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Minimal morphoagronomic descriptors for Cuban pineapple germplasm characterisation

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Abstract: A set of minimum descriptors allow for the rapid characterisation of germplasm facilitating the conservation and use of plant material. The objective of this work was to establish a list of minimum descriptors to facilitate the morphological characterisation of the *ex situ* pineapple collection in Cuba. Therefore, 48 pineapple accessions were characterised according to the morphoagronomic descriptors established by the International Board for Plant Genetic Resources (IBPGR). The data were processed by Multivariate Analysis, where a Multiple Principal Components Analysis was used for the qualitative and quantitative traits. A list with 14 minimum descriptors was proposed. The leaf's colour, the thickness of the longest leaf, the distribution of the spines, the fruit shape, the fruit colour when ripe, the flesh colour, the weight of fruit flesh, eye form, the fruit height, the fruit diameter, the fruitlet shape, the core diameter, the total soluble solids of the fruit, and the crown weight/fruit weight ratio were selected as the minimum descriptors. Because most of the descriptors refer to the pineapple's genetic improvement or commercialisation aspects, it could be a useful tool for scientists and producers.

Keywords: *Ananas comosus* var. *comosus*; genetic resources; germplasm evaluation; accessions; descriptors

The conservation of a plant's genetic diversity is an essential element to improving nutrition, achieving food security and promoting sustainable agriculture (O'Donnell, Sharrock 2018). The objective of germplasm banks is to conserve the biodiversity *ex situ* for the world agriculture. The success of this type of conservation depends on the accessibility to the accessions and the correct characterisation of the germplasm (Morales et al. 2015). Those are the most important raw materials for plant breeders and the most essential contribution for farmers.

Several germplasm collections around the world, which preserve the genetic resources of the pineapple, have been characterised according to diverse morphological and molecular markers. The discriminant power and the importance of the agronomic trait for the characterisation of the cultivars of the genus *Ananas*, has been highlighted by sever-

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al researchers. Fournier et al. (2007) took the weight of the plant and the characteristics of leaf D (number of leaves, weight, length and width) into account in a comparative study between 'MD-2', 'Flhoran 41' and 'Smooth Cayenne'. Souza et al. (2012) selected 11 quantitative descriptors for the characterisation of 89 accessions of ornamental plants of the Ananas genus from the *ex situ* collection in Brazil.

The morphological descriptors establish the useful characteristics for plant breeding programmes (Ruiz et al. 2013). The uniformity of the descriptors is an indispensable requirement for the characterisation to achieve a universal value; thus, the International Plant Genetic Resources Institute (IPGRI) has published descriptors of crops of global and regional interest. Most of these descriptor lists have the limitation of relying on a high number of traits, so the establishment of a minimum number of descriptors would facilitate the work of breeders and researchers. The objective of this work was to establish a list of minimum descriptors with enough information to facilitate the morphological characterisation of the *ex situ* pineapple collection in Cuba.

MATERIAL AND METHODS

Forty-eight pineapple accessions from the National Germplasm Bank, Bioplant Center, Ciego de Ávila, Cuba (21°47'N and 78°17'E, at 80 m a.s.l.) were evaluated in this study (Table 1). Three randomly selected plants were selected from each accession for the classification. Those accessions derive from several field trips for prospecting pineapple varieties, Cuban breeding programmes and some introductions from other countries. They have been classified in Horticultural Groups, such as those by Py et al. 1987.

