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GENETIC VARIATION OF WILD *MANIHOT* SPECIES NATIVE TO BRAZIL AND ITS POTENTIAL FOR CASSAVA IMPROVEMENT

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Wild *Manihot* species native to Brazil were collected from different localities and studied for economic characters and nature of their wild habitats. Screening for protein and hydrocyanic acid content showed two of them, *M. oligantha* Pax and *M. gracilis* Pax, to have a notably high percentage of protein combined with a low percentage of hydrocyanic acid.

Study of natural habitats has revealed a certain genetic potential in the material. It has been possible to detect, among other characteristics, resistance to drought and excessive soil aluminium toxicity and adaptation to cool temperatures.

INTRODUCTION

Wild *Manihot* species represent a potential source of many genetic characters, but, for the most part, the value of these characters in improving cassava has not been determined (Martin, 1976). For many characters, the gene pool of cassava shows a notable poverty. For example, all cassava clones investigated for protein content in the root were found to have a very low level; less than 2% (Nassar and Dorea, 1982). Resistance to African mosaic virus and meadow bug, *Pheanococcus manihotis*, is controlled by genes which do not occur in cultivated cassava (Hahn et al, 1981). These similarly lack adaptation to soil aluminium toxicity and drought conditions (Martin, 1976). The present papel describes the genetic variation of wild cassavas native to Brazil with respect to these economic characters not found in the cultigens, and assesses the potential for their use in cassava improvement.

MATERIALS AND METHODS

The description and identification of the wild *Manihot* species was based on Rogers and Appan (1973) and Nassar (1978a). Natural habitats were described, and 30 accessions of each species were examined for the following characters: (1) Tuber fonnation and their protein and hydrocyanic acid (HCN) content: tubers were taken 1 year after planting and analyzed according to Association of Official Analytic Chemists (AOAC) methods (1970). (2) Adaptation to various soil types: chemical analysis of soil was carried out according to Black et al. (1965). (3) Adaptation to various climatic conditions: annual rainfall, evaporation and temperature in natural habitats were extracted from records of Federal Meteorological Stations. (4) Seeds, cuttings or whole plants of the collected species were planted in a living collection at the Universidade de Brasilia, Brasilia.

RESULTS AND DISCUSSION

The collected wild *Manihot* species were screened rapidly for tuber formation and growth habito Results of this screening are given in Table 1.

Table 1
Tuber formation, growth habit, and other characteristics of the wild Manihot species collected.

Species	Tuber formation	Growth habit	Particular characteristic
M. oligantha Pax subsp.	+	Subshrub	Abundant cylindric tubers
M. tripartita Muell, Arg.	+	Subshrub	Abundant spheric tubers
M. anomala Pohl	+	Tall shrub	Abundant spheric tubers
M. zehntneri Ule	+	Tall shrub	Abundant conic tubers
M. gracilis Pohl	+	Subshrub	Rare tubers, grows widely all over central Brazil

M. paviaefolia Pohl	+	Subshrub	Rare tubers, occurs in poor sandy soil
M. pruinosa Pohl	+	Subshrub	Grows in limistone soil
M. falcata Roger & Appan	-	Subshrub	Grows on slopes and well drained soil
M. reptans Pax	-	Shrub	Adapted to large range of soil; exhibited different leaf shapes; hybridizes easily with other Manihot species occuring in its natural habitat
M. alutacea Roger & Appan	-	Subshrub	Grows on rocky slopes at ca. 1200m altitude
M. pentaphylla Pohl	-	Subshrub	Grows in limestone soil
M. cearulescens Pohl	-	Tall shrub	Adapted to dry areas in northeastern Brazil
M. procumbens Muell Arg.	-	Subshrub	Grows in poor soil, with high concentration of aluminium
M. stipularis Pax	-	Subshrub	Adapted to high altitude; 1450 m approx.

