GENETIC VARIATIONS AMONG PASSION FRUIT SPECIES USING RAPD MARKERS[1]

ANA PAULA DE ANDRADE AUKAR[2], ELIANA GERTRUDES DE MACEDO LEMOS[2], JOÃO CARLOS OLIVEIRA[3]

ABSTRACT - It has been evaluated the genetic variability through the use of RAPD molecular markers on the following passionflower species: *Passiflora amethystina, P. caerulea, P. cincinnata, P. coccinea, P. serrato digitata, P. foetida, P. maliformis, P. alata, P. giberti, P. laurifolia, P. macrocarpa, P. nitida, P. setacea, P. suberosa, P. ligularis, P. capsularis, P. edulis* Sims and its botanical variety *P. edulis* Sims f. *flavicarpa* Deg. In this research work, the analyses of the random amplified polymorphic DNA products (RAPD) were employed to estimate the genetic diversity and the taxonomic linkage within the species above. The total of 21 primers were used in this study which generated 270 different polymorphic products. It was possible to detect that the *Passiflora* species had shown a similarity of 17,3%, and between *Passiflora edulis* Sims and *Passiflora edulis* Sims f. *flavicarpa* a similarity of 34,35% has been found. The rate of similarity within *edulis* specie is low, making it clear that a large variability between the yellow and the purple forms exists.

Index terms: DNA, genetic diversity, passion fruit, Random Amplified Polymorphic DNA

VARIAÇÃO GENÉTICA ENTRE ESPÉCIES DE MARACUJÁ UTILIZANDO MARCADORES RAPD.

RESUMO - Foram avaliadas as variações genéticas através de marcadores moleculares RAPD, as seguintes espécies de maracujá: *Passiflora amethystina, P. caerulea, P. cincinnata, P. coccinea, P. serrato digitata, P. foetida, P. maliformis, P. alata, P. giberti, P. laurifolia, P. macrocarpa, P. nitida, P. setacea, P. suberosa, P. ligularis, P. capsularis, P. edulis* Sims e sua variedade botânica *P. edulis* Sims f. *flavicarpa* Deg. Neste estudo, a análise dos produtos da amplificação ao acaso do DNA polimórfico (RAPD) foi usada para estimar a diversidade genética e as relações taxonômicas entre as espécies. Foram utilizados 21 "primers", que produziram um total de 270 bandas polimórficas. Verificou-se que as espécies de *Passiflora* apresentaram uma média de similaridade de 17,3%, e entre *Passiflora edulis* Sims e *Passiflora edulis* Sims f. *flavicarpa*, de 34,35%. Pode-se perceber que o valor de similaridade dentro da espécie *edulis* é baixo, ilustrando a grande variação entre a forma amarela e a roxa de *Passiflora edulis*.
Termos para indexação: DNA. diversidade genética, maracujá, RAPD.

INTRODUCTION

The Passifloraceae are largely distributed around the tropics (Oliveira, 1987). Over 580 species have been described, the majority originally from tropical America, mainly, Brazil; it is composed of 12 genera; the genus *Passiflora* has the largest number of species, being ninety nine percent originally from the Americas. There is a great genetic variability and they have to be protected and conveniently handled. Snow (1993) mentions that 75 species were studied and their chromosomes were determined as 2n=12 or 18, 2n=14, 20, 24, 27, 36 e 84. The basic number of chromosome should be x =6 and x=9 in the *Passiflora* genus, for this author. Lopes (1994) also points the existence of x=10, besides the ones already mentioned above.

Research priorities to a culture related to its genetics resources has been established in technical reunions (Cunha, 1998), as follows: increase the existent genetic variability in collections, either by collecting the germoplasma, or by action that make possible the interchange among several collections, characterizing and change among several collections, characterizing and evaluating the germoplasma existing in the collections.

In Australia, the vine of *Passiflora edulis* Sims (purple passion fruit) is similar to the one of *Passiflora edulis* Sims f. flavicarpa (yellow passion fruit), except that they are generally more vigorous. The first one has a purple-reddish pigment in the shell of its fruits, stems and leaves, while the second one has a yellow-greenish bark at maturity and the flesh is more acid and aromatic, with a less market flavor compared to the purple passion fruit; the cold tolerance of the purple passion fruit is greater. The yellow passion fruit blossom occurs from 12 to 22 hours, while the purple one occurs from 6 to 12 hours (Winks et al., 1988). This difference in blossoming time may be seen as a form of reproductive isolation between them, indicating an evolution of the flavicarpa form, fitting the precepts of speciation (Futuyama, 1992). In Brazil, the blossom of the commercial and wild types of the yellow and the purple passion fruit occurs around 12 hours and remains opened until dark (Ferreira & Oliveira, 1991).

