

Evaluation of genetic relationships among *Manihot* species and determination of possible hybrid or introgressed plants between *M. leptophylla* and cassava

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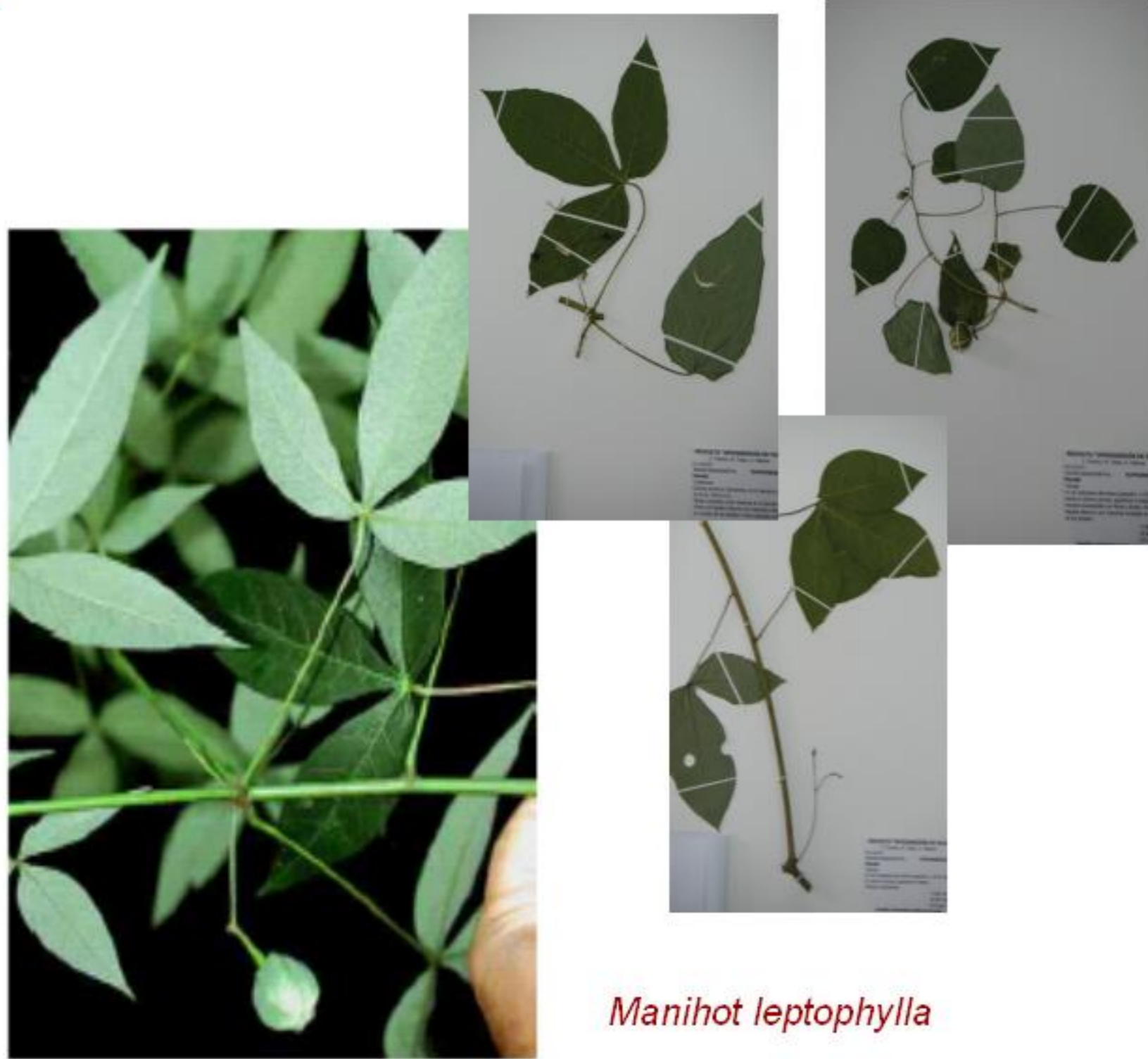
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Introduction

Studies on the genetic diversity of *Manihot esculenta* and its relation to its wild relatives (Roa et al, 1997; Roa et al, 2000; Olsen & Schaal, 1999) have generally excluded *M. leptophylla*, a species whose distribution range is reported for Ecuador, Perú, Bolivia and reaches to Belém in Brazil (Rogers and Appan, 1976). These same authors state that *M. brachyloba* and *M. leptophylla* have similar morphological characteristics and share approximately the same distributions, however *M. leptophylla*, as does the crop species, exhibits a high morphological plasticity (1) which could lead to mis-identification. Allem (2002) suggests that *M. leptophylla* could be synonymous to *M. esculenta* ssp. *flabellifolia* and therefore be part of the complex of sub-species which includes *M. esculenta* ssp. *esculenta*, *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana*.