Tuber formation pattern and protein content

Among the wild species collected from Goias state, Brazil, four species formed abundant tubers. These species were screened for tuber formation pattern, fibre and protein content. They are: *M. oligantha* Pax emend. Nassar subsp. *nestili*, collected from Cristalina; *M. tripartita* Muell., collected from Serra Dourada, municipal Goiania; *M. zehntneri* Ule, collected from Goianesia and *M. anomala* Pohl, collected from the Goiania-Inhumas road. These species differed greatly in tuber formation pattern and tuber composition. *M. oligantha* subsp. nesteli forms abundant cylindrical tubers superficially (10-30 cm below soil surface). They are dark brown with a rough surface. *M. tripartita* forms spherical tubers, deep in the ground (more than 50 cm). They are bright brown and smooth, with a creamy cortex. *M. anomala* forms superficial tubers at a depth of about 20-30 cm, oval shaped with a rough surface and light brown color with a creamy cortex. *M. zehntneri* forms cylindrical to oval tubers at a depth of about 50-70 cm which are dark brown with a rough surface and a white cortex. Protein and fibre contents are shown in Table 2.

The composition of cassava as reported in the literature is somewhat variable. This variation comes from the fact that bitter cultivars differ from sweet ones, not only in the amount of HCN they contain, but also in the proportions of nutrients. According to Bolhuis (1953) cultivars with roots containing less than 50 mg of HCN per kg are considered sweet. However, many reports state that crude protein content ranges from 2.2 mg/kg in sweet to 2.7 kg/kg in bitter cultivars and fibre ranges from 3.1 to 10.3% (Anonymous, 1968). Notably high percentages of protein occur in wild species in comparison to cultivated cassava. Some reports have referred to a protein percentage in some cassava cultivars as high as 6 or 7% (Anonymous, 1968; Amman, 1920). Since this estimation of protein was based on total nitrogen, it must be viewed with caution, because it is not certain whether the breakdown products of cyanogenic glucosides enhance the total nitrogen content or not. Narty (1969) showed that the hydrolytic products of glucosides are incorporated into amino acids for protein synthesis in cassava. Therefore, it is not unlikely that the reported cultivars with high nitrogen content were simply bitter cultivars with a high glucoside content. One wild species attracting attention is *M. oligantha* subsp. *nestili*, due to its high protein combined with a very low level of HCN (Nassar, 1978b). The author saw cows and horses eat greedily the vegetative parts and tubers of this species when grazing in its natural habitat. In the literature, two other wild *Manihot* species have been reported to have high protein content: *M. melanobasis* (Jennings, 1959) and *M. saxicola* (Lanjouw, 1939) but, as there is no reference to their HCN content, it is not possible to say to what extent crude protein estimates were affected by hydrolytic products of glucosides. It seems logical to find wild cassava with a high protein content, since selection for cultivation has aimed at increased tuber size and decreased fibre content without paying attention to protein content. This could have led to discarding protein-producing genes from the cultivated varieties.

Hydrocyanic acid content

Tubers of the five species, namely *M. tripartita*, *M. anomala*, *M. zehntneri*, *M. oligantha* subsp. *nestili*, which form abundant tubers, and *M. gracilis*, which grows widely in Central Brazil, were analysed for HCN content (Table 3). HCN in fresh unpeeled tubers fell within a range of 238 mg/kg in *M. tripartita* to 62 mg/kg in *M. oligantha* subsp. *nesteli*.

On a dry matter basis, the results were similar, except that *M. anomala* had the highest HCN content. HCN content in cassava tubers is reported to vary between cultivars. Analysis of about 100 cultivars for HCN content by Raymond et al. (1941) gave an average of 158 mg/kg fresh whole tuber with a maximum value of 434 mg/kg. Little information is available on HCN content in roots of wild *Manihot* species. Bolhuis (1953) reported 430mg/kg HCN in fresh roots of *M. saxicola*, but this is probably the only wild *Manihot* species in which HCN content has been estimated. He considered that the high HCN content represented an obstacle to the use of this spacies in breeding cassava, despite its high protein content. Bolhus (1953) stated that the minimum lethal dose of hydrocyanic acid for a human being is 50-60mg. However, chronic toxicity due to the continuous intake of small amounts of HCN is considered more important than acute toxicity because of its association with many diseases (Oke, 1973).