In the present study, the descriptors recommended by the IBPGR (1991) for the characterisation of the cultivated pineapples were used. Those items are contained in the fourth and sixth sections of the IBPGR, and are specifically referred to as Plant Data. The descriptors and their respective codes were: plant height (cm) (4.1.3), colour of leaves (4.1.8), length of longest leaf (cm) (D leaf) (4.1.11), middle thickness of longest leaf (mm) (4.1.13), distribution of spines (4.1.15), direction of spines (4.1.18), fruit shape (4.3.3), fruit height (cm) (4.3.5), fruit diameter (cm) (4.3.6), fruitlet shape (4.3.10), fruit colour when ripe (4.3.12), eye shape (4.3.20), number of differently oriented spirals (4.3.22), eye number in the longest spiral (4.3.27), flesh colour (4.4.2), weight of fruit flesh (g), (6.4.4), core diameter (cm) (6.4.6), crown length (cm) (6.5.5), total soluble solids of fruit flesh (°Brix) (6.8.2), and acids in fruit flesh (%) (6.8.4). The plant diameter (cm) and crown weight/ fruit weight ratio are not included in the IBPGR, but they were also evaluated here for their importance in the discrimination of the Cuban genotypes, as reported by Isidrón (2008).

The selection of the minimum number of descriptors was based on the Principal Components Analysis (PCA), based on the Pearson correlation matrix for quantitative data and on the Categorical Principal Components Analysis (CATPCA) for qualitative characteristics (Table 2). Using a direct selection (Jolliffe 1973), any descriptor which had a higher absolute weight coefficient (eigenvector) in the principal component of the lower eigenvalue was discarded, starting from the last component and ending with that which possessed an eigenvalue of less than 0.60.

For the qualitative characteristics "Distribution of the spines", it was necessary to introduce modifications to the List of Descriptors for the pineapple (IBPGR 1991), since some of the accessions studied in this work showed ranges of variation that were not included in the list. These characteristics were assigned a specific number to be included as an intermediate state of the characteristics. Identification of the horticultural groups was undertaken through a cluster analysis, using the matrix of Euclidean Distances to the Square generated by the CATPCA and the correlation matrix obtained from the PCA. Ward's method was used for the ascending hierarchical aggregation. The data was processed using the statistical software SPSS ver. 21 and the resampling values were determined with the statistical software PAST ver.2.12 (Hammer et al. 2001).

RESULTS AND DISCUSSION

Analysis of qualitative and quantitative traits. The results of the CATPCA for the qualitative traits evaluated are shown in Table 2. The variability observed was 82.71%, which was explained by the first two components: the first extracted 61.06% and the second 21.68%. This showed that the first two components explained much of the variation in the germplasm bank. Only the thorns direction descriptor contributed values lower than 60%. The external colour of the fruit, the distribution of the spines, the shape of the eyes, the shape of the fruit

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Table 1. The pineapple cultivar of (*Ananas comosus* (L.) Merr) conserved *ex situ* in Cuba

