GENETIC STRUCTURE AND MATING SYSTEM OF LOCAL VARIETIES AND WILD POPULATIONS OF ANNATTO (*BIXA ORELLANA L.*) IN BRAZILIAN AMAZONIA AND CENTRAL BRAZIL USING MICROSATELLITE MARKERS

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FAPESP # 2012/08307-5 | Term: Aug 2012 to Jan 2015

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Figure 1. Annatto (Bixa orellana) grown in the germplasm bank of Instituto Agronômico (IAC) (Dequigiovanni, 2013)

The annatto (*Bixa orellana L.*) has great economic importance because it is the only source of a natural pigment, bixin, used as a natural dye in the pharmaceutical, textile, dairy, food, beverage, paint and cosmetic industries. This project aims to study the genetic diversity and structure, as well as the reproductive system of local varieties and wild populations of annatto in Brazil. For that, microsatellite primers will be developed for the species by means of a microsatellite-enriched genomic library. Local varieties of annatto will be collected along two axes in the Amazon Basin - north-south (Rio Mamore, Madeira, Negro and White) and west-east (Rio Solimões and Amazonas), in the surroundings of Belém, in Central Brasil (Cuiabá - Porto Velho), as well as wild populations collected in the states of Amazonas, Rondônia and Roraima, to detect possible centers of diversity. The mating system will be studied with a hierarchical design of fruit and individuals from wild and cultivated populations of annatto. Various parameters of genetic diversity and population structure will be estimated. Bayesian analyses, plus cluster and principal coordinate analyses will be conducted in order to evaluate the relationship among the accessions and populations. For the reproductive system study, 10 plants will be used of a wild population and a local variety population, where 20 progenies will be evaluated per plant. From this analysis, multilocus and single locus outcrossing rates will be estimated, as well as other related parameters. Information about genetic diversity and structure and the reproductive system will help to guide future prospections for breeding programs, as well as plan for the *in situ* and *ex situ* conservation of annatto in Brazil.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Annatto (*Bixa orellana*) (Figure 1), which belongs to the family Bixaceae, is a tropical crop originated from the Americas, most probably from the Amazon region. The project aims to evaluate, using molecular markers, a collection of 200 samples of annatto (*Bixa orellana*) obtained in home gardens of riverine communities of agriculturists in the Amazon along the rivers: upper Rio Madeira; Rio Mamore; Rio Madeira-Purus, near Manaus; Rio Negro, near Barcelos; Rio Solimões; Rio Amazonas; and Rio Branco in Roraima. A total of 375 samples were also collected during the project in the State of Rondônia, including 193 cultivated varieties collected in 22 farmers households, 167 wild accessions, not domesticated, and 18 materials where it was not possible to determine the stage of domestication. The 200 samples obtained in the States of Amazonas and Roraima were collected by researchers from the National Institute of Amazonian Research (INPA), and the remainder by ESALQ/USP researchers in the State of Rondônia. For each population leaves, were sampled from several individual plants and geographic coordinates registered. In Rondônia, samples were collected in the municipalities of Corumbiara, Pimenteiras do Oeste, Cerejeiras, Cabixi, Rolim de Moura, São Domingos do Guaporé, São Francisco do Guaporé, Ji-Paraná, Ariquemes, Rio Crespo and Porto Velho. Interestingly, the wild accessions of annatto were all observed occurring at the edge of small streams, always in open areas where plants were exposed to the sun for most of the day. Another collection is scheduled to be held in the State of Para, near the city of Belém, to make the sampling more representatives. However, excessive rainfall in the region is delaying the timing of such collections. The annatto samples collected will be evaluated with microsatellite markers. For this, microsatellite primers were developed for the species *Bixa orellana* through an enriched genomic DNA library. A total of 24 primers were developed, and they will be used to evaluate the collected samples up to December 2014. The project is being conducted with the collaboration of researchers from INPA and Federal University of Amazonas, both in Manaus, AM, where part of the genetic analyses will be held, from the Agronomic Institute (IAC), in Campinas, SP, from Pólo Apta Centro Sul (APTA) and from ESALQ/USP, both in Piracicaba, SP. One of the objectives of the project is to assess the level of genetic variability among the sampled regions. Another aim is to compare the level of genetic diversity among wild and cultivated varieties of annatto, with molecular markers, and also to compare the reproductive system of both cultivated and wild varieties. Morphological differences were observed among the annatto varieties collected, such as fruits or capsules with yellow, yellowish red, red, green and pink colors. Fruits were also observed with different shapes, such as flat capsules and heart-shaped capsules. And the main difference observed between cultivated and wild varieties is the fruit size, with capsules of wild varieties of much smaller size in comparison with cultivated varieties, and lower number of seeds in the wild varieties.

MAIN PUBLICATIONS

Dequigiovanni G. Desenvolvimento de locos de microssatélite para a caracterização da diversidade genética de acessos de urucum (*Bixa orellana L.*). Dissertação. Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 2013.

Dequigiovanni G, Ramos SLF, Zucchi MI, Bajay MM, Pinheiro JB, Fabri EG, Bressan EA, Veasey EA. Isolation and characterization of microsatellite loci for *Bixa orellana*, an important source of natural dyes. *Genet. Mol. Res*. In press.

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