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Molecular, morphological and agronomic characterization of the sweet potato (*Ipomoea batatas* L.) germplasm collection from Mozambique: Genotype selection for drought prone regions

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ABSTRACT

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important root crops in Mozambique, ranking in the 3rd position, after cassava and maize. Within the scope of the national and regional strategies/initiatives, we have used a multi-analysis approach to characterize the national sweet potato germplasm collection at two different levels: i) genetic, morphological and agronomic diversity; and ii) agronomic potential (storage root yield, vine weight, biomass, harvest index and dry matter content) toward drought tolerance. This collection, composed by 44 accessions, comprises 28 genotypes cultivated in three different provinces of Mozambique (Gaza, Inhambane and Zambezia), nine from other African countries (Kenya, South Africa, Uganda and Zimbabwe), one from the United States of America, and six from CGIAR research centers (IITA and CIP). According to our results, the Mozambican germplasm bank presents a high level of diversity, comparable to those from the collections of the primary centers of origin and South Africa, therefore constituting of a good source of agronomic traits for breeding. Regarding drought tolerance, six Mozambican genotypes (Admarc, Chingova, Nhacoongo-1, Xihetamakote, Nwanatuyo, and Chissicuana-2), one from Uganda (NASPOT-5), one from Zimbabwe (Moz_white), one from Kenya (SPK 004), and one from the USA (Resisto) seem to have the highest potential to be used in regions with frequent drought seasons and in future breeding programs. The results showed that such integrated analysis can be used to successfully characterize the genetic material in terms of suitability to drought-prone regions, therefore helping sweet potato crop management, with economic and food security impacts.

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important root crops in the world, particularly in Sub-Saharan Africa (SSA) where its cultivation area covers around 3 million hectares with an estimated annual production of ca.13 million tons (Low and van Jaarswels, 2008). This commodity is highly productive with a low demand of inputs and labor. Additionally, it tolerates recalcitrant growth conditions, is enriched in vitamins (A, C, D and E) and provides more edible energy than all other food staples. Altogether, these aspects make this crop suitable and attractive to farmers with limited resources (Woolfe, 1992; Elameen et al., 2008; Low et al., 2009; Sreekanth et al., 2010). In fact, over the last decade sweet potato has become increasingly important in SSA, where it is expanding faster than all other major food crops (Low et al., 2009; Walker et al., 2011).

In Mozambique, sweet potato is the 3rd most important staple after cassava and maize (Walker et al., 2006; FAOSTAT, 2010), with an estimate production of 920,000 tons in 2010 (FAOSTAT, 2010). The country appears at the bottom of the Human Development Index list and is one of the most vulnerable to natural disasters and climate changes (MICOA, 2007; UNICEF, 2007). Within this context, and given the potential of sweet potato to alleviate hunger and malnutrition, in 2000 the International Potato Center (CIP) established a partnership with the Agricultural Research Institute of Mozambique (IIAM) to launch a Program aiming the dissemination of the beta-carotene rich Orange Fleshed Sweet Potato (OFSP). Among others, this program included the characterization of the sweet potato germplasm collection from Mozambique, a prerequisite for the rational use and conservation of the available genetic resources (de Vicente et al., 2006; Fraleigh, 2006).

The standard characterization of crop genetic resources includes conventional approaches such as the use of descriptor lists of morphological characters or the evaluation of the agronomic performance, complemented by molecular techniques (Gepts, 2006; Khoury et al., 2010). While morpho-agronomic characterization facilitates the efficient utilization of germplasm collections in a breeding program, providing direct useful information about the genetic relationships and

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specific traits of agronomic importance (Pagnotta et al., 2009; Khoury et al., 2010; Elameen et al., 2011; Laurie et al., 2013), molecular marker-based characterization constitutes a powerful complementary tool, producing more accurate data on genetic distances in a genotype × environment independent way (Pagnotta et al., 2009; Malviya et al., 2012). Nowadays, molecular markers are an essential component for the devise of ex situ and in situ conservation strategies. Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeats (ISSR), and Random Amplified Polymorphic DNA (RAPD) have been successfully used to monitor and characterize the genetic diversity of sweet potato germplasm collections (Connolly et al., 1994; Zhang et al., 1998, 2000; Hu et al., 2003; Elameen et al., 2008; Prakash et al., 2008; Veasey et al., 2008; Arizio et al., 2009; Lin et al., 2009; Moulin et al., 2012) and can now be employed in routine procedures for DNA fingerprinting and to evaluate genetic diversity in this species.

In this paper we report on the molecular, morphological and agronomic characterization of the sweet potato germplasm collection from Mozambique (deposited at IIAM), including the analysis of the productivity performance of each variety cultivated under irrigation and drought (rainfed) conditions. It should be pointed out that drought is one of the major concerns in the country, building strong restrictions to crop productivity. This way, we provide useful information to assist the management of the core collection, but most importantly, for the selection of the best, less risky, accessions for drought prone areas as well as for breeding toward drought resistance. The work was conducted under the context of the actual scenario of Mozambique (and SSA in general) regarding the vulnerability to natural disasters, food insecurity and malnutrition, and the national program for OFSP dissemination. It is in line with the *Action Plan for the Reduction of Absolute Poverty* (PARPA II, 2006), the *Strategy and Action Plan for Biological Diversity Conservation in Mozambique* (Estratégia e Plano de Acção para a Conservação da Diversidade Biológica de Moçambique, 2003) and with the *Sweetpotato Action for Security and Health in Africa* (SASHA), an initiative designed to improve the food security and livelihoods of poor families in SSA by exploiting the untapped potential of sweet potato.

