

Case of escape in cassava, *Manihot esculenta* Crantz

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ABSTRACT. Two cassava escapes were collected from cultivated fields near natural habitat in Bolivia. They are described morphologically and analyzed cytogenetically in this study. It is suggested that they are the product of backcrosses of cassava interspecific hybrids with the cultigen itself, and that selective conditions have developed in which certain forms of cassava segregates have adapted to grow wild in natural habitats near cassava fields. These segregates may hybridize with cultivated cassava upon coming in contact with such varieties. Because these escapes have incorporated useful genes from the wild into their genetic structure, they could be used for cassava improvement since their genetic barriers with other forms of cassava are very weak.

Key words: Cassava escapes; Interspecific hybrids; Backcrosses; Natural selection

INTRODUCTION

Plant escape is a process in which cultivated species may develop phenotypes similar to wild varieties under certain selective conditions. These forms are adapted to grow wild in natural habitats, and will cross readily with cultivated species growing in close proximity (Jones, 1967). However, it should be noted that escapes cannot multiply into new species (i.e., form a population with unified characters that propagate sexually with each other and are isolated from adjacent populations), although they may hybridize with wild relatives that occur nearby. This may be followed by recurrent backcrosses with cultivated forms, producing types very similar to cultivated varieties. Because escapes have incorporated genes from wild forms into their genetic structure, traits such as improved resistance to disease and insects, or high protein or carotenoid content, may be useful in plant breeding. Thus, they are considered valuable resources for plant improvement.

This phenomenon has been reported extensively in some cultivated plants, including maize, sorghum, and cotton (Barnaud et al., 2009), but it has not yet been documented in other crops. The present study is the first report of its occurrence in cassava, *Manihot esculenta* Crantz. Added interest regarding this phenomenon concerns its usefulness for plant breeding programs, as escapes encompass genes acquired from the wild that are rarely found in cultivated varieties. These genes can be easily transferred between and among varieties because of weak genetic barriers in both escape and cultivated forms. To this end, in his review, presented numerous examples of how the use of escapes have resolved several plant breeding problems, including development of improved disease resistance in tomato, potato, sweet potato, sunflower, and many other cultivated crops.

MATERIAL AND METHODS

In this study, two accessions (supposed cassava escapes) and a cassava cultivar (UnB 032), maintained in the living collections at Universidade de Brasília experimental station, were examined cytogenetically for meiosis and mitosis. Additionally, two accessions collected from natural habitats in Bolivia were analyzed morphologically - these accessions are referred to as Escape 1 and Escape 2.

During cytogenetic analysis, meiotic chromosome behavior was observed in flower buds fixed in Carnoy's solution, and then smeared and stained with carmine (Nassar et al., 1995). Configuration of 10 meiotic metaphase chromosomes was analyzed. Ten young, male buds stained with 1% carmine were selected for normality counts and micronuclei recognition tests as indicators of abnormal chromosome assortment for use as an infertility parameter. Carmine-stained pollen was counted for viability evaluation pollen that was homogeneously stained, and had a regular shape and size was considered viable (Nassar et al., 1995).

Morphological characteristics of the supposed escapes, *in situ* plants, and voucher specimen were evaluated. At least two exsiccates of wild species were examined at the herbarium EMBRAPA Recursos Genéticos e Biotecnologia - CENARGEN; Instituto Nacional de Pesquisas da Amazônia; Herbario Nacional de Bolivia; Universidad Mayor de San Andrés, La Paz, Bolivia; Missouri Botanical Garden, Missouri, USA; New York Botanical Garden, New York, USA; and the Herbario del Oriente Boliviano.

RESULTS AND DISCUSSION

Cassava and escape meiotic behavior

Escape taxa evaluated exhibited a diploid chromosome number ($2n = 36$) and a regular configuration in metaphase I (Table 1). Meiotic metaphase I consisted predominantly of bivalents, although, rarely, some univalents were observed.

Table 1. Meiotic evaluation, tetrad normality, and pollen viability of cassava and two *Manihot* escapes.