Morphological plasticity

1



Manihot leptophylla

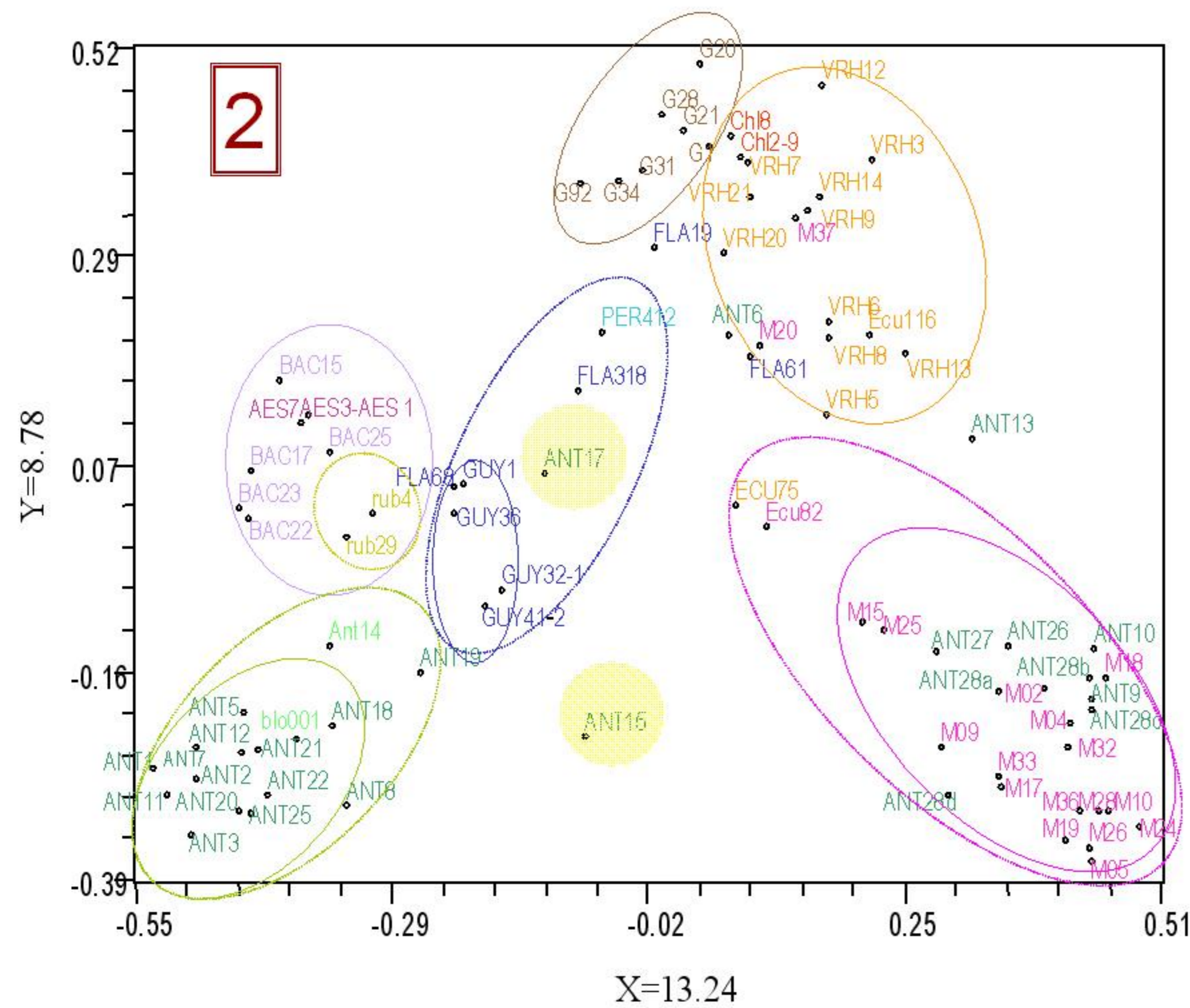


Manihot brachyloba

Although the establishment of *M. flabellifolia* as the direct ancestor to cassava is consistent in various studies (Roa et al, 1997; Roa et al, 2000; Olsen & Schaal, 2001; Olsen & Schaal, 1999; Allem, 2002), the wide diversity seen in this crop species may not be explained by a single domestication event, evermore when introgression between cassava and different wild relatives has been previously reported (Morillo, 2002; Allem, 2002) and at the same time the difference in the genetic base of sweet and bitter cassava cultivars has also been established at the molecular level (Chywona-Karlun, 2001; Narváez-Trujillo & Second, 2003).

