South African Journal of Botany 113 (2017) 270–276

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Assessment of genetic relationship in *Musa* using male flower descriptors and molecular markers

C.I. Cruz-Cárdenas [1], M. Youssef [2], R.M. Escobedo-GraciaMedrano *

Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, A.C., Calle 43, # 130, x 32 y 34, Col. Chuburná de Hidalgo, CP. 97205 Mérida, Yucatán, Mexico

ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form 5 July 2017
Accepted 12 September 2017
Available online xxxx

Edited by SO Amoo

Keywords:
AFLP marker
Bananas
Male flower
Morphological descriptors
SRAP marker

ABSTRACT

The genetic relationship among twelve *Musa* accessions was assessed using male flower descriptors and two molecular markers: sequence related amplified polymorphism (SRAP) and amplified fragment length polymorphism (AFLP). Both systems were able to discriminate among accessions by showing good separation based on cluster analysis and by generating several unique markers for certain genotypes. The similarity matrices generated by the molecular markers presented a highly significant correlation and both molecular markers were significantly correlated with the morphological data. Among the male flower descriptors, the compound tepal basic colour was cream or white in *Musa acuminata* (A-genome) accessions, pink in *M. balbisiana* (B genome) and yellow in *Musa ornata* and *Musa schizocarpa*. Moreover, the compound tepal pink pigmentation was unique in all accessions having the B-genome (*M. balbisiana*, diploid (AB) and triploid (AA and ABB) hybrids), while both yellow-tinted free tepal and white ovary basic colour were distinct for *M. ornata* and *M. schizocarpa*. On the other hand, SRAPs and AFLPs showed several specific bands for certain accessions, with 31 and 25 bands, respectively, specific for *M. ornata*, and 8 and 25 bands, respectively, specific for *M. schizocarpa*. In addition, 2 and 4 bands were common between *M. ornata* and *M. schizocarpa*, and 9 and 4 shared among accessions having a B-genome: *M. balbisiana*, and triploid and diploid hybrids (AB and ABB, genomes), generated by each of the two markers, respectively. These specific markers for morphological and molecular descriptors are very important and could be helpful in *Musa* genotyping and genetic diversity assessment.

1. Introduction

In 2012, total world production of banana and plantain (*Musa* ssp.) reached approximately 102 and 37 million tons, respectively, and was produced by approximately120 countries (FAOSTAT, 2014).

The genus *Musa* L. (*Musaceae* family) consists of almost 75 recognized species. Previous work, based on morphology, divided the genus into sections: Australimusa, Callimusa, Rhodochlamys, and Eumusa (Cheesman, 1947), and *Musa* sect. Ingentimusa (Argent, 1976); however, this subdivision into five sections has since been questioned. Recently, Häkkinen (2013), based on reported molecular phylogenetic studies, reconstructed the subdivision of *Musa* species into two sections from the only two monophyletic clades recovered, which correspond to species nested in one clade with the basic chromosome number of n = x = 11, and species in a second clade with x = 10/9/7 (Li et al., 2010). The first one is section *Musa*, which includes all species related to pre-classified Eumusa and Rhodochlamys, and the second, Callimusa, comprising species of the two remaining sections (Australimusa and Callimusa). *Musa acuminata* Colla (A genome) and *Musa balbisiana* Colla (B genome) are the most widely geographically represented wild diploid species (n = x = 11); both are believed to be the progenitors of the great majority of diploid and polyploid edible bananas (De Langhe et al., 2010). Nevertheless, the genetic contribution of the S genome of *Musa schizocarpa* N.W. Simmonds, as well as that of the T genome of *Musa textilis* Née, in the formation of a number of edible and rare cultivars, respectively, has been described by various authors (Carreel et al., 1994; Roux et al., 2008; Hippolyte et al., 2012).

Several approaches have been used, and indeed are still being used, for the assessment of genetic diversity in *Musa*. Some of these approaches include morphological markers which are known to be simple, easy and informative. Previous studies focused on the use of morphological characteristics in *Musa*, such as bunch characters (Swennen and Vuylsteke, 1987; Putra et al., 2010), vegetative characters (Pascua and Espino, 1987; De Langhe et al., 2005; Wang et al., 2010; Nzawele et al., 2013), male flower descriptors (Sebasigari, 1987; Nunes de Jesus et al., 2009; Wang et al., 2010; Rodrigues et al., 2012) and male inflorescence and fruit (De Langhe et al., 2005; Putra et al., 2010; Rodrigues et al., 2012; Nzawele et al., 2013). Male flower descriptors have been reported as one of the most important qualitative morphological

* Corresponding author.
E-mail address: escobedo@cicy.mx (R.M. Escobedo-GraciaMedrano).
[1] Present address: Centro Nacional de Recursos Genéticos, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Blvd., de la Biodiversidad # 400, CP. 47600 Reptilian de Morelos, Jalisco, Mexico.
[2] Present address: Genetics Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.

https://doi.org/10.1016/j.sajb.2017.09.011
0254-6299/

Table 1
Musa accessions used in this study.

