

2 **Cassava, *Manihot esculenta* Crantz genetic resources:**
3 **a case of high iron and zinc**

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9 **Abstract** Cassava hybrids from interspecific crosses
10 with *Manihot cearulescens* Pohl, *M. pseudoglaziovii*
11 Pax and Hoffmann and *M. dichotoma* Ule showed a
12 very high iron and zinc content in both roots and leaves,
13 e.g. 98.15 mg kg⁻¹ in roots of the interspecific hybrid
14 cassava-*M. cearulescens* versus 12.17 mg kg⁻¹ in a
15 cassava cultivar. This promising results show the
16 potential of wild *Manihot* species for micronutrient
17 enhancement of cassava.

18 **Keywords** Interspecific hybrids · Iron · Leaf ·
19 Root · Zinc

20
22 **Introduction**

23 Cassava provides about 70% of daily calories to
24 200 million people (Nassar and Ortiz 2007). Its
25 ability to adapt to poor soils and long periods of

dry spell, as well its host-plant resistance to some
pests and pathogens makes cassava an important
famine reserve to poor people in the developing
world. Furthermore, its tuberos roots can remain in
the soil until harvest, thus storage facilities are not
needed (Nassar 2007).

The roots of cassava are extremely rich in
carbohydrates; likely to be second to sugarcane
among crop plants. Cassava can produce
250 × 10³ cal ha⁻¹ daily, which is higher than that
of rice (176 × 10³) or wheat (110 × 10³) (Charles
et al. 2005). Despite cassava being a valuable source
of energy, most of the current cultivars are deficient
in proteins, fat, some minerals, vitamins and essential
amino acids (Nassar and Sousa 2007).

Mineral deficiency has been considered a kind of
malnutrition and named as "hidden hunger" by the
World Health Organization (WHO). Iron (Fe) and
zinc (Zn) are two micronutrients that have capture the
attention of nutritionists. About 2 billion of the world
population, mainly women and children, suffer from
Fe and Zn malnutrition (WHO 2002). Iron is
important for formation of hemoglobin, a large
number of enzymes, metabolic reactions, the regula-
tion of cell growth and immunity, and for the proper
function of the liver. Zinc is vital for physical and
mental development, immune system, vision and
fertility. This element also appears to be necessary for
the functioning of more than 300 enzymes in the
human body. Its deficiency has been linked to vitamin
A underutilization (Khush 2001).

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57 Cassava can play an important role in food 62
 58 security by providing micronutrients in the same 63
 59 way it does with energy poor populations that have 64
 60 limited access to foods derived from animals. Various 65
 61 reports have emphasized variability in the rich 66
 genetic resources of cassava and its wild *Manihot*
 relatives from Brazil, the country of origin of this
 crop (Nassar 2006a, b; 2007, Nassar and Ortiz 2008).
 The aim of this research was therefore to survey Fe
 and Zn content in accessions, cultivars, and a wild

Table 1 Cassava accessions, cultivars, *Manihot* species, and interspecific hybrids

Indigenous cultivars Amarela 1, Amarela 2, Amarela 4, Amarela 5, Branca Santa Catarina Vermelha 1, *Manihot zehntneri* Ule, hybrids from *M. anomala* Pohl × Cassava, *M. cerulecens* × Cassava, *M. glaziovii* Muell. Arg × Cassava—Polyploid type, *M. grahamii* Hooker × Cassava, *M. grahamii* × Cassava 2, *M. pilosa* Pohl × Cassava accession 1076, *M. pohlii* × Cassava, *M. pseudoglaziovii* Muell. Arg. × Cassava—Diploid type and *M. pseudoglaziovii* × Cassava—Polyploid type, Hybrid *Manihot esculenta* Pax × cassava (namely ICB 300)—Polyploid type, ICB 300 Progeny 9, Cassava cultivar UnB 60 Diploid type, Cassava cultivar UnB 60 Polyploid type, Cassava cultivar UnB 530 Diploid type, Cassava cultivar UnB 530 Polyploid type, Cultivar UnB 031 Diploid type, Cultivar UnB 031 Polyploid type, Cassava cultivar UnB S1, Cassava cultivar UnB 307, Cassava cultivar UnB 567

Table 2 Iron contents in cassava and *Manihot* materials (mg kg⁻¹ dry weight)