Modern taxonomists insist that all characters must have the same weight on the classification construction (Priest & Austin, 1993). That's an important premise since the taxons definition should be based on several similarities and not simply created as the result of the taxonomic intuition, it shows a level of similarity or unsimilarity also called genetic distance that can be calculated among taxons. This method put together techniques of molecular markers with computer programs.

The current study intended to evaluate the genetic distance among some species of Passiflora, as well as to accurately compare the species of Passiflora edulis Sims and Passiflora edulis Sims f. flavicarpa Deg, for the current botanical classification of the yellow passion fruit, considered as a variety of the purple passion fruit and to characterize the germoplasma present in the germoplasma bank from FCAV/UNESP.

MATERIAL AND METHODS

Plant material

All the species studied were originated from the germoplasma bank of the Plant Science Department, UNESP- Jaboticabal Campus.

DNA extraction technique

Young leaves were collected in the upper part of adult plants cultivated in the field and used for DNA extraction. The plants were apparently healthy and the DNA extractions were based in the method proposed by Saghai-Maroof et al (1984).

DNA amplification (RAPD)

The Amplification reaction volumes were ol 20 µl, each containing 20 ng of genomic DNA, 2µl of 10X PCR buffer (GIBCO), $MgCl_2$ 1,5mM, 0,4µl of dNTPs (10mM), 1,5 unities of Taq DNA polimerase (GIBCO), 0,25mM of primer, milli Q filtered water q.s.p. 20µl. The tubes were placed in a thermocicler apparatus (PTC-100 Programmable Thermal Controller- MJ Reserch, Inc.) and submitted to a cycle of: 94°C for 1 minute, 92°C for 1 minute, 35°C for 1 minute, 72 °C for 2 minutes, followed by 40 cycles starting in step 2 to 4 and 72°C for 5 minutes. It was analyzed 88 random primers of 10 pairs of bases obtained from the

1 (Trabalho 012/2002). Recebido: 06/02/2002. Aceito para publicação: 10/10/2002.
2 Depto. de Tecnologia, FCAV-UNESP, Jaboticabal - SP, Brazil.
3 Depto. de Fitotecnia, FCAV-UNESP, Jaboticabal - SP, Brazil.
Correspondence: E. G. M. Lemos - Rod. Prof. Paulo Donato Castelani, Km 5 - 14870-000 - Jaboticabal S.P. – Brazil - E-mail: egerle@fcav.unesp.br

University of British Columbia (RAPD Oligo Project) - Biotechnology Laboratory. The amplified samples were submitted to gel electrophoresis in agarose at 1,5%, in 1X TBE buffer, in the Horizon 11-14 (Gibco BRL) system, with a tension of 80V, for approximately two hours, stained with ethidium bromide. The results were analyzed with a photo documentation equipment (Gel Doc- 1000 - Bio Rad) (Figure 1).

Data analysis

Through banding analysis produced by each primer; giving parameter 1 for the presence of band and zero for the absence, it was possible the construction of a binary matrix, analyzed by the Multivariate Analysis System program (NTSYS-pc). The dendrogram was constructed by the Hierarchic Clustering Method UPGMA- "Unweighted Pair Group Method with Arithmetic Mean" [9] through the SHAN program of NTSYS "Numerical Taxonomy and Multivariate Analysis System" – (Rohlf & Slice, 1992) To compute the similarity matrix was used the coefficient of Jaccard (Legendre & Legendre, 1983).

