 0.0786	0.2497 0.1195	0.0609 0.1300	0.3221 0.1910	0.3473 0.2505	0.2988 0.2228
(0.0533) 7 Smolt Folco Hom						0.0690	0.2203	0.2819	0.2057	0.2612	0.1568	0.4339	0.3556	0.3958
(0.0584)							0.0700	0.1842	0.2202	0.2673	0.1236	0.3426	0.4447	0.3658
8. Medium False Horn								0.0238	0.0437	0.1114	0.0797	0.2371	0.2359	0.1602
(0.0911) 9. Giant False Horn								0.1990	0.0847	0.2540 0.1170	0.2356 0.1607	0.2619 0.2619	0.2686	0.4791 0.2378
(0.1186)									0.1702	0.2514	0.2388	0.4695	0.4244	0.4301
10. True Horn										0.0429	0.1628	0.2510	0.2637	0.2139
11. Unstable 'Bise Egome 2	ŝ									1001.0	0.0000	0.1816	0.2168	0.1441
												0.2962	0.3759	0.3094
Bananas 12. Dessert												0 0693	01113	0.1062
(0.1985)												0.3606	0.4659	0.2795
13. Cooking (0.1909)													0.0596 0.2818	$0.1401 \\ 0.3655$
14. Other (0.1992)														0.1547 0.2832

Table 1 Range of the phenotypic distance index (lowest value in upper line and highest value in lower line) based on quantitative morphological descriptors between

 
 Table 2
 Analysis of variance between and within Musa clusters for the phenotypic distance index based on quantitative morphological descriptors

Source of variation	Mean square	Expected mean square
Between clusters Within clusters	0.130579*** 0.001380	$ \begin{aligned} &\sigma_{\rm W}^2 + {\rm n}\sigma_{\rm C}^2 \\ &\sigma_{\rm W}^2 \end{aligned} $

\*\*\* Indicates that the source of variation was significant according to respective *F*-test at P < 0.001, while n is the weighted mean for the total number of comparisons within each cluster (i.e., 72.611)

French plantains '76.18' (Nigeria) and 'N'Jock Kon' (Cameroon), the giant French plantains 'Osabum' (Ghana) and 'Ebare Egome' (Nigeria), the medium French plantains 'Px3' (Ivory Coast) and 'Amou' (Cameroon), the medium French Horn plantains 'Ntanga 1' and 'Mbang Okon' (both from Nigeria), the medium False Horn plantains 'Borodehene' (Ghana) and 'Agbagba' (Nigeria), and the True Horn plantains collected in Ghana ('Osakro', 'Asamienu' and 'Asamiensa'). Hence, the results confirmed that the same landrace could have been originally collected with different vernacular names or codes.

The phenotypic distance index between other putative duplicates was larger than that calculated between each of them and another plantain landrace from the same taxonomic cluster. For example, the giant French Horn plantains '3-Vert' (Ivory Coast) and 'Batard' (Cameroon) have been considered as duplicates. However, the phenotypic distance index between each landrace and the other giant French Horn plantain 'Ngomba' (Cameroon) was smaller (Table 3), which suggested that both landraces, although showing a similar phenotype for the qualitative descriptors, may have different genotypes for the quantitative descriptors. Similar observations were recorded for medium French plantains '74.79' (Nigeria) vs. 'Cantebalon' (Cameroon), and '76.25' vs. 'Akpakpak' (Nigeria), and the medium False Horn plantains '78.12' vs. 'Mbirinyong' (Nigeria), and 'Borodewuio' (Ghana) vs. 'Obubit Ukom' (Nigeria). The French plantains considered as putative duplicates had a lower phenotypic distance with 'Egjoga", a third Nigerian landrace. Likewise, the respective False Horn plantains considered as putative duplicates had a higher phenotypic resemblance (of their quantitative characteristics) to the Nigerian landrace 'Ngok Egome" than between themselves. There were similar cases in which the putative duplicates had a closer phenotypic diversity index to a third accession, but the putative duplicate landraces showed a distinct qualitative descriptor with respect to this third accession. For example, the medium French plantain 'Ntanga 3' differs by a distinct unripe peel colour from the putative duplicate 'French Sombre' (Cameroon) and 'Obubit Ntanga 2' (Nigeria). Similarly, the giant French plantain 'Ntanga 5' (from Nigeria) and its putative Ghanaian duplicate 'Apen Pa' have a distinct subhorizontal bunch orientation with respect to the closest Ghanaian cultivar 'Osabum'.