Material	Root		Leaf	
	Minimum	Maximum	Minimum	Maximum
Amarela 1	16.75	28.25	63.25	97.75
Amarela 2	–	13.25	–	55.75
Amarela 4	10.75	12.25	82.00	88.50
Amarela 5	11.00	12.25	61.00	73.25
Branca Santa Catarina Vermelha 1	12.75	22.00	61.25	75.50
<i>Manihot zehntneri</i>	20.50	32.50	45.25	58.00
<i>M. anomala</i> × Cassava	–	51.25	–	105.50
<i>M. cerulecens</i> × Cassava	16.50	19.25	70.50	84.25
<i>M. glaziovii</i> × Cassava Polyploid type	69.75	91.25	64.50	86.75
<i>M. grahamii</i> × Cassava	–	25.50	–	60.75
<i>M. pilosa</i> × Cassava accession 1076	28.00	85.50	66.75	70.75
<i>M. pohlii</i> × Cassava	–	33.00	–	50.00
Hybrid Restituição (<i>M. pseudoglaziovii</i> × Cassava)	54.25	61.25	78.50	92.25
Hybrid <i>M. pseudoglaziovii</i> × Cassava Diploid	–	18.00	–	100.00
Hybrid <i>M. pseudoglaziovii</i> × Cassava Polyploid	24.25	26.75	60.00	79.75
Hybrid <i>M. oligantha</i> × Cassava (ICB 300) Polyploid type	–	32.00	–	136.00
ICB 300 Progeny 9	26.75	29.50	64.25	244.25
Cassava cultivar UnB60 Diploid	16.00	38.00	59.25	76.50
Cassava cultivar UnB60 Polyploid	–	16.25	–	65.75
Cassava cultivar UnB530 Diploid	23.50	32.00	68.75	74.00
Cassava cultivar UnB 530 Polyploid	18.25	18.25	62.75	83.75
Cassava cultivar UnB 031 Diploid	15.25	23.50	63.00	78.25
Cassava cultivar UnB 031 Polyploid	–	21.25	–	95.00
Cassava cultivar UnB S1	–	11.50	–	236.75
Cassava cultivar UnB 307	15.75	24.75	72.75	74.75
Cassava cultivar UnB 567	13.00	15.50	65.00	134.75
	14.50	32.00	64.25	87.75

67	from Brazil as well as some interspecific <i>Manihot-</i>	work are part of the living Collection of Cassava,	78
68	cassava hybrids available from the University of	placed in the Biological Experimental Station of the	79
69	Brasilia.	University of Brasilia. They were collected from	80
		Amazon, Para, Minas Gerais, Mato Grosso do Sul,	81
		and Goias States.	82
70	Materials and methods		
71	<i>Cassava</i> and interspecific hybrids materials	Method for analyzing micro-nutrient	83
		concentrations	84
72	Table 1 lists the materials used for determining Fe	The samples of these cassava materials were ana-	85
73	and Zn contents. Seven of them are indigenous	lyzed twice. The results of the preliminary analysis	86
74	cultivars (Amarela 1, 2, 4 and 5, Branca Santa	provided means for selecting those materials for the	87
75	Catarina and Vermelha 1) and a wild species	second phase of analysis. The roots were cleaned	88
76	(<i>M. zehntneri</i> Ule) The 27 accessions, cultivars,	with distilled water and dried in an air oven at 70°C	89
77	species and interspecific hybrids analyzed in this	after skin removal. The leaves were also cleaned and	90

Table 3 Zinc content ranges in cassava and *Manihot* materials (mg kg⁻¹ dry weight)

Material	Roots		Leaves	
	Minimum	Maximum	Minimum	Maximum
Amarela 1	7.25	8.00	12.50	15.00
Amarela 2	–	5.25	–	17.00
Amarela 4	8.75	9.50	15.75	26.50
Amarela 5	9.75	11.75	32.50	38.75
Branca Santa Catarina	7.00	8.75	12.00	12.00
Vermelha 1	6.25	10.00	18.25	21.75
<i>Manihot zehntneri</i>	–	16.00	–	15.00
<i>M. anomala</i> × Cassava	11.75	15.00	17.50	20.25
<i>M. cearulecens</i> × Cassava	7.50	11.50	16.25	17.75
<i>M. glaziovii</i> × Cassava—Polyploid type	–	11.00	–	48.25
<i>M. grahamii</i> × Cassava	11.00	11.50	12.75	20.00
<i>M. pilosa</i> × Cassava accession no. 1076	–	9.75	–	18.75
<i>M. pohlil</i> × Cassava	13.00	17.75	73.75	280.00
Hybrid Restituição (<i>M. pseudoglaziovii</i> × Cassava)	–	13.50	–	26.00
<i>M. pseudoglaziovii</i> × Cassava Diploid type	12.25	13.75	24.75	29.75
<i>M. pseudoglaziovii</i> × Cassava Polyploid type	–	7.25	–	25.25
Moligantha Pax × Cassava (ICB 300) Polyploid type	12.75	14.25	11.25	13.00
ICB 300 Progeny no. 9	6.00	8.50	11.50	15.75
Cassava cultivar UnB 60 Diploid type	–	5.00	–	33.50
Cassava cultivar UnB 60 Polyploid type	9.50	12.00	19.00	21.50
Cassava cultivar UnB 530—Diploid type	10.25	10.75	18.25	20.25
Cassava cultivar UnB 530 Polyploid type	8.25	10.50	14.00	15.00
Cassava cultivar UnB 031 Diploid type	–	9.50	–	19.25
Cassava cultivar UnB 031 Polyploid type	–	9.75	–	22.50
Cassava cultivar UnB S1	9.00	12.50	20.00	56.25
Cassava cultivar UnB 307	8.00	11.00	24.50	25.75
Cassava cultivar UnB 567	8.50	12.25	21.75	22.00

