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Diversity in oil content and fatty acid profile in seeds of wild cassava germplasm

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ABSTRACT

Cassava (*Manihot esculenta*) is the only commercial species of the *Manihot* genus, cultivated for its starchy tuber roots. However, cassava seeds are known to be rich in oils and fats, there are scant reports on the content and properties of oil from cassava seeds and its wild relatives. Wild *Manihot* species usually produce a higher number of seeds with a large diversity in shape and weight. Seeds of 106 accessions belonging to 12 species of *Manihot* from the collection of Embrapa Cassava and Fruits were evaluated for oil content by NMR and fatty acids composition by gas chromatography. The oil content ranged from 17.2% (*M. caerulescens*) to 30.7% (*M. flabellifolia*) and the species clustered into eight different groups based on the oil content. Five fatty acids were found in all species with the average content of the fatty acids being: linoleic (C18:2) 61.5%; oleic (C18:1) 20.0%; palmitic (C16:0) 12.3%; stearic (C18:0) 4.5%; and linolenic (C18:3) 1.7%. The content of fatty acids varied significantly between species as well as between accessions within a species. The highest content of linoleic acid was in seeds of *M. peruviana*, *M. pseudoglaziovii*, *M. cecropiaefolia*, *M. flabellifolia*, *M. glaziovii* and *M. carthaginensis* (average of 65%); and the highest level of oleic acid was in *M. caerulescens*, *M. esculenta*, *M. anomala*, *M. dichotoma* and *M. tomentosa* (average of 23%). The collection of Embrapa's *Manihot* germplasm is a valuable source for cassava breeding programs, containing a large variability in seed size, oil content and fatty acid composition. The oil from seeds of wild *Manihot* species may be equally valuable for industrial uses as oil from seeds of other Euphorbiaceae species.

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1. Introduction

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is cultivated for its starchy roots. Worldwide, it is the sixth most important source of calories, the fifth in South America and third in Africa (FAO, 2007) in the human diet. It is found over a wide range of edaphic and climatic conditions and grows on soils with low-fertility considered marginal for most crops, low annual rainfall and with prolonged periods of drought during a dry season (4–6 months) (El-Sharkawy, 2004). The genus *Manihot* contains 98 species but only *M. esculenta* is cultivated. In this genus, 20% of the species are native to North America (southern Mexico) while 80% occur in South America, mainly in central and northeastern region of Brazil (Rogers and Appan, 1973; Nassar, 1978, 2000, 2003, 2007). Cassava (*M. esculenta*) is vegetatively propagated by mature stem cuttings (stakes) and does not produce a large amount of sexual seeds. Wild species, on the other hand, are naturally propagated through seeds, which are known to be rich in oils and fatty acids (Teixeira, 1987). Other genera of Euphorbiaceae such as *Ricinus* and *Jatropha* (Akbar et al., 2009) are also known for their production of oils, which are used in medicine, cosmetics, industrial applications, and have tremendous potential as a feedstock for biodiesel production. In northeastern Brazil, roots and shoots of some wild shrubby cassava species (*M. caerulescens*, *M. dichotoma*, *M. glaziovii*, and *M. pseudoglaziovii*) known as "maniçobas" or "mandiocas-bravas" (Allem et al., 1999; Teixeira, 1987) are used for animal feed. In some northeast semi-arid areas of Brazil, seeds of these species are randomly consumed by humans due to their oily endosperm which tastes like Brazil nuts (*Bertholletia excelsa*) (Allem, 2002a and personal communication from local people of "Brotas de Macaúbas"

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municipality, Bahia State, 2006). Local communities in Nigeria use oil extracted from cassava seeds for treatment of infections caused by opportunistic skin pathogenic microorganisms (Popoola and Yangomodou, 2006).

To preserve wild *Manihot* genetic resources and increase their utilization in the improvement of cultivated cassava, Embrapa Cassava and Fruits, Cruz das Almas, Bahia, Brazil established a collection of *Manihot* species in 2005. Since then, the collection continued to expand with new accessions obtained from various regions including the biomes "Caatinga" (Thorny Forest) in the semi-arid region of northeast Brazil and "Cerrado" (Savannas) region in central-west Brazil. Currently, the collection has approximately 680 accessions belonging to 20 species, maintained under field condition (Alves et al., 2011). In addition, approximately 64,000 open pollination seeds from over 370 populations have been collected and are maintained under 5 °C and 25% RH. Reports describing oil content and fatty acids composition in *Manihot* are scant. The objective of this study was to characterize the oil content and the fatty acid profile in seeds of selected accessions of 12 *Manihot* species maintained by the Embrapa germplasm collection.

2. Materials and methods

2.1. Plant material

The oil content, fatty acid composition and seed weight were analyzed in mature seeds of 106 accessions that belong to 12 species of the genus *Manihot*. The seeds were collected during 2008–2009 in various regions of Bahia from wild populations. The collected seeds were stored for approximately two years in a refrigerator (5 °C, ca. 25% RH) in paper bags until analyzed for 100-seed weight, oil content and fatty acid composition. The analysis was done in three replicates per accession.

