# Morphological and genetic diversity of camu-camu (*Myrciaria dubia, (Kunth) McVaugh) in Peruvian Amazon*

Šmíd Jan[1], Lojka Bodan[1], Kalousová Marie [1], Mandák Bohumil[2], [3], Bílý Jan [2], Houška Jakub [4]

[1] Dep. of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, CULS, Kamýcká 129, CZ-165 00, Prague, Czech Rep.; [2] Dep.of Ecology, Faculty of Environmental Sciences, CULS, Kamýcká 129, CZ-165 00, Prague, Czech Rep.; [3] Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Rep.; [4] Dep.of Soil Science and Soil Protection, Faculty of Agrobiology, Food and Natural Resources, CULSPrague, Prague, Czech Rep.

## Introduction

Camu-camu (*Myrciaria dubia*, (Kunth) McVaugh) is currently **one of the most important fruit species** which is grown not only in the Peruvian Amazon, but also in Brazil, Colombia or Bolivia (Figure 1, 2, 3). Plantations in the larger extent were established until two decades ago and a large part of the production is still obtained by collecting of fruits from the nature (Figure 4). The economic importance of camu-camu lies in the **high content of Vitamin C** in its fruits that is reported to be in the range between **877 and 3,133 mg/100g of pulp**, thus considered as **one of the richest source of Vitamin C from all plants**. As there is still limited information about genetic diversity of that species, thus the primary objective of this study was to evaluate morphological and genetic diversity among cultivated and wild populations in Peruvian Amazon.

Figure 1. Various stages of ripeness. Second fruit from left is in stage which is the most suitable for collection for vitamin C.

Figure 2. Open flower and flower buds.

Figure 3. Cross section of pulp with visible seeds and very thin skin.

Figure 4. Plantation of camu-camu near Iquitos city.

## Methodology

In total we have sampled **13 populations**; 10 wild populations in Iquitos region, three cultivated populations in Pucallpa in Peruvian Amazon (Figure 5). For genetic analysis, the leaf tissue samples were collected from eight to ten individuals from each population (**n=126**), and for morphological data were collected from five trees of each population (**n=65**). To assess the genetic diversity, we used **seven microsatellite primers** that were developed from available DNA sequences (Table 1). The DNA was extracted from the dried leaves using modified **CTAB method**. Using various genetic software, major indexes of variability were detected and dendrograms of relatedness of populations and individuals were created.


Figure 5. Map of north Peru with the study sites marked in red .

Table 1. Microsatellite primers designed and used in our study.

| SSR | 5‘ Forward primer | 3‘ Reverse primer | Ta(°C) | Size (bp) | Motif |
|---|---|---|---|---|---|
| MDI003 | GCATAAATAACCCCGCGGTCTC | GTACAGCTCCCAGCAGGAGT | 59 | 156 | (CT)6(AG)14 |
| MDI004 | GCCTTCCAGACCCTTTTGC | GTTCTTGAACCGGGACGC | 56 | 397 | (CTT)10 |
| MDI006 | GCTCTCTCTCTGAGTACCTGAAAC | CTTTCACGCAAGACCGACG | 56 | 195 | (CT)5(CT)6(CT)12(TCG)11 |
| MDI007 | TCTCGAGAGCTTTCCTCGGAG | AGTACTTCACTCTGTCCGGCC | 58 | 245 | (CTT)10 |
| MDI009 | CGAAGTCCTGACCTGTTCTGAGTT | GCAGACCAGCGAGTTTACACC | 59 | 363 | (GA)18 |
| MDI010 | CGATCGCTGCCCTTTCTG | GGTTCGGGAGGGTAGGAG | 56 | 95 | (CTT)12 |
| MDI015 | CTGTACCTGCATCGATGGTG | CGTTCTAATCCGCCATTATTCGTC | 56 | 305 | (TC)12 |

## Results and discussion

The statistical analysis did not reveal statistically significant differences for most of the morphological descriptors, except for the fruit parameters. The trees from wild populations had **higher fruit and pulp weight variation** and their fruits contained less seeds compared to cultivated populations (Table 2). The **observed heterozygosity** was 0.347 and 0.404; **expected** 0.516 and 0.506; **inbreeding coefficient** was 0.328 and 0.200 for wild and cultivated populations, respectively (Table 3). **Wild populations could be divided** according to the dendrogram **into two completely different groups** (Figure 6). In cultivated populations, their approximate origin was determined. All the results indicated a **high genetic variability** but also a **high degree of inbreeding**. One possible explanation of high genetic variability is that distances between the populations are large and populations are isolated from each other by tropical rain forest. Migration of this species through the forest is less probable because seeds are mainly dispersed by the water. Explanation of higher inbreeding coefficient could be following. Oxbow lakes, where the plants grow are relatively small and the populations are dense. Thanks to this to the crossbreeding and low gene flow occurred.