The occurrence of species with low HCN level is a valuable discovery. The spacies *M. oligantha* Pax emend. Nassar subsp. *Nestili*, with its notably low HCN content, can be considered a useful parent.

Table 2
Average protein and fibre content of wild *Manihot* species on a percent dry matter basis.

Species	Crude Protein (%)	Crude Fibre (%)
M. oligantha Pax subsp. *nestili*	7,10 ±0,58	26,67 ± 4,86
M. tripartita Muell. Arg.	6,88 ±1,48	33,48 ±6,36
M. anomala Pohl	3,74 ± 0,63	23,44 ±4,82
M. zehntneri Ule	3,06 ±0,82	21,52 ± 4,82

[a]Four replicates of 20 tubers of each species were analyzed.

Table 3
Hydrocyanic acid content of unpeeled tubers of wild *Manihot* species

Species	HCN content in fresh root (mg/kg)	HCN content on dry matter basis (mg/kg)
M. tripartita Muell. Arg.	281,1a	657,2b
M. anomala Pohl	192,2a	1026,3a
M. zehntneri Ule	125,8a	504,2b
M. oligantha Pax emend.	97,2c	291,2c
Nassar subsp. *nestili*	62,3d	183,2d

Means within a column followed by the same letter are not significantly different by Duncan's Multiple Range Test (P= 0,5).

Growth habit and natural habitats

Results of screening wild *Manihot* species for growth habit and natural habitat are present in Table 1. Of particular interest in *M. paviaefolia*, which forms tubers, has limited vegetative growth and is adapted to very poor soil. As a parent in cassava breeding programs, it offers potential for additional adaptation to poor soil conditions.

M. reptans readily forms hybrids with other species in its natural habitat, producing intermediate forms. Collections made by Ule in 1892 were restricted to the Northern border of Minas Gerais, close to Goias (Rogers and Appan, 1973), but the author has found it now widespread over most of Goias. In the last 80 years this species may have expanded its geographical distribution and ecological range through genetic variation and interespecific hybridization. In our samples of *M. reptans*, leaf shape was found to vary widely, reflecting the extent of hybridization with other *Manihot* species. For example, *M. reptans* from Goias Velho was distinguished by bright red leaf veins, a characteristic growth habit, flower and inflorescence morphology. Donations of genes from different environments could allow this spacies to expand rapidly over the whole state of Goias. Harlan (1961) gave an example of *Helianthus annuus* (the annual sunflower) which has acquired a vast gene pool due to the formation of hybrids with at least six other Helianthus species.

M. pruinosa forms tubers with about 3.8% protein by dry weight as compared to cassava with 2%. As seen from Table 4, *M. pruinosa* and *M. pentaphylla* may represent a source of adaptation to limestone soils. The adaptation to high altitude of *M. alutacea* makes it a good candidate for breeding programs concerned with producing cultivars tolerant to low temperature *M. falcata* may provide the potential for breeding cultivars with limited vegetative growth which are adapted to well-drained soil.

Adaptation to climatic conditions

Study of annual rainfall, evaporation and temperature ranges in natural habitats of wild cassavas showed some species of particular interest. They are *M. caerulescens* Pohl collected from Araripina, state of Pernambuco, and from Posse, state of Goias: shrubs 1-3 m tall, with a deeply extended root to 2 m underground. They rarely form tubers; out of 20 plants examined only 2 had formed tubers. Tubers were intermittent, at depths exceeding 50 cm; external color was brown, surface smooth and cortex white. Protein content was 3.9% on a dry matter basis and HCN content was 125 mg/kg unpeeled fresh root. Chemical analysis of soil showed it to be very poor (Table 4).

