

TheoBRomics

Interaction between Theobroma grandiflorum and Moniliophthora perniciosa: Association Studies and Functional Genomics

ABSTRACT

The cupuassu tree, Theobroma grandiflorum (Willd. ex Spreng.) Schumm., is a fruitful species native to the Amazon, which belongs to the Malvaceae family and has similar characteristics to cacao (T. cacao L). This plant has huge economic potential due to the multiple uses of its fruit pulp and almonds. Several products are manufactured from its pulp: juices, ice creams, liqueurs, jellies, creams and sweets (Calzavara et al., 1984). Almonds have a fine fat, composed mainly of oleic and stearic acids (Vasconcelos et al., 1975), from which can be obtained a product similar to chocolate, called cupulate (Ribeiro et al., 1992). Moreover, the cupuassu pulp has a good level of vitamin C (23.6 mg/100 g), pectin (0.39%), protein (8.1%) and traces of various minerals (Ribeiro et al., 1992). Among the native tropical fruits of the region, the cupuassu shows the best conditions for industrial exploitation (Costa et al., 2003) with the advantage that it can be grown in an agroforestry system, making it a kind of sustainable farming, thus assuming a significant socioeconomic and environmental importance to the region. Cupuassu tree, like cacao, is affected by the witches' broom disease, caused by the fungus Moniliophthora perniciosa, resulting in a large reduction in fruit production (Benchimol, 2004). Unlike cocoa, which has its genome sequenced and vast information on functional genome (Argout et al. 2008; Argout et al. 2011), the cupuassu breeding program is incipient and lacks knowledge about the plant molecular genetics. Some recent initiatives have resulted in increasing knowledge in molecular genetics, with the generation of omic data, identification of ESTs from fruits and development of SSR molecular markers based on those (Santana et al., 2012).

However, cupuassu still lacks information to enable the development of tools that speed up the selection procedures and streamline the breeding program of the species, combining time-saving with reduced research costs. Therefore, it is necessary: i) to increase knowledge about the associations between genomic and phenotypic characteristics; ii) to generate omic data in great quantity; iii) to evaluate functionally the genes identified.

This proposal has been outlined comprising 4 main actions:

- 1. Genome wide association studies (GWAS).
- 2. RNA-seq and bioinformatics analyses.
- 3. Expression analysis of candidate genes.
- 4. Functional studies of candidate gene by transformation of model plant.

Thus, this project proposes to develop cupuassu genotyping using GBS (genotyping by sequencing), identifying thousands of SNPs, and expand the cupuassu database by RNA-seq, identifying genes and small RNAs involved in the response to the pathogen M. perniciosa. It also proposes to evaluate functionally some genes (e.g. genes with function related to plant-pathogen interaction, genes present in the QTLs regions), using RT-qPCR and in situ hybridization, as well as, genetic transformation of a model plant (tomato var. Micro-Tom).

Information on cupuassu genetics and the development of molecular markers to identify witches' broom resistant varieties will be of major interest in the breeding program of this species, as well as cacao. In addition, the characterization of genes involved in plant-pathogen interactions is also of great interest. In this sense, our work will result in powerful tools to develop witches' broom-resistant and productive varieties, which are crucial for commercial production of these tropical fruits.

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