

Molecular identification of pollen donor plants on a progeny of Cambona-4 female matrix of maté (*Ilex paraguariensis* St. Hil. – Aquifoliaceae)

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ABSTRACT. A progeny of Cambona-4, a female maté plant (*Ilex paraguariensis*) selected by its agronomical characteristics and mild flavor, was evaluated using RAPD markers in order to identify its male parents. Using RAPD markers specifics of each one of the four potential males, the paternity of 84 out of 107 offsprings was confirmed. The majority of the offsprings (83.3%) were ascribed to pollen donor A, while male plants B, C, and D represented 11.9, 4.8, and 0%, respectively. The highly desirable agronomical characteristics and product quality of Cambona-4/pollen donor A offsprings, identified by RAPD markers, lead to the planting of an orchard Cambona-4 and pollinator A to obtain bi-clonal commercial seeds.

Key words: *Ilex paraguariensis*, maté, RAPD, paternity.

RESUMO. Identificação molecular de plantas polinizadoras em uma progênie da matriz de erva-mate (*Ilex paraguariensis* St. Hil. - Aquifoliaceae) Cambona-4. Uma progênie da matriz feminina de erva-mate (*Ilex paraguariensis*) denominada Cambona-4, selecionada pelas suas características agrônômicas e sabor suave, foi avaliada por marcadores RAPD para a identificação dos respectivos parentais masculinos. Usando-se fragmentos específicos de RAPD de cada um dos quatro polinizadores potenciais, a paternidade de 84 de 107 descendentes foi confirmada. A maioria dos descendentes (83,3%) foi atribuída ao polinizador A, enquanto os polinizadores B, C e D representaram 11,9; 4,8 e 0%, respectivamente. As características agrônômicas e de qualidade do produto, oriundas da progênie Cambona-4/polinizador A, identificado pelos marcadores RAPD, direcionaram o plantio de um pomar a partir do cruzamento destas para obtenção de sementes comerciais biclonais.

Palavras-chave: *Ilex paraguariensis*, erva-mate, RAPD, paternidade.

Introduction

Ilex paraguariensis St.-Hil. (Aquifoliaceae), commonly called maté, is native to South America, and been found in the southern States of Brazil, Paraguay, Argentina and part of Uruguay. Maté is an evergreen 12-30 m tree, which can live for up to 100 years. It is an obligatory out-crossing, dioecious, insect-pollinated, and diploid ($2n = 40$) species (NIKLAS, 1987).

Leaves and green branches of *I. paraguariensis* are commercialized as dried and ground leaves for 'chimarrão' and 'tererê', traditional beverages in Brazil, Uruguay, Argentina and Paraguay, or as canned drinks, soluble teas, and essences. The production of maté is in constant growth and is currently ca. 1.4 million t/yr, of which 550,000 t are produced in Brazil (CANSIAN et al., 2008).

Most of *I. paraguariensis* commercialized in Southern Brazil came from native orchards, but the cultivation of this species is increasing due to the expansion of the internal and external market, and the reduction of native areas. In general, *I. paraguariensis* breeding programs focus on agronomical characters as plant height, leaf production, and product quality, among others (SIMEÃO et al., 2002; CANSIAN et al., 2008; TECHIO et al., 2009). However, due to the lack of genetic information and improvement programs, the seeds in use to establish new plantations are usually of poor quality. The standard has been the formation of average to low yielding stands. Wendt et al. (2007) characterized the genetic variability among progenies of maté from different provenances by using RAPD markers, and the

results suggest the possibility of obtaining higher genetic gains through selection among progenies than among provenances.

In this context, the present work aimed to identify pollen donor plants of a progeny of a maternal plant named Cambona 4, selected by its excellent agronomical characteristics, high productivity, and mild flavor. With this work, the creation of an orchard with bi-parental progeny is intended, which yields a product with the sensory characteristics similar to the maté from native orchards that are used as source of blends for commercial mate tea. This way the production pressure over native plants is decreased, as native material is substituted by Cambona-4.

