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PARENT PAIR ANALYSIS OF CACAO TREES SELECTED IN FARMS FOR RESISTANCE TO *Moniliophthora perniciosa* USING MICROSATELLITES

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The current paternity studies, in special parent pair analysis, were accomplished with promissing selfcompatible cacao selections and others that did not cluster with Sca 6 in previous genetic diversity studies. The molecular markers used, was microsatellites, using polyacrylamide gel, run in ABI Prism 377 sequencer. For the analysis used the Cervus package. The result of this work shows that the farm selections, in spite of many, the resistance is based on Sca 6, Sca 12 and IMC 67 sources. In the other hand, this results reinforce the necessity to include other resistance sources in the Cocoa Research Center breeding program.

Key words: Paternity analysis, population genetics, *Theobroma cacao*, farm selections.

Uso de microssatélites na análise de paternidade de cacaueiros selecionados nas fazendas para resistência a *Moniliophthora perniciosa*. O presente estudo de paternidade foi realizado com seleções de cacaueiros autocompatíveis e outros que não agruparam com Sca 6 em estudos prévios de diversidade genética. Os marcadores moleculares usados foram microsatellites, usando o gel de poliacrilamida, no seqüenciador ABI Prism 377. Para a análise de paternidade usou-se o pacote Cervus. Os resultados deste trabalho mostram que as seleções das fazendas, apesar de muitas, as fontes de resistência estão baseadas em Sca 6, Sca 12 e IMC 67. Por outro lado, esses resultados reforçam a necessidade de incluir outras fontes de resistência no programa de melhoramento genético do cacaueiro do Centro de Pesquisas do Cacau.

Palavras-chave: Análise de paternidade, genética de população, *Theobroma cacao*, seleção nas fazendas.

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Introduction

The cocoa tree (*Theobroma cacao* L.) was introduced in Bahia in 1746 coming from Pará state. Although witches´ broom disease (*Moniliophthora perniciosa*) is known for long date the disease was detected in Bahia in 1989 (Pereira et al. 1989).The eradication of the diseased trees did not give the expected effect. Integrated control measurements such as: chemical, cultural and biological has been adopted concomitant with the use of resistant varieties. However, in some countries that resistance has not been maintained and it has been attributed to the variability of the fungus and the unique resistance source comes from Sca 6 and Sca 12 clones.

In Bahia, the initial planting was made with local materials, being known as "common" material, where the natural selection took place. Those materials although being adapted to local conditions are susceptible to several diseases. Later, the selections were accomplished by researchers but the genetic base is narrow (Yamada et al. 2001). To increase the genetic diversity, clones of Amazonian and other countries were introduced. Increase of the variability with those introductions was demonstrated using RAPD and isoenzymes (Yamada et al. 2001).

The variability present in the field allowed, researchers at Cocoa Research Center (CEPEC) of Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) began to select resistant material to witches´broom in areas of high infection. The first selections accomplished by CEPLAC were coded as VB (stands for vassoura-de-bruxa, in Portuguese) Other selections were made by farmers with the help of researchers and extension service people.

The studies of paternity in VB selected in farms were accomplished at the beginning of the selection work for the resistance to witches´ broom (Yamada and Lopes, 1999). The work brought important information, but it has certain limitations, considering at that time, isoenzymes that was the available markers in CEPEC, and limitations of few isozyme polymorphism in cacao. Also, for the first selections, the informations were preliminary and a lack of more detailed evaluations for yield and resistance to witches´ broom. Today, we have more material proved to be resistant with good yield and some is already recommended to farmers.

The information of paternity is important to identify other source of resistance besides Sca 6 and Sca 12. In the previous study, beyond of these two clones, IMC 67 e PA 150 were identified as the possible parents of those selections (Yamada and Lopes, 1999).

Studies in the farm selections revealed high genetic diversity (Faleiro, F.G. et al.2004, Faleiro, A.S.G. et al. 2004,Yamada et al.2005, Leal, 2004). Observing within of those selections some genotypes did not cluster with Sca 6 and Sca 12, suggesting, different source of resistance. This information would be of great importance to pyramidize genes of different sources of resistance.

The current paternity studies, in special parent pair analysis, were accomplished with promissing selfcompatible cacao selections and others that did not cluster with Sca 6 in previous genetic diversity studies.

Material and methods

Genetic material

The genetic material was divided in 3 sets for the analysis:

Candidate parent (29)

CEPEC 515, CEPEC 523, Csul 3, EET 392, EET 399, ICS 1, ICS 6, ICS 8, ICS 95, IMC 67, Ma 16, P 7, Pa 30, Pa 150, Pa 169, Pa 285, RB 39, Sca 6, Sca 12, SIAL 70, SIC 23, TSA 644, TSA 654, TSA 656, TSH 516, TSH 565, UF 168, UF 613 and UF 667.

Offspring file (9)

America 2, FSU 151, PH 16, SJ 02, VB 184, VB 311, VB 515, VB 547 and VB 900.

Genotype file (47)

Candidate parents (29) + offspring file (9) + clones CCN 10, CEPEC 42, CEPEC 86, EQX 107, RD 01, TSH 1188, VB 1117, VB1139 and VB 1151.

