

## TRACING THE DISPERSAL OF SWEET AND BITTER MANIOC IN BRAZILIAN AMAZONIA WITH CHLOROPLAST AND NUCLEAR GENETIC MARKERS

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Figure 1. Domesticated manioc is an herbaceous shrub that store large amounts of starch in its tuberous roots

Manioc or cassava (*Manihot esculenta* ssp. *esculenta* Crantz) was domesticated in the southwestern Amazon basin and currently is the main source of calories for more than 800 million people in the world, especially in the tropics. After its initial domestication, divergent selection pressures gave rise to two major groups of varieties (sweet and bitter manioc), which differ in their contents of toxic compounds (cyanogenic glucosides). Although there is some overlap, especially in Brazilian Amazonia, these groups of varieties have distinct patterns of distribution, which may be the outcome of limited processes of contact and interchange between varieties during the crop's domestication and dispersal history. The evolutionary history of a crop may be partly understood by studying the organization of its genetic diversity within and among its domesticated populations. Few studies have evaluated how the genetic diversity of manioc is organized across its geographic distribution, and there are no genomic

population studies with manioc varieties. The main goal of this research is to evaluate, with different molecular markers, the genomic and genetic diversity and structure, and the phylogeography of manioc varieties traditionally grown along the major rivers of Brazilian Amazonia. The current genetic diversity and structure will be evaluated with nuclear microsatellites markers (ncSSR), while historical patterns of the organization of genetic diversity (phylogeography) will be evaluated with chloroplast microsatellites (cpSSR). Novel information on the genomic basis for the distinction between sweet and bitter varieties, as well as possible distinct patterns of dispersal, will be generated with SNPs detected with Restriction-site associated DNA sequencing (RAD-seq). We expect to contribute to the understanding of manioc's dispersal across the Amazon basin after its initial domestication, as well as to the understanding of the genomic basis for the differentiation between sweet and bitter manioc. This information may help identify centers of genetic diversity of the crop, which in turn may be useful for the management and conservation of its genetic resources, and also for manioc's numerous breeding programs.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A total of 38 municipalities along five major rivers of the Brazilian Amazon basin (Madeira, Negro, Branco, Solimões and Amazonas) in four different states (Amazonas, Pará, Rondônia and Roraima) were visited. A total of 542 manioc varieties were sampled (307 bitter, 219 sweet, and 17 non-designated), and also 28 plants of *Manihot esculenta* ssp. *flabellifolia*, the wild relative of manioc. Due to the high costs of the RAD-seq technique, 87 individuals were selected for SNP discovery, which is being carried at the moment. The current genetic diversity and structure is also being evaluated. Ten ncSSR markers were tested, and all were polymorphic. Results of a preliminary trial with 68 individuals showed that the ncSSR revealed high values of genetic diversity, with an average of five alleles per locus (ranging from two to eight), and average expected heterozygosity of 0.630, ranging from 0.098 to 0.793 over loci. These preliminary results show the potential of these ncSSR markers for detecting genetic variation of manioc varieties from different localities. For the phylogeographic analysis, ten universal cpSSR loci for dicotyledonous species were tested, and three of them were polymorphic. The analyses showed that the groups of sweet and bitter varieties had similar levels of genetic and haplotypic diversity, and that both were more diverse than the group of wild plants, probably because only three municipalities were visited where wild plants could be collected. There were no chlorotypes (combinations of different cpSSR alleles) associated specifically with bitter or sweet varieties, nor to the major river basins. Instead, the most frequent chlorotype was shared by sweet and bitter varieties. All wild plants, except one, shared another chlorotype that differed by two alleles from the cultivated varieties' most common chlorotype. Due to these results, the genetic divergence found between the groups of sweet and bitter varieties was very low ( $F_{ST} = 0.001$ ), in contrast to that found between the groups of cultivated varieties in relation to the wild plant group ( $F_{ST} = 0.76$ ). The low level of genetic divergence between sweet and bitter manioc, and the absence of associations of chlorotypes with groups of manioc varieties, is probably due to the features of the traditional management employed by the communities of farmers in Amazonia. This management permits gene flow between manioc plants in the fields, and also genotype flow, since exchange of varieties among different farm families, communities, and municipalities is a common and widespread practice of traditional manioc cultivation in Amazonia. We expect a significant increment of information about the genetic diversity present in our manioc sample once the screening with ncSSR and SNP markers detected with the RAD-seq technique are completed. Our chosen ncSSR loci revealed considerable variation within a subset of the sample. The forth-coming RAD-seq essays, which typically reveal hundreds to thousands of markers with SNP variation, should expand this variation significantly, so we may expect that the final results of this project will provide robust evidence on how genetic diversity of manioc varieties is organized across different localities.

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