ID	Cultivar	HG	Provenance	ID	Cultivar	HG	Provenance
1Cu (009)	Red Spanish M 35	Spa	Cuba (Radiation 35Gy)	64Cu (081)	Red Spanish	Spa	Cuba (Colombia)
3Cu (004)	Red Spanish	Spa	Cuba (Sgto[4]. de Cuba)	68Cu (084)	Red Spanish	Spa	Cuba (Arabos)
5Cu (086)	Red Spanish	Spa	Cuba (Villa Clara)	61Cu (003)	Red Spanish Colorada Caney	Spa	Cuba (Caney)
15Cu (005)	Cabezona	Spa	Cuba (Holguín)	2Cu (030)	Champaka	Cay	Brazil
16Cu (075)	Spanish Morada	Spa	Cuba (Holguín)	6Cu (019)	Barón de Rothschild	Cay	Cuba (Granma)
26Cu (044)	Red Spanish	Spa	Cuba (Florencia)	11Cu (029)	Mocaena	Cay	Cuba (INCA)
30Cu (001)	Red Spanish pinareña	Spa	Cuba (Ciego de Ávila)	21Cu (018)	Smooth Cayenne serrana	Cay	Cuba (Morón)
33Cu (008)	Red Spanish un borde liso	Spa	Cuba (Caney)	22Cu (109)	MD-2	Cay	Costa Rica
37aCu (012)	Red Spanish P3R5	Spa	Cuba (Somaclonal var.[3])	42Cu (038)	Mocaena	Cay	Cuba (Baracoa)
37bCu (012)	Red Spanish P3R5	Spa	Cuba (Somaclonal var.[3])	24Cu (037)	Cayenne the Hawai	Cay	Hawai
39Cu (027)	Red Spanish	Spa	Cuba (Cienaguilla)	38Cu (016)	Smooth Cayenne	Cay	Cuba (Cienaguilla)
40Cu (010)	Red Spanish	Spa	Cuba (Ceiba Agua)	50Cu (072)	Smooth Cayenne	Cay	Cuba (Rodas)
41Cu (082)	Red Spanish	Spa	Cuba (Baracoa)	52Cu (042)	Smooth Cayenne	Cay	Cuba (Ceiba del Agua)
43Cu (093)	Ocaena	Spa	Cuba (Baracoa)	67Cu (121)	Smooth Cayenne	Cay	Cuba (Nueva Gerona)
44Cu (017)	Red Spanish	Spa	Cuba (San Cristóbal)	7Cu (040)	White pineapple	Per	Cuba (Sgto. de Cuba)
45Cu (046)	Red Spanish	Spa	Cuba (Rosario)	14Cu (133)	White pineapple	Per	Cuba (Bolondrón)
46Cu (118)	Red Spanish	Spa	Cuba (Viñales)	17Cu (039)	White pineapple serrana	Per	Cuba (Morón)
47Cu (077)	Red Spanish	Spa	Cuba (Aguada)	35Cu (025)	Cubana	Per	Cuba (Baracoa)
48Cu (073)	Red Spanish	Spa	Cuba (Abreus)	49Cu (071)	White pineapple	Per	Cuba (Rodas)
51Cu (070)	Red Spanish	Spa	Cuba (Rodas)	60Cu (041)	Cubana del Caney	Per	Cuba (Caney)
53Cu (134)	Red Spanish	Spa	Cuba (Bolondrón)	70Cu (065)	White pineapple	Per	Cuba (Baracoa)
59Cu (007)	Red Spanish Caney	Spa	Cuba (Caney)	9Cu (033)	Puerto Rico	–	Puerto Rico
63Cu (023)	Red Spanish	Spa	Cuba (Niceto Pérez)	12Cu (099)	Hybrid CBCE-054	–	Cuba (Plant breeding)
64Cu (081)	Red Spanish	Spa	Cuba (Colombia)	13Cu (098)	Hybrid CBCE-021	–	Cuba (Plant breeding)

ID – number used to identify the material, in parenthesis: the entry number in the germplasm bank; HG – horticulture group; Spa – Spanish; Cay – Cayenne; Per – Pernambuco; var. – variety; Stgo.– Santiago

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Table 2. The percentage of variability explained for the qualitative traits of the pineapple

Components	Eigenvalues	
	Explained variance (%)	Accumulated (%)
CP 1	61.06	61.06
CP 2	21.68	82.71
Variables	Relative contribution (%)	
	CP 1	CP 2
Colour of middle leaves	0.353	0.735
Flesh color	0.873	0.131
Fruit colour when ripe	–0.915	0.004
Direction of spines	0.257	0.541
Distribution of spines	–0.095	0.922
Eye form	0.931	0.009
Fruit shape	0.919	0.075
Fruitlet shape	–0.982	–0.031

The underlined letters indicate a higher contribution

and the depth of the eyes were selected to conform the List of Minimum Descriptors. These characteristics, according to Bartholomew et al. (2010), should be taken into account in the identification of pineapple cultivars.