Taxon (<i>M. esculenta</i>)	Examined cells	Univalents		Bivalents		Normality (%)	Total count (N)	Pollen viability (%)	Total count (N)	Meiotic regularity
		Range	Average	Range	Average					
UnB 032	10	0	0	18	18	94.2	608	94.5	3014	Highly regular
Escape 1	10	0-2	0.4	17-18	17.8	92.8	443	98.9	3755	Regular
Escape 2	10	0-2	0.4	17-18	17.8	93.7	607	94.5	3014	Regular

As noted in Table 1, the escapes exhibited a similar chromosome configuration to the cassava cultivar that was evaluated. Tetrad normality and pollen viability confirmed the regular configuration observed in metaphase I. Morphological examination, combined with observation in natural habitat, of Escapes 1 and 2 clearly revealed the following descriptive characteristics:

Escape 1

Shrub, 2-5 m in height; thickened roots, with stems branching off from a woody base; absent petiole scars; abundant branching; strongly decumbent branches with membranous appearance, dark green and glaucous leaves, with scattered trichomes; deciduous stipules, semi-foliaceous, ovate-lanceolate, <2 mm compose, covered with trichomes; petioles, 3-10 (-15) cm, compose, trichomatous and membranous at the base; gently and delicately constructed membranous blade, strictly 3-lobed, often 1-to-2-lobed at base of inflorescence, elliptic-lanceolate, central lobe with attenuated apex, entire margin; lateral lobules obviously reduced; veins covered with dispersed trichomes, median large inflorescence, 15-30 cm compose, central panicle with 3-4 racemes; bracts and bracteoles deciduous, semi-foliaceous, ovate and covered with trichomes, <1 mm, entire margin; flowers, thin pedicels with delicate appearance, 2-5 mm in staminate flowers and 15-30 mm in female flowers; staminate flowers, short-tubular, 8-10 x 4-6 mm, tube 4-6 mm long; petals/straight lobes (not reflex), 4-6 mm long, whitish outer surface with purple longitudinal lines, inner surface with purple tint in the center and white toward the apex; open female flowers, petals/free lobes, straight and strongly reflex longitudinally on the outer surface and inner surface green with purple tint in the central portion; stigma 3-lobed, with moderately lobed lobes, white in color; fruits globose, 1.2-1.4 cm diameter, strongly glaucous, prominent ribs, sometimes with purple tint at the top; elliptical seed 7-9 x 4-6 mm, caruncle slightly flattened with slightly prominent angle towards the edge (Figure 1A-J).

Escape 2

Shrub decumbent, 4-6 m in height; very short roots, cylindrical, with brown-bleached skin and white flesh; stems 2-3 emerging from a woody base, with prominent petiole scars,