2.2. Seed weight

The average 100-seed weight was expressed in grams per 100 seeds. The seeds weight was established for 75 accessions in three replications (Table 1).

Table 1
100-Seed weight for 12 species of *Manihot* germplasm maintained at the Embrapa, Brazil collection.

Species of *Manihot*	*n*	100-Seed weight (g)			
		Mean	SD	min	max
M. anomala	8	11.0f	2.7	5.3	15.6
M. caerulescens	3	86.1a	13.3	60.5	105.0
M. carthaginensis	1	20.1e	3.5	16.3	23.1
M. cecropiaefolia	3	6.6g	1.2	3.9	7.9
M. dichotoma	8	47.5b	32.6	11.7	101.4
M. esculenta[a]	6	11.9f	2.1	7.3	16.0
M. flabellifolia[a]	10	12.7f	2.2	6.8	16.6
M. glaziovii	5	32.7c	9.8	19.7	52.2
M. irwinii	6	7.0g	1.1	4.4	8.6
M. peruviana[a]	10	13.6f	2.0	7.6	17.2
M. pseudoglaziovii	8	25.4d	3.8	16.8	33.6
M. tomentosa	7	6.6g	1.7	3.0	9.5
All species	75	20.8	21.5	3.0	105.0

*Means are values of three replicates of each accession (*n*). Means within columns with the same letter are equal by Scott–Knott's multiple comparison procedure ($P < 0.05$). SD – standard deviation.

[a] Taxonomically, these taxa are considered as subspecies of *M. esculetna*; *M. esculenta* subsp. *esculenta*; *M. esculenta* subsp. *flabelifolia*, *M. esculenta* subsp. *peruviana* (Olsen and Schaal, 2001).

2.3. Oil content

Oil content was determined in seeds for all 106 accessions of the 12 *Manihot* species (Table 2) by pulsed nuclear magnetic resonance (NMR) (Bruker Minispec MQ20), with a 0.47 T permanent magnet maintained at 40 °C and provided hydrogen nuclei with a resonance of 20 MHz. The instrument was calibrated and checked with standards of known oil contents from hexane extracted oil from *Manihot* seeds. The 106 accessions were run in four replicate sampled randomly. Five to six seeds for each replicate were placed into an 18 mm test tube and preheated to 40 °C before analysis. The pulsed NMR analytical method was set for 16 scans which reports grams of total oil. Oil content was calculated on moist seed basis due to limited seed quantity.

2.4. Fatty acid composition

Out of 106 accessions evaluated for oil content, 75 were also evaluated for fatty acids composition. The 75 accessions were selected based on the oil content established by pulsed NMR (Conway and Moffett, 1963). Gas chromatography (GC) of fatty acid methyl esters (FAMEs) was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector (FID) and an auto sampler/injector. Analyses were conducted on a SP 2380 30 m × 0.25 mm i.d. (Supelco, Bellefonte, PA). Saturated C8-C30 FAMEs provided standards which were used to make FAME assignments. SP 2380 analysis were conducted as follows: column flow 1.4 mL/min with helium head pressure of 20 psi; split ratio 50:1; septum purge of 4 mL/min; programmed ramp 170–190 °C at 4 °C/min, 190–265 °C at 20 °C/min; injector and detector temperatures set at 250 °C.

Fatty acid methyl esters were made by placing a portion of the seeds, after the shells were crushed and kernels mixed, into a 4 dram vial. Potassium hydroxide/methanol solution 5 mL (0.25 M) was added to the vial and the seeds ground for 20 s with a Modular Homogenizer System (Cole-Parmer Instrument Company, Vernon Hills, IL) fitted with a 10 mm diameter shaft. The vial was then sealed with an aluminum lined cap and placed in a heating block maintained at 65 °C. After one half hour the vial was removed and allowed to cool and 5 mL of hexane and 5 mL of saturated sodium chloride solution were added to the vials. The contents of the vial were mixed thoroughly; the layers were allowed to separate. A 0.25 mL aliquot from the top hexane layer containing the methyl esters was removed by Pasteur pipette and diluted up to 2 mL with hexane in a GC vial. The sample was then injected (1 μL) on the GC using the conditions described above. All 75 accessions samples were run in three replications.

2.5. Statistical analysis

Differences between and within species were tested by ANOVA and the means were separated in clusters using Scott–Knott's multiple comparison procedure by applying SISVAR software (Ferreira, 2008). The statistical procedure was selected because it places means in distinguished groups without result overlapping.

3. Results and discussion

3.1. Seed weight

Seeds of the 12 *Manihot* species showed large diversity in size and shape (Fig. 1) and in the average of 100-seed weight ranged from 6.6 to 86.1 g (Table 1). The smallest seeds were observed in *M. cecropiaefolia*, *M. tomentosa*, and *M. irwinii* (average 6.6–7.0 g). The average 100-seed weight of the cultivated species (*M. esculenta*)