**Table 3** Phenotypic distancebetween putative duplicates andlowest value of both landraces (orcultivars) with a third accesionwithin the same cluster.(Alternative landrace name withlowest value for pair comparisonin brackets)

Pair comparison	Distance	Lowest value
Dessert banana		
74.10 vs. Red	0.0693	0.0693 (same)
French plantains		
74.79 vs. Cantebalon	0.0610	0.0390-0.0439 (Egjoga)
76.15 vs. Mbi Egome 3	0.0423	0.0423 (same)
76.18 vs. N'Jock Kon	0.1015	0.1015 (same)
76.25 vs. Akpakpak	0.0994	0.0543-0.0563 (Egjoga)
French Sombre vs. Obubit Ntanga 2	0.0997	0.0546-0.0663 (Ntanga-3)
Ntanga 5 vs. Apem Pa	0.1687	0.0891-0.1393 (Osabum)
Osabum vs. Ebare Egome	0.0814	0.0814 (same)
Px3 vs. Amou	0.0469	0.0469 (same)
Ukom Ntanga vs. Obino l Ewai	0.0774	0.0317-0.0540 (Cantebalon)
French Horn plantains		
3-Vert vs. Batard	0.0545	0.0422-0.0542 (Ngomba)
Ntanga 1 vs. Mbang Okon	0.0297	0.0297 (same)
False Horn plantains		
78.12 vs. Mbirinyong	0.0719	0.0398-0.0657 (Ngok Egome)
Borodehene vs. Agbagba	0.0520	0.0520 (same)
Borodewuio vs. Obubit Ukom	0.0557	0.0409–0.0511 (Ngok Egome)
Okoyo Ukom (D) vs. Okoyo Ukom	0.0238	0.0238 (same)
Horn plantains		
Osakro vs. Asamienu	0.0429	0.0429 (same)
Osakro vs. Asamiensa	0.0774	0.0429–0.0774 (Asamiensa)
Asamienu vs. Asamiensa	0.0745	0.0745 (same)

Most of the medium and the small French plantain landraces were grouped together as shown in the dendrogram resulting from the average linkage analysis for Musa triploid accessions (Fig. 1). Similarly, the small and medium False Horn landraces (except 'Kiogo') were in the same cluster. All cooking bananas, except the tall cultivar 'Nzizi', were grouped together in this dendrogram. 'Popoulou' (the interspecific banana from the Maia Maoli banana set) was in between the medium French plantains 'French Sombre' and 'Madre del Platanar', whereas the other interspecific banana 'Pome' was closer to the bananas derived from intraspecific crosses of M. acuminata. All the giant French plantains and the False Horn plantains (except '76.22') were respectively clustered together but separated from their medium or small counterparts. The tall banana cultivar 'Lacatan' (collected in Nigeria) was closer to 'Gros Michel' than to the other 'Cavendish' bananas. Some of the putative duplicates (e.g. '74.10' vs 'Red', '76.18' vs. 'N'jock Kon', 'Amou' vs. 'Px-3', 'Asamienu' vs. 'Osakro') were together in the dendrogram.

The dendrogram (Fig. 1) suggests, in descending order, ten small clusters in this triploid Musa germplasm: small and medium French plantains (plus 'Popoulou'), small and medium False Horn plantains (plus 'Osoboaso', the only small French Horn plantain, and the giant False Horn plantain landrace '76.22'), medium French Horn plantains (including the unstable plantain 'Bise Egome 2'), giant French Horn plantains, giant False Horn plantains (plus the medium landrace 'Kiogo'), True Horn plantains, cooking banana cultivars (except 'Nzizi'), all dessert bananas, giant French plantains, and the tall cooking banana cultivar 'Nzizi' (alone). There was an undefined pattern of geographical variation among plantain landraces within each of these small clusters; i.e., landraces collected in the same country belonging to similar set were not always grouped together.

## Discussion

The overall diversity for quantitative descriptors (measured by Wright's  $\phi_{FS}$  as 0.566) among the originally defined taxonomic *Musa* clusters was very close to the allozyme diversity, calculated with Nei's statistics (Nei 1973), for selfing crop species (0.588) (Hamrick and Godt 1997). Self-pollinated species have little gene flow among populations via pollen, which explains the higher population differentiation exhibited by these species. Similarly, most triploid *Musa* accessions are almost male sterile, and the few male-fertile triploids have scarce pollen production (Dumpe and Ortiz 1996). Hence, the variation observed within each taxonomic cluster could arise from the mutations accumulated throughout the cultivation of this crop after its domestication.