Table 4 Iron (Fe) and zinc (Zn) content ranges of six selected cassava cultivars, interspecific hybrids and a wild species (mg kg⁻¹ dry weight)

Material	Root Fe		Leaf Fe		Root Zn		Leaf Zn	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
S1	7.50	19.50	72.00	77.25	5.50	9.00	27.00	71.00
Cassava cultivar UnB31, Polyploid type	10.50	13.75	193.25	199.50	6.75	7.00	27.75	29.50
Cassava cultivar Amarela 2	10.25	14.50	57.25	62.75	4.50	7.00	21.75	23.50
Hybrid <i>M. pseudoglaziovii</i> × Cassava Diploid type	11.50	24.75	57.25	64.25	10.25	13.75	38.25	48.75
<i>M. cerulecens</i> × Cassava	62.94	124.88	58.75	89.75	7.66	18.98	18.75	21.50
<i>Manihot zehntneri</i>	39.75	47.75	93.75	97.50	18.75	18.75	16.75	18.00

91 dried as per the roots. The dried rhizome and leaves
92 were ground using a laboratory Wiley mill. Wet
93 digestion using a mixture of sulphuric, nitric and
94 perchloric acids (1:10:2) was employed for chemical
95 analysis, and Fe and Zn were determined by atomic
96 absorption spectrophotometry (Allen 1989).

97 Results and discussion

98 Table 2 provides the results of the preliminary Fe
99 content analysis in cassava and *Manihot* materials.
100 Roots showed a great variability in Fe content: from
101 10.75 mg kg⁻¹ in Amarela 4–91.25 mg kg⁻¹ in
102 *M. cerulecens* × *Manihot* hybrid. There was also a
103 great variation of Fe leaf content: from 45.25 to
104 244.25 mg kg⁻¹.

105 The wild species *Manihot zehntneri* showed the
106 highest Zn content (16 mg kg⁻¹) in the roots. The Zn
107 leaf content ranged from 11.25 mg kg⁻¹ in Hybrid ICB
108 300 Polyploid to 280 mg kg⁻¹ in *M. pohlii* × *Manihot*
109 (Table 3). Above results led to select six cassava and

Manihot materials with the aim to assess them
110 statistically. The materials included cultivars (Amarela
111 2 and Amarela 5), interspecific hybrids (S1, Clone 031
112 Polyploid and *M. cearulecens* × *Manihot*), and the
113 wild species *M. zehntneri* (Table 4), which showed the
114 highest Fe or Zn root and leaf levels in the preliminary
115 analyses.
116

There were significant differences ($P < 0.05$) for
117 both Fe and Zn content among the selected materials
118 (Table 5). The interspecific hybrid *M. cearules-*
119 *cens* × *Manihot* showed on average the highest
120 content of root Fe (98.15 mg kg⁻¹), which was
121 significantly above that of cassava cultivars
122 (12.17 mg kg⁻¹). The same interspecific hybrid had
123 higher Zn root content (12.15 mg kg⁻¹) than cassava
124 cultivars (7 mg kg⁻¹). The Clone 031 Polyploid
125 (offspring of the interspecific hybrid of *M. dichotoma*
126 with cassava) showed significantly higher Fe leaf
127 content (196.83 mg kg⁻¹) than cultivar Amarela 2
128 (60 mg kg⁻¹). S1—a selection derived from the
129 offspring of an interspecific hybrid of cassava with
130 *M. pseudoglaziovii*—had 67.58 mg kg⁻¹ of Zn leaf
131

Table 5 Iron (Fe) and zinc (Zn) average contents in leaf and root samples of cassava and *Manihot* materials

Material	Leaf		Root	
	Fe	Zn	Fe	Zn
Cassava cultivar UnB S1	74.96 c	47.63 a	15.29 c	7.79 bc
Cassava cultivar UnB 031 Polyploid type	196.83 a	28.67 ab	12.83 c	6.92 bc
Cassava cultivar Amarela 2	60.00 c	22.42 ab	12.17 c	5.58 c
Hybrid <i>M. pseudoglaziovii</i> × Cassava. Diploid type	61.67 c	43.33 ab	16.71 c	11.88 b
Hybrid <i>M. cerulecens</i> × Cassava	73.05 c	21.25 ab	98.15 a	12.15 b
<i>Manihot zehntneri</i>	95.63 b	17.38 b	42.75 b	18.75 a

Means follow by same letters are not significantly different at $P \leq 0.05$ as per Tukey's Multiple Comparison Test

- 132 content vis-à-vis 22.42 mg kg⁻¹ of cassava cultivar
133 Amarela 2.
- 134 The wild species *M. cearulescens*, *M. dichotoma*
135 and *M. pseudoglaziovii* appear to be rich for Fe and
136 Zn sources, which can be transferred to interspecific
137 hybrids and inherited by their derived offspring.
138 These results show therefore the potential of wild
139 *Manihot* species to improve both Fe and Zn contents
140 in the cassava gene pool, which can provide better
141 food to fight malnutrition, especially in resource-poor
142 populations.
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