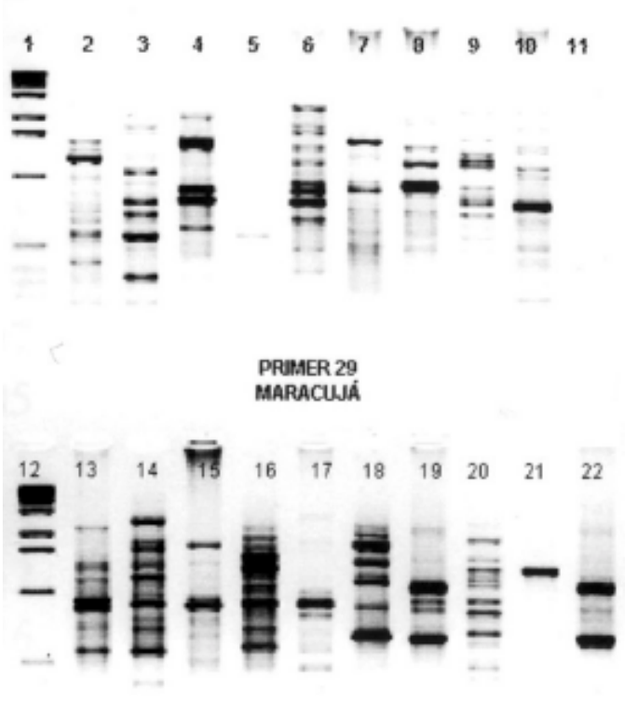

FIGURE 1- Banding patterns of 19 different passion fruit species in a simultaneous PCR using primer 29. The DNA concentration was 20 ng in 20µl reaction mixture.1-Mr standards (1 Kb), 2-*Passiflora amethystine*, 3-*P. caerulea*, 4- *P. cincinnata*, 5- *P. coccinea*, 6- *P. serrato digitata*, 7- *P.edulis* Sims f. *flavicarpa*, 8- *P. edulis* Sims, 9- *P. foetida*, 10- *Pmaliformis*, 11- negative control, 12-Mr=standards (1 Kb), 13- *P.alata*, 14-*P. giberti*, 15-*P. laurifolia*, 16-*P. macrocarpa*, 17-*P.nitida*, 18-*P.setacea*, 19-*P.suberosa*, 20-*P.ligularis*, 21-*P. capsularis*, 22 *P. murcielago*.

RESULTS AND DISCUSSION

Among the 88 primers tested, 21 were selected, and provided the best amplifications. Therefore, these primers were used in the similarity analysis among species. All PCR reactions were carried out in triplicate, and the markers generated in all three reactions were analyzed. From the 21 selected (Table 1), 270 RAPD amplification products (markers) were stably generated, all of them were polymorphic, reflecting the genetic diversity within *Passiflora*, and were therefore included in the analysis. It was also noted that within the *Passiflora edulis* Sims species, the quantity of similar fragments between the yellow and the purple passion fruit was not outstanding, showing a broad genetic basis between them.

According to the study, the primers that turned out to be more specific and able to differentiate the passion fruit species were as follows: 1, 5, 29, 19, 30, 52, 53 and 54. And those that differentiated the yellow passion fruit from the purple were those numbered: 5, 30, 52, 53 and 54. It can be observed that the species *Passiflora amethystina* presented smaller similarity averages, revealing an ampler genetic basis compared to the other species.

It is interesting to point out that the species *Passiflora edulis* Sims and *Passiflora edulis* Sims f. *flavicarpa* Deg are grouped side by side in the dendogram (Figure 2), which is common to occur among related species or within species. Concerning the similarity degree, however, a low value was obtained (34,35%). As we are dealing with the same species, this value should have been greater if a codominant marker is used.

A larger variability was observed studying the genotypes from Australia, being that the difference in blossoming period lead to a possible speciation of this cultivar. Comparing similarity averages obtained within the species *Passiflora edulis* we've found results inconsistent with the usual taxonomic classification, as has occurred with similar study conducted with mango. The similarity among the species was smaller (17,9%), when compared with the similarity within the *Passiflora edulis* Sims species (34,35%).

Several studies have been done using RAPD to verify the polymorphism level in fruits. In a study done by Graham and Mcnicol (1995) two wild species of black raspberries were analyzed *R. occidentalis*, a indigenous species from North America, and *R. leucodermis*, similar to *R. occidentalis*, but that only occurs from British Columbia to California. *R. occidentalis* and *R. leucodermis*, with their slightly difference of geographical locations, grouped together on the dendogram with 50% similarity. Salla et al. (2002) verified that in between the major and the minor of genetic similarity found among acerola varieties was respectively 95% and 57%. In the examples above the level of polymorphism obtained was minor when compared to the *Passiflora* genus.

It would be interesting to develop a similar work with plants from other sources, from Brazil as well as from other countries, to verify the degree of diversity of the purple and the yellow passion fruit. Bhat et al (1995) also demonstrated the complementary nature of different types of molecular markers in gene banks for germoplasma characterization, identification and classification. We have suggested the f-AFLP technique in order to confirm this large genetic variability found within the *Passiflora* genus, as well as, for a possible taxonomic reavaliation.

We may conclude that the study provides a useful tool to discriminate the genotypes, given that some plants of the genus are mistaken in their identity, being the species better characterized in this way.