The distinct clustering of giant plantains, with respect to their medium-small counterparts within each set, suggests that tall landraces may be the ancestors of the other accessions showing the same inflorescence morphology. This hypothesis seems to be supported by reports that a single recessive mutation negatively affects plant height in plantains (De Langhe 1964; Ortiz and Vuylsteke 1995). Early farmers may have selected medium to small plants to avoid lodging caused by strong winds. Giant plantains have heavier bunches but longer cycling than medium plantains (Ortiz and Langie 1997). This finding explains why these landraces with medium plant height are the most productive per unit time, which make them more attractive for human selection. Hence, there are more medium than giant plantains in the French and False Horn sets.

The putative duplication of triploid banana and plantain accessions was confirmed for 50% of the pair comparisons using the phenotypic distance index based on quantitative descriptors (Table 3). Although Swennen (1990) did a comprenhensive comparison of African plantain landraces to detect duplication in this germplasm, his work was based mostly on qualitative descriptors. Gross morphological changes in qualitative descriptors are easy to be seen; however, replicated field trials are required to detect significant quantitative variation. We suggest that the elimination of putative duplicates in *Musa* gene banks must be based on a twostep process. Germplasm curators may identify putative duplicates based on qualitative descriptors assessed in unreplicated plots and then confirm their observations by comparing quantitative descriptors recorded in replicated field experiments. An alternative method will be the comparison of putative duplicates using the DNA markers which have recently become available in Musa (Faure et al. 1993; Howell et al. 1993; Jarret et al. 1994; Kammer et al. 1992).

The dendrogram of clusters resulting from the phenotypic distance index for Musa triploid accessions (Fig. 1) suggests that earlier reports for the clustering of plantain landraces according to inflorescence morphology were correct (Tezenas du Montcel et al. 1983; Swennen et al. 1995). However, the within clustering of small and medium French or False Horn plantains, based on the total number of leaves, was not completely reliable as shown by the position of the small landraces with respect to their medium counterparts in the dendrogram. The descriptor total number of leaves, although exhibiting significant polymorphism between triploid plantains, which explains its high broad-sense heritability (0.86), may be affected by the environment and the genotype-by-environment interaction (Ortiz and Vuylsteke 1998).

The results suggest that the heterogeneous interspecific triploid bananas should be assigned to specific clusters rather than all these cultivars being classified in a single taxonomic cluster, as earlier defined by the qualitative descriptors. For example, the medium French plantain 'Madre del Platanar' was next to 'Popoulou' (from the Maia/Maoli banana cluster) in the dendrogram developed with the average linkage cluster analysis. This was not surprising since 'Madre del Platanar' sometimes exhibits a bunch resembling that of 'Maqueño' (Cardenosa 1953), another cultivar from the Maia Maoli taxonomic cluster. Similarly, the interspecific banana cultivar 'Mattui' was closer to the 'Cavendish' bananas than to any of the other interspecific banana. 'Mattui' was earlier assigned to the AAB banana cluster based on qualititative descriptors (R. Swennen, personal communication). This result also reinforces the importance of considering both qualitative and quantitative descriptors for germplasm clustering.

A core collection (Brown 1989) may simplify the management and enhance the utilization of genetic resources in Musa. This Musa core collection should consist of a representative subset of all the accessions available in different gene banks in order to capture most of the genetic variability in the whole section Eumusa of the genus Musa. The lack of a geographical pattern of variation within each plantain set suggests that this factor should not be considered during the sampling of this taxonomic cluster for the development of a Musa core collection. The International Network for the Improvement of Bananas and Plantains (a programme of the International Plant Genetic Resources Institute) has the largest Musa collection. This in vitro collection, based at the Katholieke Universiteit of Leuven (Belgium), consists of 1089 Musa accessions (diploid, triploid, and tetraploid landraces and artificial hybrids) (INIBAP 1997). A core collection consisting of 110 accessions (approx. 10% of the whole gene bank) may contain most of the genetic diversity in Musa. Such a core collection will be an important entry point for taxonomic or genetic research, and it may become an important source of desired alleles for Musa breeders. Based on our analysis, we propose that at least 1 plantain landrace for each set plus 1 or 2 extra accessions of the medium False Horn and French plantain sets be chosen for this Musa core collection. Similar analysis will be required within each of the Asian diploid and triploid Musa germplasm clusters to select the respective accessions for the core collection, which may also consist of diploid M. acuminata and M. balbisiana accessions, and interspecific and intraspecific triploid and tetraploid cultivars.

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