An extensive review of herbarium specimens indicated that only specimens from the western coast of Ecuador and the southwestern part of Colombia are morphologically similar to the *M. leptophylla* type reported from the Manabí Province of Ecuador. Specimens of this species available from the Amazon Basin are morphologically distinct from those from the West leading us to believe that there may be a misidentification of specimens, given the plasticity of *Manihot* species. In this case, specimens from the eastern side of the Andes more probably correspond to *M. brachyloba* than to *M. leptophylla*, which would be restricted to the lowlands on the western side of the Andes in Ecuador and Colombia. Molecular marker studies using AFLPs (Narváez & Second, 2002) clearly indicated that *M. leptophylla* is genetically very distant from cassava and from *M. flabellifolia*.

The objectives of the on-going project are to establish the genetic relationships of *M. leptophylla* to central and south American *Manihot* species and to evaluate possible introgression between this species cassava in order to establish its possible participation in a distinct domestication event, that could eventually be the genetic basis of the sweet and bitter differentiation.



Manihot esculenta ssp. *esculenta* (sweet-eastern side of the Andes), *Manihot esculenta* ssp. *esculenta* (sweet-Western side of the Andes), *Manihot esculenta* ssp. *esculenta* (bitter French Guiana), *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana*, *M. brachyloba*, *M. leptophylla*, *M. baccata*, *M. rubricaulis*, *M. aesculifolia*, *M. chlorostica*.

materials and methods

Up to the moment 15 of a total of 50 selected SSR loci (Mba et al, 1999) have been analyzed in a set of samples including:

- *M. leptophylla* from coastal Ecuador (and presumed hybrids)
- *M. esculenta* sweet cultivars from Napo (Amazon basin) and Manabí (Western Coast) in Ecuador
- *M. esculenta* bitter cultivars (French Guiana)
- *M. flabellifolia* from French Guiana
- *M. flabellifolia*, from Brasil (CIAT germplasm bank through MTAs)
- *M. aesculifolia* from Central América (CIAT germplasm bank through MTA)
- *M. chlorostica* from Central América (CIAT germplasm bank through MTAs)
- *M. rubricaulis* from Central América (CIAT germplasm bank through MTAs)
- *M. brachyloba* from Ecuador



* Principal Coordinate Analysis using NTSYS base on the Dice (Sorensen) genetic distance coefficient was used to establish relationships among species and cultivars. FST pair-wise comparison between geographic populations were used to establish their genetic differentiation.

results and discussion

Principal coordinate analysis on the complete data, with a total of 188 alleles for the 15 SSR loci analyzed, shows a clear genetic differentiation between all *Manihot* species.

The two axis of variation separates cassava from most other *Manihot* species, with a distinct geographical differentiation (west and east of the Andes) and also a genetic distinction between sweet and bitter cultivars. The sweet cultivars originated from the Pacific coastal area appear as the most distant from bitter cassava. Most Centralamerican species form a discrete group from which *M. chlorostica* is excluded while it includes *M. baccata*. Nevertheless, the third axis of variation (not shown) separates *M. baccata* from *M. aesculifolia* and *M. rubricaulis*. The third axis of variation also differentiates *M. chlorostica* from cassava varieties, however interpretation of this data is still not clear and should be further supported with a higher number of SSR loci and a phylogenetic analysis. *M. esculenta* subsp. *flabellifolia* and *M. esculenta* subsp. *peruviana*, which formed a mixed group, were the closest to the crop. This information supports the findings that Brazil is the center of origin of cassava. *Manihot leptophylla* cannot be considered a synonym to *M. flabellifolia* and is actually the most distant to cassava. Samples that were encountered as feral forms of cassava and in this study were considered as putative hybrid or introgressed forms actually fit tightly into the sweet cassava pool from the Ecuadorian west coast; however, two samples (Ant 17 and Ant15) collected at *M. leptophylla* populations collection sites are intermediate between groups (2) and may therefore be considered as introgressed or hybrid forms. Confirmation of this presumption may be obtained when data is completed for all 50 SSR loci; additionally, a new sampling of plants from these populations has been undertaken to expand the sample size of possible introgressed populations.

Up to the moment no species specific markers have been determined that may be used for tracking gene flow or indicating introgression. However, pairwise comparisons of the genetic differentiation estimator (FST) indicate the following:

Manihot leptophylla has a higher genetic differentiation with bitter cassava cultivars from French Guiana (FST = 0.22) while being genetically most related to sweet cassava cultivars on the Pacific Coast. (Fst = 0.19).

M. flabellifolia from French Guyana is more genetically more differentiated from cassava, than *M. flabellifolia* accessions acquired through CIAT, which are mainly from Brazil.

M. leptophylla and *M. flabellifolia* with Brazilian origin are more genetically similar to sweet cassava cultivars than *M. flabellifolia* from French Guiana.

These results indicate that bitter cassava cultivars have a gene pool distinct from sweet cultivars which is still unexplained by the present theory on the origin of cassava

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