The results of the PCA for the evaluated quantitative traits are shown in Table 3, where 69.46% of the total variability observed is explained by three components; the first extracted 37.17%, the second 21.13% and the third 11.16%. The descriptors present:

Table 3. The percentage of variability explained for the quantitative traits of the pineapple

Components	Eigenvalues		
	Explained variance (%)	Accumulated (%)	
CP 1	37.17	37.17	
CP 2	21.13	58.30	
CP 3	11.16	69.46	
Variables	Relative contribution (%)		
	CP 1	CP 2	CP3
Acids in fruit flesh (%)	0.498	–0.341	0.283
Plant height (cm)	–0.534	0.469	–0.282
Middle thickness of longest leaf D (cm)	0.625	0.220	–0.592
Plant diameter (cm)	0.132	0.838	–0.199
Core diameter (cm)	0.734	0.107	–0.042
Fruit diameter (cm)	0.854	0.062	0.067
Crown length (cm)	0.144	0.160	0.663
Length of longest leaf D (cm)	–0.405	0.549	–0.284
Fruit height (cm)	0.562	0.457	0.487
Number of differently oriented spirals	0.055	0.594	0.119
Eyes number in the longest spiral	–0.394	0.620	0.479
Fruit flesh weight (g)	0.357	0.614	0.129
Crown weight/fruit weight ratio	0.224	–0.426	0.755
Total soluble solids of fruit flesh (°Brix)	–0.268	–0.253	0.787

The underlined letters indicate a higher contribution

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Table 4. The percentage of variability for the minimum descriptors of the pineapple

Components	Eigenvalues		
	Explained variance (%)	Accumulated (%)	
CP 1	36.47	36.47	
CP 2	27.05	63.52	
CP 3	12.44	75.99	
Variables	Relative contribution (%)		
	CP 1	CP 2	CP 3
Middle thickness of longest leaf D (cm)	<u>0.650</u>	0.542	–0.111
Colour of middle leaves	<u>0.633</u>	0.473	0.158
Flesh color	–0.432	<u>0.820</u>	0.021
Fruit colour when ripe	<u>0.832</u>	0.407	0.045
Core diameter (cm)	0.211	<u>0.665</u>	0.408
Fruit diameter (cm)	0.142	<u>0.842</u>	0.115
Distribution of spines	<u>0.609</u>	–0.329	–0.062
Eye form	<u>–0.919</u>	–0.010	0.035
Fruit shape	<u>–0.855</u>	0.348	0.085
Fruit height (cm)	–0.176	<u>0.718</u>	–0.279
Fruit flesh weight (g)	0.068	0.459	<u>–0.780</u>
Fruitlet shape	<u>0.767</u>	–0.548	–0.132
Crown weight/fruit weight ratio	0.203	0.029	<u>0.892</u>
Total soluble solids of fruit flesh (°Brix)	–0.917	–0.089	0.107

The underlined letters indicate a greater contribution

fruit diameter, plant diameter, core diameter, total soluble solids of the fruit flesh and the crown weight/fruit weight ratio, with values around 80%; in addition, descriptors were selected with values higher than 60%.

Selection of the minimum descriptors. The first analysis with the morphoagronomic descriptors selected to establish the min.number of descriptors allowed for the eight lowest contributions to be discarded. The PCA with the selected descriptors explained 75.99% of the total variability observed with three components (Table 4); the first extracted 36.47%, the second 27.05% and the third 12.44%; the rest were discarded.

The 14 minimal descriptors selected (Table 5) contributed to the variability values higher than 60%, which demonstrates their discriminant power. They represent 13% of the 115 descriptors for the characterisation of the pineapple proposed by the IBPGR. A similar number of minimum descriptors was selected by Silva et al. (2017) for the cassava and twice that value to describe four species of *Capsicum* (Silva et al. 2013). In the pineapple, Delgado-Huertas and Arango Weisner (2015) also

Table 5. The minimum descriptors for the morphoagronomic characterisation of the pineapple

Qualitative descriptors			Quantitative descriptors
Fruit	Eye form		Total soluble solids of fruit flesh
	Fruit shape		Fruit diameter
	Fruitlet shape		Core diameter
	Fruit colour when ripe		Fruit height
	Flesh colour		Fruit flesh weight
			Crown weight/fruit weight ratio
Leaves	Distribution of spines Colour of middle leaves	**Vegetative**	Plant height