No.	Section	Species/accession name	Subspecies/subgroup	Genome	Name	Abbreviation	ITC[a]
1	Musa	*M. acuminata*	*malaccensis*	AAw	Malaccensis	MAL	0399
2	Musa	*M. acuminata*	*banksii*	AAw	Banksii	BAN	0623
3	Musa	*M. acuminata*	Unknown	AAcv	Pisang Berlin	BER	0611
4	Musa	*M. acuminata*	Unknown	AAcv	No.110	No.110	0413
5	Musa	*M. acuminata*	*malaccensis*	AAAcv	Grand Naine	GRN	–
6	Musa	*M. acuminata*	*malaccensis*	AAAcv	Yangambi-KM5	YAN	1123
7	Musa	*M. balbisiana*	*balbisiana*	BBw	Balbisiana CICY	BAL	[b]
8	Musa	*M. acuminata* × *M. balbisiana*		AB	Kunnan	KUN	1034
9	Musa	Cooking banana	Pisang Awak	ABB	Pisang Awak	AWK	0213
10	Musa	Prata ana	Pome	AAB	Prata ana	PRA	0962
11	Musa	*M. schizocarpa*		SS	Schizocarpa	SCH	0599
12	Musa	*M. ornata*	ornata	–	Ornata	ORN	–

[a] International Transit Code.
[b] Accessions collected from Teapa, Tabasco, Mexico.

descriptors in *Musa* (Ortiz, 1997). On the other hand, several molecular markers have been used to assess the genetic diversity in *Musa*, including RAPD (Crouch et al., 2000; Ferreira et al., 2004), VNTR (Crouch et al., 1999), RFLP (Careel et al., 2002; Nwakanma et al., 2003), diversity array technology (DArT) (Risterucci et al., 2009), AFLP (Loh et al., 2000; Wong et al., 2001; Ude et al., 2003; Youssef et al., 2011), and SRAP (Phothipan et al., 2005; Youssef et al., 2011; Xie et al., 2012; Valdez-Ojeda et al., 2014).

Evaluation of the diversity, based on phenotypic characterization alone, may not be entirely reliable since the traits are limited in number and influenced by cultivation and growth environment (Ahmed et al., 2011). The combination of molecular and phenotypic markers could overcome the limitations of the latter. Additionally, a standard descriptor list should include reliable morphological traits and DNA markers capable of describing the diversity of the crop and its wild relatives (Ortiz and Swennen, 2014). In this regard, a number of studies have been carried out using both markers to evaluate *Musa* genetic variability (De Langhe et al., 2005; Miri et al., 2009; Nunes de Jesus et al., 2009; Rodrigues et al., 2012).

Evaluation of *Musa* genotypes, including wild species and edible hybrids from different species, improves the understanding of genetic diversity in the genus and facilitates the selection of genotypes for subsequent breeding and DNA-technology approaches. Thus, the objective of this study was to assess the genetic diversity in *Musa* accessions of a collection including wild species, cultivars and hybrids using morphological descriptors of male flowers, SRAP and AFLP molecular markers.

2. Materials and methods

2.1. Plant materials

Twelve *Mus*a accessions were collected from the *Musa* germplasm collection of the Scientific Research Centre of Yucatan (CICY — for its acronym in Spanish) held at the research station of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP-for its acronym in Spanish) in Uxmal (Yucatan, Mexico). These consisted of wild species of *M. acuminata*, *M. balbisiana*, *M. schizocarpa*, *Musa ornata* Roxb., commercial cultivars and hybrids (Table 1).

2.2. Morphological characterization

Twelve morphological characters were evaluated among the tested *Musa* accessions, including male flower and ovary lengths (cm). Ten qualitative morphological descriptors were assessed using numerical categories according to IPGRI (1996) (Table 2).

2.2.1. Morphological data analysis

The morphological evaluation was performed using a randomized complete block design (RCBD) with six replications for each accession. Analysis of variance was conducted for male flower, and ovary lengths and averages were compared by Duncan's multiple-range test using M-STATC micro-program (Nissen, 1984). For the qualitative descriptors, each one was demonstrated by numerical categories (Table 2). The cluster analysis was based on the simple matching coefficient (for multistate data) using "SimQual" tool in NTSYS-PC version 2.20 (Applied Biostatistics Inc.). The sequential, agglomerative, hierarchical and nested clustering parameters (SAHN) program using the un-weighted pair group method with arithmetic means (UPGMA) in (NTSYS) was used to generate the dendrogram. The representativeness of the dendrogram was evaluated by estimating the cophenetic correlation using Mantel's matrix correspondence test (Mantel, 1967).

2.3. Molecular analysis

2.3.1. DNA extraction and quantification

Total genomic DNA from the 12 *Musa* accessions was extracted from 100 mg of frozen young cigar leaves following the protocol from Dellaporta et al. (1983) with some modifications (Youssef, 2012). DNA

Table 2
List of male flower descriptors used in this study.

No.	Descriptors	Categories			
		1	2	3	4
1	Male flower dominant colour	White	Cream	Pink/pink-purple	–
2	Compound tepal basic colour	White	Cream	Yellow	Pink/pink-purple
3	Compound tepal pigmentation	Very little	Rust-coloured spots	Presence of pink	–
4	Lobe colour of compound tepal	Cream	Yellow	Orange	Green
5	Free tepal colour	Translucent white	Opaque white	Tinted with yellow	Tinted with pink
6	Ovary basic colour	White	Cream	Yellow	Green
7	Ovary pigmentation	Very little or not visible	White red-purple	–	–
8	Ovary shape	Straight	Arched	–	–
9	Male flower shape	Straight	Arched	–	–
10	Number of stamens	Five	Variable (5 or 6)	–	–