Table 4

Analisis of soil from natural habitats of six *Manihot* spp.

Species	pH	Ca^{2+}+Mg^{2+} (meq/kg)	P (ppm)	K^{+} (ppm)	Al^{3+} (meq/kg)
M. paviaefolia	4,9	2	-	16	4
M. pruinosa and *M. pentaphylla*	5,5	191	1	136	-
M. cearulescens	5,2	1	1	9	2
M. procumbens	4,9	2	0	18	5
M. stipularis	5,0	3	1	28	6

According to standards laid down by the Estadual commission for soil fertility, Goias, Brazil, the common good soil is that which has: Ca 2+ + Mg 2+, 2-5 meq/kg; P, 10 ppm; K+, 50 ppm ; Al 3+, 0,1meq/kg.

A fascinating aspect of the ecology of *M. caerulescens* is its habitat in the Western part of Pernambuco and South Ceara, which are among the most semi-arid of the world tropics. The mean average rainfall of this region is about 500 mm, with a high evaporation capacity and high temperature. This unfavourable climate, coupled with poor soil, suggests that this species is capable of affording a potential source of resistance to drought.

It seems likely that adaptation of *M. caerulescens* to this arid region depends on its deeply growing root system. However, this species has some distinct characters distinguishing it from other *Manihot* species. For example, it has very large ribbed fruit, 4-6 times the normal size of Manihot fruit. The author was informed by local inhabitants that seeds are eaten in times of famine. It has a wide range extending from Northeastern to Central Brazil. Some biotypes of this species have apparently spread through this area. They tolerate a wide range of environmental conditions varying from severe drought in the regions of Araripina, Picos and Grato, in Pernambuco, Piaui and Ceara state respectively, to a considerable amount of moisture at Posse in Goias state (Table 5).

Table 4
Mean monthly precipatation, evaporation and temperature in natural habitats of *M. cearulescens*

Month	**Precipitation (mm)**		**Evaporation Pontential (mm)**		**Temperature (ºC)**	
	Picos	**Posse**	**Picos**	**Posse**	**Picos**	**Posse**
Jan	98,8	286,2	149,1	129,0	26,3	22,5
Feb	162,3	89,4	138,4	129,3	25,9	22,2
Mar	130,3	68,5	124,8	264,9	25,5	25,3
Apr	31,4	117,5	120,1	167,9	25,4	23,5
May	12,6	16,4	122,4	224,3	24,5	22,5
Jun	4,1	17,6	121,9	230,9	24,3	22,0
Jul	1,1	0,7	121,2	364,1	24,1	21,9
Aug	1,3	20,2	137,7	352,3	26,4	22,8
Sep	3,2	26,3	147,0	269,1	27,8	24,5
Oct	17,9	195,2	160,6	202,4	26,1	24,1
Nov	32,2	204,3	150,3	135,6	26,0	23,9
Dec	61,7	344,1	145,8	128,0	26,2	24,9
Total	556,7	1386,4				

Data from Picos and Posse federal meteorological stations.

M. stipularis Pax collected from Brasilia, a very short subshrub, ca. 20 cm tall, does not form tubers, has a woody root and grows on rocky banks. Soil analysis indicates a poor soil (Table 4). This species is characterized by dioecious flowers. This character, together with its very short height, distinguishes it from other *Manihot* species. *M. stipularis* was collected from an altitude of about 1450 m in one of the highest regions of Brazil. This species may offer genes for adaptation to coolness.

M. procumbens Muell. Arg. collected from Corumba is a procumbent subshrub (ca. 40 cm high) with a large woody root and yellow latex, found growing in a very poor soil. It shows potentiality for tolerance to soil aluminium toxicity and absence of major elements.

The fact that wild *Manihot* species hybridize easily because of their weak interspecific barriers (Nassar, 1984) must have contributed to the vast variation within this group and the evolution of the large number of species in the genus *Manihot*.

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