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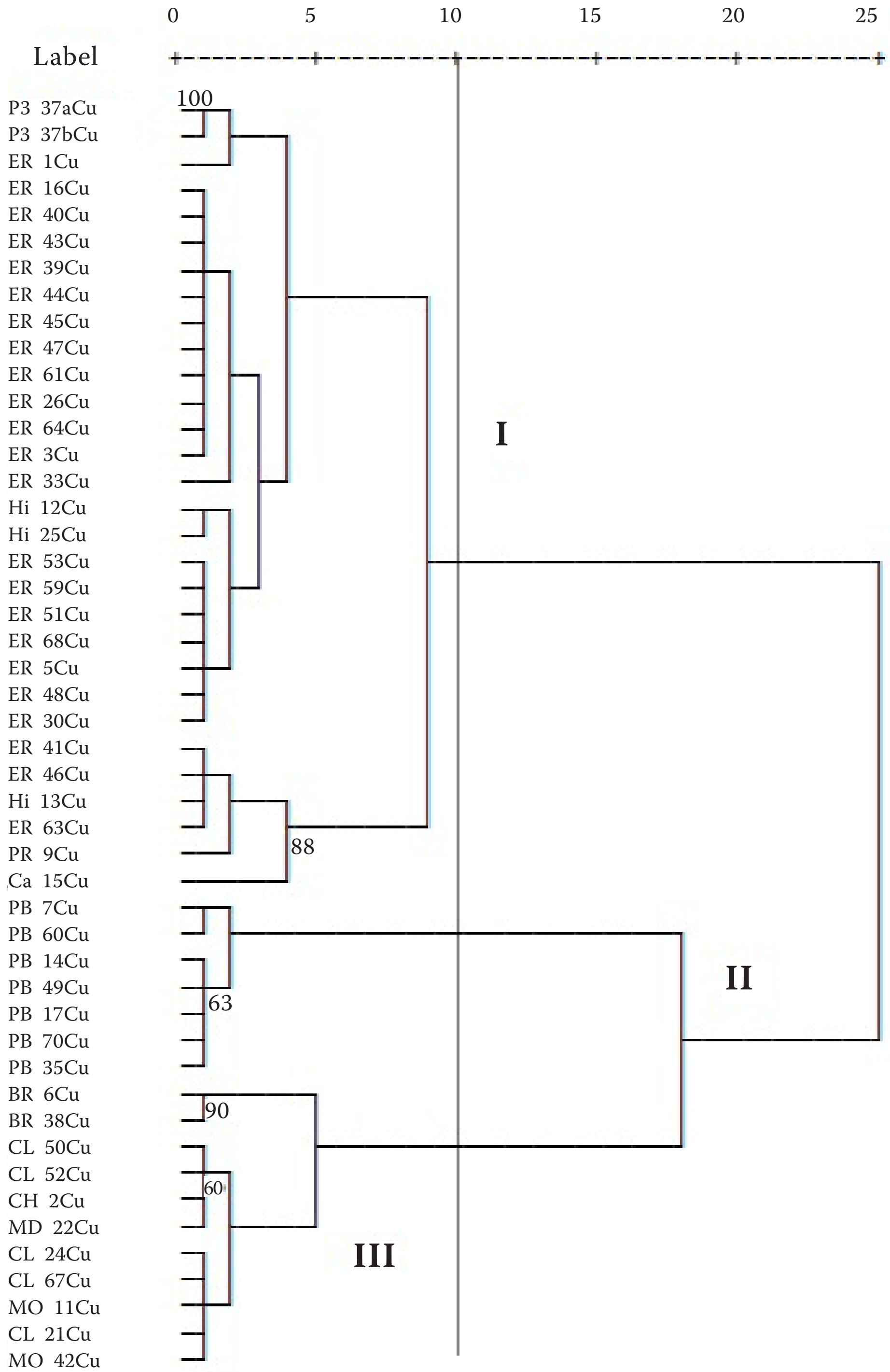

Figure 1. A dendrogram formed with the minimum descriptors from the Euclidean distance of 48 accessions from the *ex vitro* pineapple collection in Cuba

Bootstrap values greater than 60% are represented

determined the discriminant power of the colour of the middle leaves, the fruit shape, and the distribution of the spines and fruit height.