2. Materials and methods

2.1. Plant material and trial location

Forty four accessions (Table 1) introduced in Mozambique with the reasoning of being drought tolerant have been evaluated. These included: i) 28 accessions collected in three provinces of Mozambique: Gaza (17), Inhambane (9), and Zambezia (2); ii) 9 accessions from other African countries: South Africa (4), Zimbabwe (3), Kenya (1) and Uganda (1); iii) one accession from the United States of America; and iv) 6 accessions from CGIAR research centers: International Institute of Tropical Agriculture (IITA) (2) and *Centro Internacional de las Papas* (CIP) (4).

Field trials were conducted during two cropping seasons, 2006/2007 and 2007/2008, at the Umbeluzi Research Station (26° 03′ South latitude, 32° 23′ longitude East and 12 m above sea level), district of Boane, province of Maputo, South of Mozambique. The site has a semi-arid to dry climate with a mean annual precipitation of 679 mm, average temperature ranging from 23 °C to 26 °C during the rainy season and 17 °C to 23 °C during the dry season, daily evaporation between 2.8 and 7.2 mm, and annual evaporation of 1857 mm (Gomes, 1996). Information on the water status of Umbelúzi (Supplementary Table S1) provides evidence that it is a suitable site for testing drought tolerance.

2.2. Morphological and agronomic characterization

Morphological characterization was performed in three replicates of each genotype, in a randomized split plot design and comprised a total of 16 characters, including 6 quantitative and 10 qualitative traits (Table 2), selected from the sweet potato descriptors (Huamán, 1991). All qualitative traits were assessed using a continuous scale, thus being suitable to be collectively used with the quantitative traits in the multivariate analysis.

The agronomic characterization was conducted in a split plot design with two water regimes, namely irrigation and rainfed (dry) conditions and three replications. The genotypes were assigned randomly to different plots and non-randomized irrigations levels were allocated. Five main attributes were measured, namely the total storage root yield, weight of the vines, biomass, harvest index (HI) and dry matter content (DM).

Table 1
Identification of the variety and geographical origin corresponding to each of the 44 sweet potato accessions from the germplasm collection characterized in this study.

Variety name	Origin	Accession ID
Nhacutse 5	Gaza (Xai-Xai)	3
Nwaracu	Gaza (Xai-Xai)	4
Nwazambane	Gaza (Xai-Xai)	5
Nwamanhiça	Gaza (Xai-Xai)	11
Nhacutse 3	Gaza (Xai-Xai)	13
Tuang-Thuang	Gaza (Xai-Xai)	18
Nhacutse 2	Gaza (Xai-Xai)	25
Xiadla xa kau	Gaza (Chókwé)	28
Nhacutse 4	Gaza (Xai-Xai)	31
Nwamazambe	Gaza (Chókwé)	34
Nwamonguane	Gaza (Xai-Xai)	36
Chulamete	Gaza (Macia)	37
Nwaxitsimbwane	Gaza (Chókwé)	40
Cacilda	Gaza (Chókwé)	41
Nwanatuyo	Gaza (Chókwé)	42
Xihetamakote	Gaza (Chókwé)	44
Ximitakwatse	Gaza (Macia)	46
Chissicuana 2	Inhambane (Morrumbene)	2
Nhacoongo 1	Inhambane (Nhacoongo)	8
Chissicuana 3	Inhambane (Morrumbene)	21
Jogó	Inhambane (Morrumbene)	27
Xitsekele	Inhambane (Homoine)	29
Chissicuana 1	Inhambane (Morrumbene)	30
Jogó 2	Inhambane (Morrumbene)	32
Mafambane	Inhambane (Homoine)	35
Xiphone	Inhambane (Morrumbene)	39
Admarc	Zambezia	14
UNK-Malawe	Zambezia	24
Tacna	South Africa	1
Mafutha	South Africa	10
ST 87-030	South Africa	16
Atacana	South Africa	19
Cordner	Zimbabwe	52
Chingova	Zimbabwe	53
Moz white	Zimbabwe	56
SPK 004	Kenya	58
NASPOT 5	Uganda	6
Resisto	USA	47
TIS 9265	IITA	45
TIS 2534	IITA	54
440203	CIP	17
Jonathan	CIP	48
CN 1448-49	CIP	50
Lo 323	CIP	57

CIP — International Potato Center; IITA — International Institute of Tropical Agriculture.

2.3. RAPD analysis

Total genomic DNA was extracted from leaves as described in Taura et al. (2001). Average yield was calculated spectrophotometrically (SmartSpec™ 3000, Biorad) and DNA samples were stored at −20 °C until use. RAPD assays (Williams et al., 1990) were performed as described in Taura et al. (2001). PCR reactions were carried out in a final volume of 25 μl containing 50 ng of genomic DNA, 1× PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 pmol primer and 0.5 U of *Taq* DNA polymerase (Invitrogen). PCR amplifications were done in a thermal cycler (iCycler, Biorad),