Microsatellites

The DNA extraction of cocoa follow the methodology of Faleiro et al.(2002). The microsatellites primers used were CIRAD 7, CIRAD12, CIRAD15, CIRAD35, CIRAD40 and CIRAD 60 (Table1).

Table 1. Microsatellites primers used, its sequence, and respective number of polymorphic alleles.

Loci	Sequence of primers F and R (5' and 3')	Number of polymorphic alleles
CIRAD 35	TTTCCTTGTATTGACCTA ATATAAACACACTTCAGAGAT	5
CIRAD 12	TCTGACCCCAAACCTGTA ATTCCAGTTAAAGCACAT	7
CIRAD 15	CAGCCGCCTCTTGTTAG TATTTGGGATTCTTGATG	11
CIRAD 7	ATGCGAATGACAACTGGT GCTTTCAGTCCTTTGCTT	11
CIRAD 40	AATCCGACAGTCTTTAATC CCTAGGCCAGAGAATTGA	11
CIRAD 60	CGCTACTAACAAACATCAAA AGAGCAACCATCACTAATCA	10

* Isolated and characterized by LANAUD et al. (1999).

The amplifications reactions were prepared for a total volume of 15 µl, including Tris-HCl 10 mM (pH 8.3), KCl 50mM, $MgCl_2$ 2.4 mM, 150 mM for each one of desoxinucleotides (dATP, dTTP, dGTP and dCTP), 1.5 pM of primers F marked and 1.5 of primers R marked, a unit of enzyme Taq polymerase and 30 ng of DNA.The amplifications were made in termocycler using the following program:4 minutes at 94° C + 10 cycles (30 sec at 94°C + 60 sec at 60°C - 1°C to each cycle + 90 sec at 72° C) + 30 cycles(30sec at 94 °C + 60 sec at 48° + 90 sec at 72° C + 6 minutes at 72 °C. Then, the temperature of the samples was reduced to 4° C.

For loading buffer in polyacrylamide gel were used 250 µL of formamide, 50 µL of gene scan size standard ROX 500 and 25 µl of loading buffer for 100 samples. The multiplex system and details of methodology are described in Yamada et al.2007.

Statistical analyses

For the parent pair analysis used the Cervus package (Marshall et al.1988, Kalinovski et al.2007). For allele frequency analysis and simulation of parentage analysis, used genotype file data, of recorded alleles, and for parent pair analysis (sexes unknown) also used this data and ID of candidate parents and offspring file.

Results and discussion

The 6 microsatellites primers generated 55 alleles. The results (Table 2) shows the possible parents pair for each one of 9 selections. The major contributions as parent of 9 selections appears UF 168 (4 times), SIC 23 (3 times), UF 667, TSH 565, and ICS 8 (2 times). As once time appears IMC 67, TSA 656, TSA 654, Pa 150, Sca 12, UF 613 and TSA 644. For SJ 02 exists 2 sets of potential parent pair because of the same lod score, probably the most probable parent is SIC 23 x TSH 565, considering pods color. The clone TSH 565 was suspected as parents of compatible selection (Yamada et al. 2005).In this studies the Scavina 6 clone do not appear in none of the cases as possible parent and the explanation can be the use of selections that did not cluster with Scavina 6 in genetic diversity studies (Faleiro, F.G. et al.2004, Faleiro, A.S.G. et al. 2004). Besides they would be the grandchildren of Scavina 6 (TSH = Sca 6 x ICS 1 and TSA = Sca 6 x IMC 67) and occur in 5 out of 9 possibilities. Then, this can be the explanation why they did not cluster with Scavina 6 in genetic diversity studies. IMC 67 and Sca 12 can contribute to the resistance in smaller proportion. The unexpected results were PH 16 and VB 184 that both parents ICS 8 and UF 168 is unknown as resistant material.

The clones EET 399, EET 392, RB 39, CSul 3, and Ma 16, known as resistant to witches´broom, in spite

Table 2. Farm selections of cacao trees resistant to *Moniliophthora perniciosa,* potential parent pairs and respective lod score.

Selections code and number	Potential parent pair	LOD score
FSU 151	UF667-TSA 654	(2.39) (1.69)
SJ 02	1) SIC 23-Pa150	(1.88) (2.94)
	2)SIC 23-TSH565	(1.88) (2.94)
VB 311	UF 667-TSH 565	(-1.38) (-1.27)
VB 515	Sca12-UF 613	(-1.19) (1.57)
VB 547	TSA644-SIC 23	(-8.06) (-1.94)
América 2	TSA656-UF168	(2.34) (4.46)
PH 16	ICS8-UF168	(1.6) (4.77)
VB 184	ICS8-UF168	(7.63) (2.58)
VB 900	IMC67-UF168	(-1.09) (6.43)

LOD = decimal logarithm

of they did not participate in hybrid formation, in the past, were included in this work because they could be the parents of open pollinated seeds of the germplasm that were distributed to farmers.

Conclusions

The result of this work shows that the farm selections, in spite of many, the resistance is based on Sca 6, Sca 12 and IMC 67 sources. In the other hand, this results reinforce the necessity to include other resistance sources in the Cocoa Research Center breeding program.

Acknowledgments

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