Identification of duplicate accessions within a sweetpotato germplasm collection using morphological characterization and AFLP markers

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Abstract

The International Potato Center (CIP) sweetpotato germplasm collection consists of 8,018 accessions. In 1988 CIP Annual Report, it was reported about 66% of the Peruvian collection represent duplicates. This high percentage of duplicates is also anticipated in the Latin American collection. The cost of maintenance of duplicates is high because they are maintained both in greenhouse and in vitro collections. For this reason the rationalization is necessary through identification and elimination of duplicates.

Three hundred and sixty accessions collected from Perú grouped into 119 synonym groups were studied. The morphological and molecular characterization were done using 33 morphological descriptors according to descriptors for sweetpotato (CIP, AVRDC and IBPGR, 1991) and seven AFLP primer combinations following procedures of Vos et al. (1995) with IRDye in LICOR 4300 System. Cluster analysis for morphological and molecular data was based on Average taxonomic distance and Jaccard coefficients, respectively and the unweighted pair-group method using an arithmetic average (UPGMA) algorithm with NTSYS-pc version 2.1.

The result showed that the 360 accessions studied can be reduced to 119 unique genotypes and the remaining 241 accessions consist of 197 duplicates and 44 require further evaluation.

Keywords: *Ipomoea batatas*, morphological characterization, AFLP, duplicate accessions.

Introduction

The sweetpotato germplasm collection consists of 8,017 accessions. In the 1988 CIP Annual Report, it was reported about 66% of the Peruvian collection represent duplicates. This high percentage of duplicates is also anticipated in the Latin American collection. The maintenance cost of duplicates is high because they are maintained both in greenhouse and in vitro collections. For this reason the rationalization in the identification of duplicates is very important. We are using firstly morphological characterization in the field using the international standard descriptors and then to complement with the molecular characterization using AFLP markers.

Materials and methods

Germplasm studied

Three hundred and sixty accessions collected from Perú grouped into 119 synonym groups were used in this study. The plant material was obtained from screenhouses at San Ramon and La Molina and CIP in vitro Genebank in Lima, Peru. All expected duplicates were grouped by their similarities of morphological descriptors by cluster analysis, (Huamán, 1997).

Morphological characterization

All accessions were planted with 2 replications and characterized with 30 morphological descriptors according to descriptor for sweetpotato (CIP,AVRDC and IBPGR) in May- June in la Molina field with a Pocket PC. The list of morphological descriptors were:

1	Plant type	16	Petiole pigmentation
2	Ground cover	17	Petiole length
3	Vine internode diameter	18	Storage root shape
4	Vine internode length	19	Storage root surface defects
5	Predominant vine color	20	Storage root cortex thickness
6	Secondary vine color	21	Predominant skin color
7	Vine pubescence	22	Intensity of predominant skin color
8	General outline of the leaf	23	Secondary skin color
9	Leaf lobes type	24	Predominant flesh color
10	Leaf lobe number	25	Secondary flesh color
11	Shape of central leaf lobe	26	Distribution of secondary flesh color
12	Leaf size	27	Storage root formation
13	Leaf vine	28	Latex production storage roots
14	Mature leaf color	29	Oxidation in storage roots
15	Inmature leaf color	30	Twining

Molecular characterization

Leaf samples. At four months of sowing, five to seven (100 mg.) healthy young leaves of each accession were collected from plants in the field. These leaves were placed in a tube containing CTAB 2X buffer and transferred into icebox.

Extraction of DNA. Genomic DNA was isolated from young fresh leaf tissue using a modification of the CTAB method (Doyle & Doyle, 1987 as modified by NCSU, 1990). Quantification and quality of the DNA extracted were checked in 1% agarose gel using lambda DNA standard as control. DNA samples were diluted to 100 ng/ul for AFLP analysis.