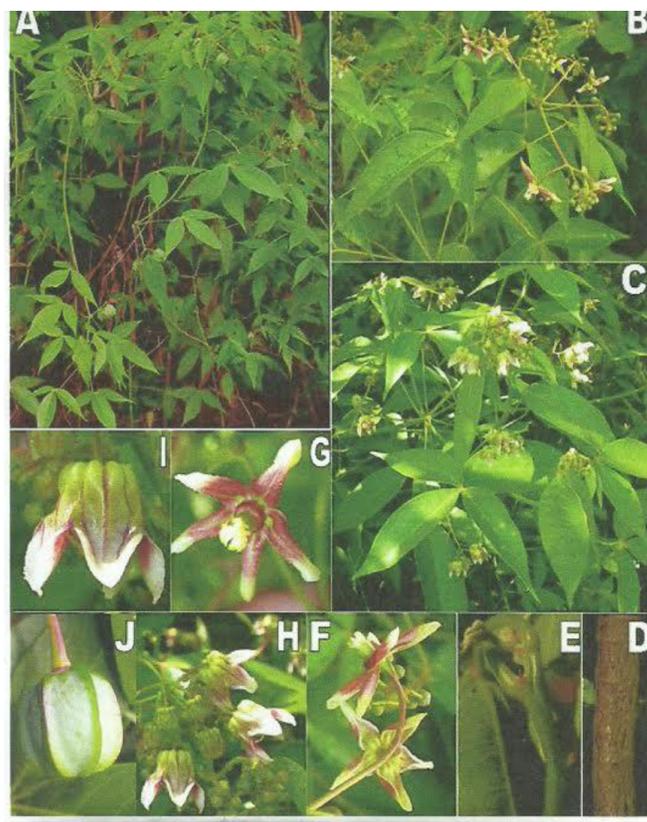


Figure 1. *Manihot esculenta*, Escape 1. Morphological characteristics labeled alphabetically, as follows: **A.** growth habit, semi-erect with decumbent branches; **B. G.** female flowers; **C. I.** male flowers; **D.** stem and cortex; **E.** stipules pubescent in young shoots; **F.** female inflorescence; **H.** male inflorescence; **J.** fruits with wings.

0.5 mm; abundant branching; strongly decumbent branches, trichomes scarce on young parts, margined leaves, soft green adaxial face yellow and glossy, abaxial face green and glaucous, pubescent on both sides; stipules deciduous, sheetlike, linear-lanceolate, <2 mm compose, trichomes scarce, foliaceous, blade 3-5-lobed or rarely uni-lobed at the base, obovate, central lobe lanceolate to acuminate-attenuated apex, entire margin; side lobes obviously reduced, veins covered with fine and simple, scattered trichomes; middle inflorescence large, 10-20 cm, with central panicle with 3-4 racemes covered with simple trichomes, <1 mm compose, entire margin; flowers pubescent, thin pedicels, 5-9 mm long in staminate flowers and 10-20 mm in female flowers; flowers staminate, short, campanulate, 6-8 x 5-6 mm, tube 3-4 mm long; petals 3-4 mm long, outer surface sometimes whitish with purple pigmentation in the junction with the pedicel, inner surface all white; open female flowers, tepals free, straight, 2 heavy along reflex, external and internal white surface; stigma 3-lobed, heavily-lobed lobes, white color; fruits globose, 1.2-1.6 (-1.8) cm diameter, strongly glaucous, with thin, sharp, prominent ribs, with yellowish tint at the top; elliptical seed 8-10 x 4-6 mm, slightly prominent caruncle, flattened and slightly angled towards the edge (Figure 2A-J).

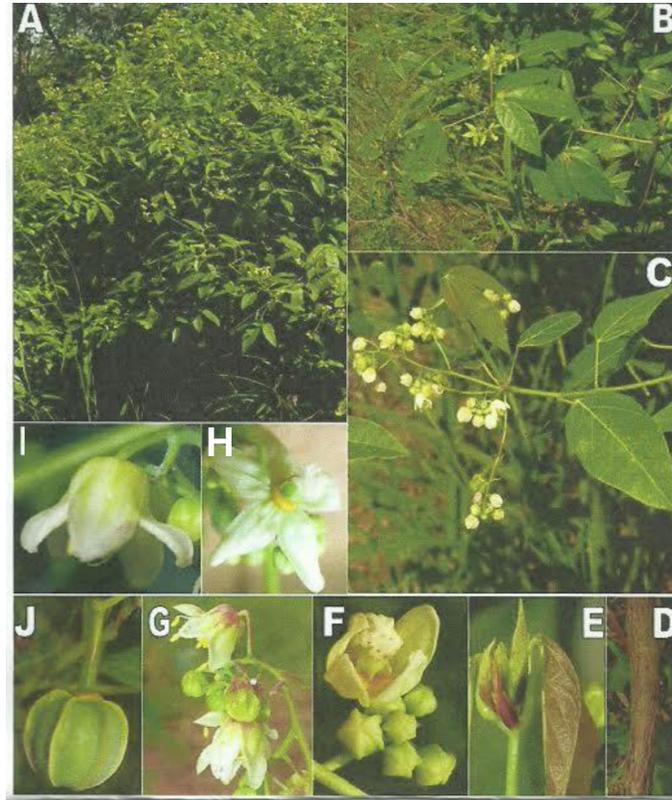


Figure 2. *Manihot esculenta* Escape 2. Morphological characteristics labeled alphabetically, as follows: **A.** growth habit, semi-erect with decumbent branches; **B. I.** female flowers; **C. H.** male flowers; **D.** stem and cortex; **E.** stipules pubescent in young shoots; **F.** female inflorescence; **G.** male inflorescence; **J.** fruit with wings.