Material and methods

Plant samples

Young leaves of 107 two-year-old plants of the progeny of Cambona-4, the maternal tree Cambona-4, and four potential pollen donor plants designated as A, B, C, and D, were collected, identified and stored at -80°C until DNA extraction. The male plants A, B, C and D were placed 14.8, 17.5, 19 and 23.8 m, respectively, from the maternal tree Cambona-4. It is worth noting that the next male plant was more than 200 m from this group.

DNA extraction and RAPD amplification

The DNA was extracted from 150 mg of young leaves using the procedure described by Gauer and Cavalli-Molina (2000). The DNA was quantified at 260 nm and checked for integrity in 0.8% agarose gel.

The amplification of DNA followed the method described by Gauer and Cavalli-Molina (2000), which uses a thermal cycler (model PTC 100, MJ Research INC., Watertown, MA). A total of 120 primers from kits OPA, OPB, OPF, OPH, OPY and OPW from Operon Technologies Inc. (Alameda, CA) were used considering the high reproducibility of the fragments (SANTOS et al., 2007). Amplification products were separated by horizontal electrophoresis in 1.4% agarose gels containing 1 µg mL⁻¹ ethidium bromide at 90 volts. Fragments were visualized under UV light and photographed in a GEL-PRO system (Media Cybernetics, Silver Spring, MD). Phage Lambda DNA was used as molecular weight marker.

Plant identification was performed through verification of the presence or absence of bands, considering those present in progeny plants, in only one of the pollen donors, and necessarily absent in the other paternal plants and in the maternal plant.

The offspring was considered originated from a pollen donor when at least six fragments characteristic of a male plant were present in its profiles.

Results and discussion

Initially 120 primers were assayed against Cambona-4 and the potential donor plants (A, B, C and D) to select those who amplified specific markers to be used in the evaluation of the Cambona-4 progeny. Twenty-four primers that yield 26 well-defined, reliable and specific bands were selected. The potential pollen donor plants were identified by the following markers: pollinator A (OPA18₈₅₀, OPB06₅₀₀, OPF06₉₅₀, OPF08₉₀₀, OPF09₈₃₀, OPH₄₀₀, OPH14₇₉₀, and OPW03₅₄₀), pollinator B (OPA10₂₀₀₀, OPB17₁₈₀₀, OPF07₁₁₅₀, OPF08₈₆₀, OPW18₇₀₀, and OPY08₂₈₀), pollinator C (OPF02₁₃₀₀, OPF03₁₀₅₀, OPF05₈₈₀, OPF16₅₀₀, OPW17₂₄₀, and OPW18₇₇₀), and pollinator D (OPF07₂₀₀, OPF20₁₂₀₀, OPH17₃₂₀, OPW07₈₄₀, OPW13₁₄₀₀, and OPW15₁₅₅₀). An example of the patterns obtained with OPA18 indicating the pollen donor A specific marker 850 pb is present in Figure 1.

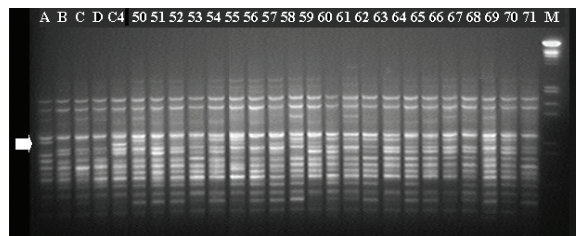


Figure 1. RAPD pattern obtained with OPA-18 primer, showing a specific fragment of pollen donor A, absent in the other paternal plants and present in several offsprings (A, B, C and D = pollen donors; C4 = maternal plant Cambona-4; 50 to 71 = progenies; M = molecular weight marker Lambda, clived with Hind III and Eco RI).