AFLP procedure. AFLP markers were obtained using 7 primers combinations (Table 1) marked with IRDyes (Infrared dyes) and visualized in a LI-COR 4300 Hightroughput System. The AFLP protocol used follow the procedure described by Vos et al. (1995) with the modifications listed below. The DNA was digested using two restriction enzymes EcoRI / MseI. AFLP adapters for both restriction enzymes were then ligated to the restriction fragments. Subsequently, template DNA was pre-amplified using primer combinations based on the sequence of the adapters but 3' – extended without selective nucleotide (EcoRI + 00/MseI + 00).

The second amplification reaction used primers marked with IRDye* infrared dyes.

PCR was completed in a PTC-100 thermocycler programmed for 1 cycle of 30sec at 94ºC; 30sec at 65ºC; 2min at 72ºC foll1owed by 12 cycles in which the annealing temperature decrease 1.0ºC per cycle, followed by 30 cycles of 30sec at 94ºC; 30sec at 56ºC; 2min at 72ºC.

Data analysis

Morphological characters. The morphological data were recorded using a 0-9 scale for all 30 descriptors. Cluster analysis of the morphological data was performed with NTSYS-pc version 2.1 (Rohlf,1993) based on Average taxonomic distance coefficient (DIST) and the unweighted pair-group method using an arithmetic averages (UPGMA)

DNA markers

The AFLP profiles generated by the 7 primer combinations were scored for the presence (1) or absence (0) of each fragment and missing data (9). Only those fragments with high intensity were counted. Scores were recorded using SAGA MX GT Generation 2 Software. The NTSYS –PC software package, version 2.1 (Rohlf 1993) was used to compute a matrix using Jaccard coefficients, based on this matrix we perform cluster analysis with the option UPGMA (Unweighted pair-group method) producing a cluster dendrogram.

Table 1. AFLP primer combinations for identification of duplicates in sweetpotato

Primer Combinations	Primer Name	Sequence (5'-3')	Annealing temperature (°C)
E32-M48	E32-AAC	GACTGCGTACCAATTCAAC	56
	M48-CAC	GATGAGTCCTGAGTAACAC	56
E35-M36	E35-ACA	GACTGCGTACCAATTCACA	56
	M36-ACC	GATGAGTCCTGAGTAAACC	56
E35-M48	E35-ACA	GACTGCGTACCAATTCACA	56
	M48-CAC	GATGAGTCCTGAGTAACAC	56
E36-M50	E36-ACC	GACTGCGTACCAATTCACC	56
	M50-CAT	GATGAGTCCTGAGTAACAT	56
E38-M61	E38-ACT	GACTGCGTACCAATTCACT	56
	M61-CTG	GATGAGTCCTGAGTAACTG	56
E39-M49	E39-AGA	GACTGCGTACCAATTCAGA	56
	M49-CAG	GATGAGTCCTGAGTAACAG	56
E42-M35	E42-AGT	GACTGCGTACCAATTCAGT	56
	M35-ACA	GATGAGTCCTGAGTAAACA	56

Results and discussion

Morphological markers. A dendrogram showing the relationships between accessions is presented in Figure 1.

At 100% of similarity, from 226 accessions we identify 83 unique genotypes, in other group of 90 accessions we identify 36 unique genotypes and the remaining 44 accessions need further evaluation. No differences were found between the two replications.

DNA markers. The 7 primer combinations generated 270 clearly scorable polymorphic fragments for the 360 accessions. (Fig 1). At 100% of similarity, from 229 accessions we identify 80 unique genotypes, in other group of 87 accessions we identify 37 unique genotypes and the remaining 48 accessions need further evaluation.

Morphological and Molecular markers. The result showed that the 360 accessions studied can be reduced to 119 unique genotypes and the remaining 241 accessions consist of 197 duplicates and 44 require further evaluation.).