Morphology indicates that these two forms clearly display the prominent characteristics of *M. esculenta*, notably fruits with wings, typical shape, and characteristic size of cassava. Moreover, both types have an inflorescence panicle with reduced bracts and bracteoles, and leaves of similar form and type as cassava. Both are shrubs 4-5 m in height, a size that is normally acquired by cassava plants grown naturally near cassava plantations. Finally, study of the roots of Escape 2 revealed formation of rudimentary thickened roots, but with cassava-type form.

One of the most striking features of these two plant forms is the prominence of wings on the fruit, which is typical of cassava, and the type of inflorescence panicle on both forms. Of the 98 species reported by Rogers and Appan (1973), and others described later, all have spherical fruit without wings or inflorescence racemes, and no panicles, with the exception of *Manihot flabellifolia* Pohl, *Manihot tristis*, and *Manihot zehntneri* Ule. Later studies revealed that the alleged species *M. flabellifolia* maintained the closest genetic relationship (DNA) with the cultivated species (Haysom et al., 1994; Bredeson et al., 2016); thus, this species and the two others represent instances of cassava escape and are not independent species, as indicated by cytogenetic and morphological evidence.

In cassava crosses with wild species, clearly revealed a pattern of easy gene flow between *M. zehntneri* and cassava, compared to the difficulties exhibited in crosses of cassava with other wild *Manihot* species used in the experiment. Additionally, hybrids obtained from *M. zehntneri* and cassava were highly fertile with extremely regular bivalent pairing (18). This indicates that the plant form recognized as *M. zehntneri* by Ule is no more than an escape (Nassar, 1980, 2000). *M. flabellifolia* represents another case for an escape that developed through hybridization of cassava with a wild species, followed by backcrossing with the cassava cultigen itself. Recent molecular evidence by Bredeson et al. (2016) reveal that this form is the same as *M. esculenta*, confirming the assertion that cassava and *M. flabellifolia* are indeed the same species (Nassar, 2000; Nassar et al., 2008; Nassar and Ortiz, 2009).

Manihot melanobasis Müll. Arg. is another example of an escape. In addition to morphological similarities that exclude any possibility of this variety being a solid wild species, there is also feasible hybridity and the absence of barriers noted by Bolhuis (1953) and Jennings (1959). There also seems to be a strong chromosomal linkage between the genes that determine the formation of starch storing roots and those that control vegetative propagation and fruit shape, stem nodes, and inflorescence form. Domestication of *M. esculenta* brought these characteristics together in novel cassava varieties. It is further believed that fruit shape and inflorescence panicle characteristics are controlled by multiple genes, and are accumulated via natural selection over the course of thousands of years. Thus, through the domestication of plants, which combined these characteristics with starch storing in roots, these attributes became solid morphological characteristics of *M. esculenta* (Pujol et al., 2005). The great similarity of Escape 2 with cultivated cassava crops can be further attributed to being a part of a complex. In this crop-hybrid complex, individuals always hybridize with cultivated species, leading to the restoration of all lost features, such as fertility and morphological characteristics (Harlan and deWet, 1965, 1971; Rieseberg et al., 1999).

Analysis of herbarium specimens indicates that these two plant types occur in the Amazon rainforest, confirming reports of several crops known to behave in this mode in their natural habitats, including sorghum, corn, potato, sweet potato, cotton, sunflower, and others (Astley and Hawkes, 1979; Barnaud et al., 2009; Mutegi et al., 2012). Cytogenetic analysis of the two types revealed regular chromosome pairing in meiosis similar to that observed in cassava cultivars. This leads us to conclude that both types studied here are no more than variations of the cassava cultigen itself. Because they maintain morphological similarities and regular cytogenetic behavior, they cannot be considered different species. In addition, they have not developed genetic barriers with other wild species and are not isolated from cassava in any way.

Conflicts of interest

The authors declare no conflict of interest.

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