The analysis of the conglomerates (Figure 1) from the mini.number of descriptors, allowed the formation of three classes, the first integrated by the cultivars of the Spanish horticultural group, the second by the Pernambuco and the third by the Cayenne.

In general, the Spanish horticultural group presented barrel-shaped fruits, very deep and rectan-

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gular eyes; the Pernambuco group presented fruits with a pyramidal shape and medium deep and rounded eyes; and the Cayenne group presented cylindrical fruits and shallow, hexagonal eyes. The characteristics of this last group favour its use in industry for the preparation of slices and other products (Bartholomew et al. 2012).

The colour of middle leaves also contributed in determining the variability, in the second component. This descriptor supported the location of the cultivars in different horticultural groups, which were sometimes distinguished by their colouration. In the Spanish group, green leaves with red dyes predominated; in the Pernambuco group, green ones predominated, and in the Cayenne group, they were green or green with a red dye. According to Coppens d´Eeckenbrugge and Duval (1995) this characteristic is encoded by a dominant gene, which determines the dark red of the leaves of certain cultivars. Homozygous recessive genotypes showed phenotypes with varying levels of anthocyanins according to the environment, whereas the heterozygotes expressed much higher densities.

Although the distribution of the spines in the margin of the leaves is the most studied characteristic from a genetic point of view, only three of the five types of spines recognised by Kinjo (1993) were represented in the Cuban collection. They corresponded to the four types of thorns proposed by Coppens and Duval (1995). According to this classification, the Spanish and Pernambuco horticultural groups were characterised by having margins of the spiny type which were determined by the presence of an allele or a recessive allele family (s). The Cayenne group presents leaves with smooth edges corresponding to the presence of a dominant allele (S), and with spines only at the base and the end of the leaves (Py et al. 1987).

Coppens d'Eeckenbrugge and Govaerts (2015) indicate that there is confusion between the botanical forms (species and varieties) and the horticultural forms. They indicate the *Ananas comosus* var. comosus (edible pineapple), which is the correct way to name it. However, farmers tend to differentiate pineapple cultivars in traditional horticultural groups. Pineapple cultivars are grouped into five horticultural groups proposed by Py et al. (1987). Although this classification has been criticised by Duval and Coppens d'Eeckenbrug (1993), for just taking morphological characteristics into account, it is the most used by farmers to identify the cultivars.

The minimum descriptors selected here allowed for the identification and characterisation of the pineapple genetic resources in Cuba. The descriptors have been used already with different objectives, but, to our knowledge, there is no precedent for the establishment of a List of Minimum Descriptors for the cultivars. Some of the published works use a set of descriptors to characterise the genus *Ananas*, such as Duval et al. (1997) in the *ex situ* collection conserved in the CIRAD-FLIIOR of Martinique, and Santos and Ferreira (1999) in the accessions of *A. bracteatus*, *A. ananassoides* and *A. nanus*, belonging to the EMBRAPA in Brazil. On the other hand, to design a key for identifying commercial pineapple varieties, Leal (1990) selected agronomic and qualitative characteristics of the plant, flower and fruit in the Venezuelan collection. In the same collection, Paez (1998) used descriptors to differentiate the wild species of economic importance. In Cuba, Isidrón (2008) proposed a varietal description format in *A. comosus* for the varietal registry, which is useful to describe the Cuban hybrids CBCE-116 and CBCE-74.

The selection of a minimum number of descriptors is a useful tool for the morphoagronomic characterisations of plant genetic resources. The List of the Minimum Descriptors established in this work was sufficient for the characterisation of the Cuban germplasm. This list may be used in future studies of the genus *Ananas*, as well as to facilitate the work of the breeders and curators of the germplasm bank.

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