Using these markers, the paternity of 84 out of 107 (78.5%) offsprings of Cambona-4 was confirmed. Seventy plants (83.3%) were identified as offspring of pollen donor A, 10 plants (11.9%) of pollen donor B and only 4 plants (4.8%) of pollen donor C. No plants were found with the characteristic bands of pollen donor D, indicating that this plant, even when placed at 23.8 m, did not participate as pollen donor (Figure 2). These results confirm the efficiency of RAPD markers in the identification of male parents in progeny studies of plants (WERLMARK et al., 1999; HUANG et al., 2000; DONGRE; PARKHI, 2005). In *I. paraguariensis*, RAPD molecular markers have been used for the evaluation of genetic variability in natural populations (GAUER; CAVALLI-

MOLINA, 2000), and progeny genetic studies (VIDOR et al., 2002).

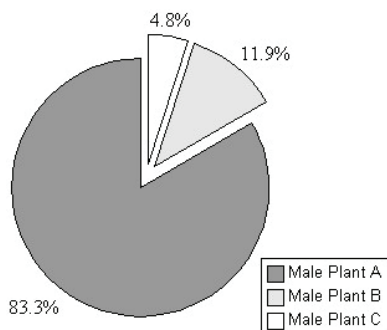


Figure 2. Pollen donor contribution on a progeny of Cambona-4 maternal plant as identified by RAPD markers.

Maté is basically insect-pollinated, although some pollen transport by the wind cannot be discarded (FERREIRA et al., 1983). Data obtained confirm the estimate of pollen flux in maté populations described by Winge et al. (1995). These authors showed that using isoenzymatic markers, 81% of pollen migrated from a distance shorter than 30 m, but 3% of the offsprings may have originated from plants more than 600 m away.

Since pollinator A and B are just 14.8 m and 17.5 m apart from the maternal plant, the high contribution of pollinator A may be attributed to flowering coincidence with Cambona-4, rather than to a position or distance effect. Pollinators C and D are within a cluster of female plants, fact that affects plant-plant movements of insects.

Due to the excellent characteristics of Cambona-4, approximately 73,000 offspring (37.73 ha) were planted in 2000 and 2001. The production of these orchards is beginning, and due the high quality, the product is attaining a commercial value up to 102% superior to that of conventional non-selected orchards.

Cansian et al. (2008) assert that the controlled crossings using selected male and female plants based on progeny tests, and the evaluation of specific crossings, aiming to set up bi-clonal orchards, is an important strategy on maté tea breeding. In this sense, Cambona-4/pollinator A offsprings identified by molecular markers showed the desired agronomical characteristics, leaf color, leaf brightness, and mild flavor of the maternal parent. Based on these results, an orchard with plants derived from stem cutting of Cambona-4 and pollinator A was formed to obtain bi-clonal commercial seeds.

Maté tea industries in Southern Brazil usually blend the native mate-tea (mild flavor) with cultivated plants (bitterer flavor) to adjust the flavor

to the customer's preference. The native mate trees are extracted from the remaining Araucária Forest still existent in the south of Parana State and some regions in Santa Catarina and Rio Grande do Sul States. The progeny of Cambona-4 may substitute the native plants for mate blends, due to its sensorial characteristics and its genetic stability. This use of Cambona-4 may decrease the demand for exploration of remaining forests, contributing to its preservation. The results of this work contributed for the award received by the project Sistema Agroflorestal Cambona 4 (2nd Brazilian Prize for Environment).

Conclusion

The RAPD marker can be adopted for large-scale screening of hybrids, but maté being heterozygous, markers diagnostic of each male parent are to be determined for each cross. Fingerprinting of seedlings at the juvenile stage by screening with RAPD markers would be practical and of economic significance in a perennial crop like *Ilex paraguariensis*, enabling the elimination of doubtful seedlings and the certification of both plants and product. The results made possible the stabilization of a peculiar genetic material and the creation of orchards for production of seeds of the first bi-parental progeny of *Ilex paraguariensis* St. Hill in Brazil.

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