TABLE 1 - Selected primers list, with their respective sequences and generated number of polymorphic fragment (NPF)

PRIMER	SEQUENCE (5' 3')	NPF
University of British Columbia		
1	CCTGGGCTTC	18
5	CCTGGGTTCC	15
12	CCTGGGTCCA	13
13	CCTGGGTGGA	12
17	CCTGGGCCTC	14
19	GCCCGGTTTA	15
20	TCCGGGTTTG	13
25	ACAGGGCTCA	11
26	TTTGGGCCCA	12
28	CCGGCCTTAA	17
29	CCGGCCTTAC	07
30	CCGGCCTTAG	05
37	CCGGGGTTTT	13
39	TTAACCGGGC	15
41	TTAACCGGGG	12
50	TTCCCCGCGC	16
51	CTACCCGTGC	11
52	TTCCCGGAGC	12
53	CTCCCTGAGC	11
54	GTCCCAGAGC	13
77	GAGCACCAGG	15

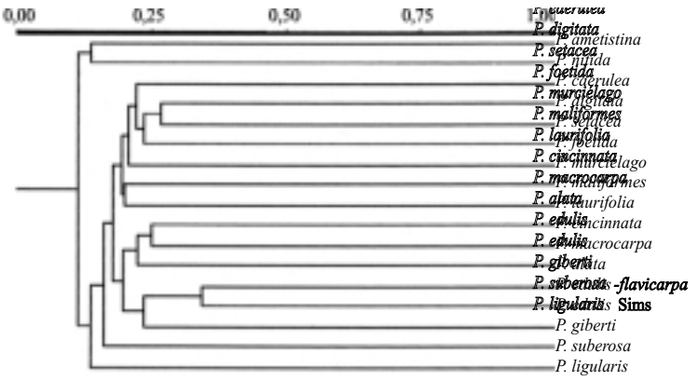

FIGURE 2 - Dendogram of passion fruit species based on 270 polymorphic markers.

REFERENCES:

BHAT, K. V.; JARRET, R. L.; RANA, R. S. DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. **Eletroforesis**, Weinheim, v.16, p.1736-1745, 1995.

CUNHA, M.A.P. da. Prioridades de pesquisa por subárea e objetivo. In: REUNIÃO TÉCNICA: PESQUISA EM MARACUJAZEIRO NO BRASIL, 1997, Cruz das Almas, BA; 1998. p.11-14.

FERREIRA, F. R.; OLIVEIRA, J. C. Germoplasma de Passiflora. In: São José, A. R.; Ferreira, F. R.; Vaz, R. L. **A cultura do maracujá no Brasil.** Jaboticabal: FUNEP, 1991.

FUTUYAMA, D. J. **Biologia evolutiva**. Ribeirão Preto: Sociedade Brasileira de Genética, CNPq, 1992. 631p.

GRAHAM, J.; MCNICOL, R. J. An examination of the ability of RAPD markers to determine the relationships within and between Rubus species. **Theorethical and Applied Genetics**, Berlin, v. 90, p. 1128-1132, 1995.

KAZAN, K., MANNERS, J. M., CAMERON, D. F. Genetric relationships and variation in the *Stylosanthes guianensis* species complex assessed by randonm amplified polymorphic DNA. **Genome**, Ottawa, v.36, p.237-60, 1993.

LEGENDRE, O. L., LEGENDRE,P. **Numerical ecology**. New York: Elsevier, 1983.

LOPES, S.C. Citogenética do maracujá, *Passiflora* spp**.** In: SÃO JOSÉ, A. R. **Maracujá, produção e mercado**. Vitória da Conquista, BA: UESB, 1994. p.19-23.

NEY M. **Molecular evolutionary genetics**. New York: Columbia University Press, 1987.

OLIVEIRA, J. C. Melhoramento genético. In: RUGGIERO, C. **Cultura do maracujazeiro**. Ribeirão Preto-S.P.: Legis Summa, 1987.

PRIEST, F.; AUSTIN, B. **Modern bacterial taxonomy**. London: Chapman &Hall, 1993. 228p.

ROHLF, F. J., SLICE, D. E**. NTSYS:** numerical taxonomy and multivariate analysis sistem. New York: Exater: Software, 1992.

SNOW, N. New chromosome reports in Passiflora (*Passifloracea).* **Systematic Botany**, Oshksoh, v.18, n.2, p.261-273, 1993.

SAGHAI-MAROOF, M. A.; SOLEMAN, R. A.; ALLARD, R. W. Ribossomal DNA spacer lenght polymorphisms in barley: Mendelian inheritance, chromossomal location, and population Dynamics. **PNAS**, v. 81, p. 8014-8018, 1984.

SCHNELL, C. M.; RONNING, R. J.; KNIGHT JR., R. J. Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. **Theorethical and Applied Genetics**, Berlin, v. 90, p. 269-274, 1995.

SALLA, M. F. S.; RUAS, C. F.; RUAS, P. M.; CARPENTIERI-PÍPOLO, V. Uso de marcadores moleculares na análise da variabilidade genética em acerola (*Malpighia emarginata* D. C.). **Revista Brasileira de Fruticultura**, Jaboticabal, v.24, n.1, 15-22, 2002.

WINKS, C. W.; MENZEL, C. M.; SIMPSON, D. R. Passionfruit in Queensland. **Queensland Agricultural Journal**, Brisbane, v. 114, p.217-224, 1988.