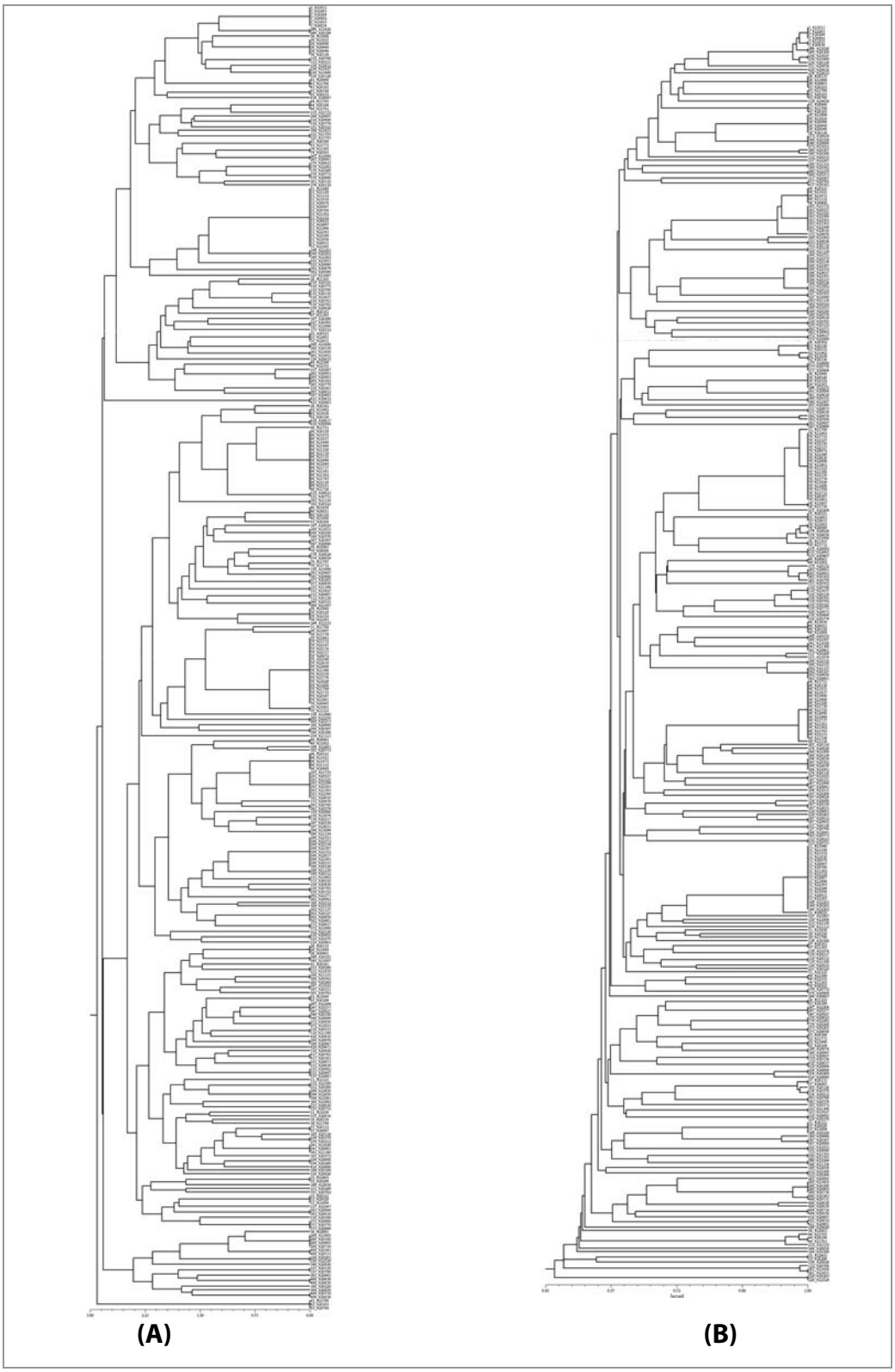

Figure 1. Cluster analysis for morphological (A) and molecular (B) characterization for the 119 synonym groups

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