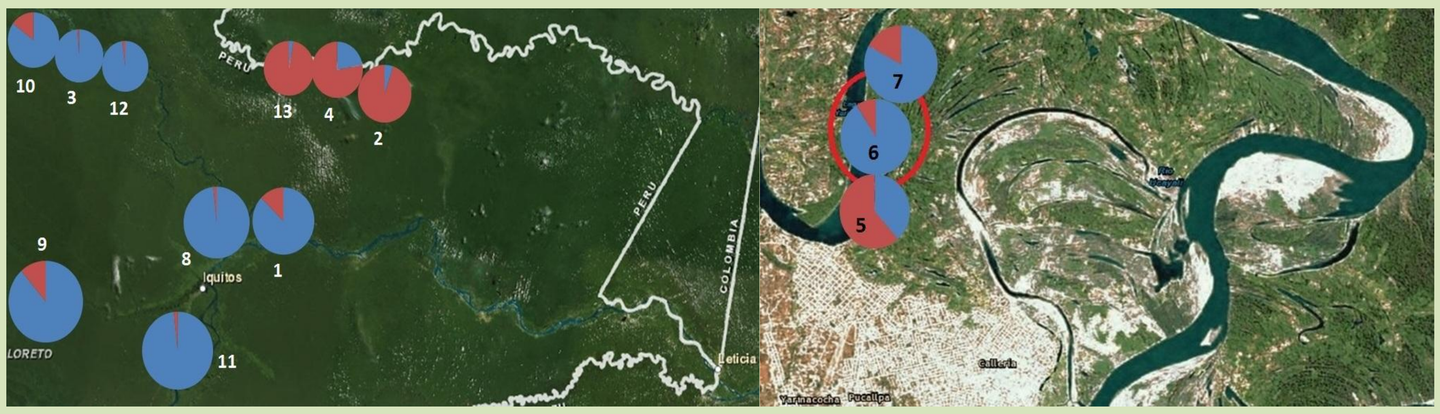


Figure 6. Graphical representation of the populations divided into two clusters. Populations 1,2, 3, 4, 8, 9, 10, 11, 12 and 13 are wild. Populations 5, 6 and 7 are cultivated.

Table 2. Quantitative morphological descriptors for the wild and cultivated populations of camu-camu, including number of samples (n), mean, median and standard deviation (SD) of each characteristic.

| Parameter | Wild population (W) | | | | Cultivated population (C) | | | |
|---|---|---|---|---|---|---|---|---|
| | N | mean | median | SD | N | mean | median | SD |
| leaf length | 50 | 9.14 | 9.21 | 0.95 | 15 | 9.05 | 9.07 | 0.79 |
| leaf width | 50 | 3.40 | 3.37 | 0.43 | 15 | 3.23 | 3.19 | 0.39 |
| length/width | 50 | 2.72 | 2.72* | 0.33 | 15 | 2.83 | 2.89* | 0.32 |
| petiole length | 50 | 0.70 | 0.63 | 0.22 | 15 | 0.82 | 0.67 | 0.42 |
| number of flowers | 17 | 4.07 | 4.00 | 1.21 | 14 | 3.66 | 3.57 | 1.16 |
| size of fruit | 9 | 2.60 | 2.59 | 0.24 | 14 | 2.54 | 2.54 | 0.19 |
| weight of fruit (g) | 9 | 11.22 | 10.82 | 2.70* | 14 | 9.82 | 9.75 | 1.74* |
| weight of seed (g) | 9 | 2.39 | 2.31 | 0.74 | 14 | 2.71 | 2.74 | 0.66 |
| weight of pulp (g) | 9 | 8.83 | 8.54 | 2.15* | 14 | 7.11 | 7.05 | 1.23* |
| number of seeds | 9 | 2.44* | 2.67 | 0.81 | 14 | 2.94* | 3.00 | 0.70 |
| diameter of trunk | 50 | 6.22 | 6.10 | 1.88 | 15 | 6.37 | 6.34 | 1.70 |

Table 3. Main measures of genetic diversity for all seven loci. (k: number of detected alleles at the locus, *Ho*: observed heterozygosity, *He*: expected heterozygosity, *HW*: significance of deviation from Hardy-Weinberg equilibrium, *Fis*: inbreeding coefficient)

| Locus | k | *Ho* | *He* | *HW* | *Fis* | Allelic richness |
|---|---|---|---|---|---|---|
| MDI010 | 9 | 0.408 | 0.680 | 0.00020 | 0.390 | 3.58 |
| MDI006 | 3 | 0.137 | 0.218 | 0.00020 | 0.390 | 1.86 |
| MDI015 | 8 | 0.421 | 0.626 | 0.00020 | 0.317 | 3.59 |
| MDI004 | 4 | 0.284 | 0.397 | 0.00020 | 0.298 | 2.48 |
| MDI009 | 10 | 0.426 | 0.661 | 0.00020 | 0.351 | 4.19 |
| MDI007 | 3 | 0.296 | 0.354 | 0.06160 | 0.160 | 2.04 |
| MDI003 | 4 | 0.527 | 0.647 | 0.00160 | 0.186 | 3.46 |
| Average | | 0.357 | 0.512 | | 0.301 | 3.03 |

## Conclusion

This study found a **low morphological variability** within and between wild and cultivated populations of camu-camu. From all detected characteristics the most distinctive parameters were fruits. For genetic evaluation, **seven primers** were developed and **showed high level of variability within and between populations**. These primers divided wild populations into two main groups and also were able to show us the origin of cultivated populations. For this reason, **these primers can be recommended for further genetic studies** of this species. The crossbreeding of the individual trees from different geographical location, that suffer from inbreeding and are genetically different, could result in heterosis effect and the resulting hybrids can possess higher genetic variability and might be more suitable for growing in plantations.

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