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RELATIONSHIP BETWEEN MOLECULAR MARKERS AND INCOMPATIBILITY IN *Theobroma cacao* L.

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The objective of the present work was to see the relationship of molecular markers and incompatibility and try to elucidate the mechanism and inheritance of incompatibility. The clone Sca 6 is selfincompatible and ICS 1 selfcompatible and it is expected that F2 of this progenies segregate for selfcompatible plants. Self pollinations were evaluated in 68 F2 plants obtained by selfing TSH 516 (SCA 6 x ICS 1). The pollinations that reached 5% of setting were considered selfcompatible. The markers utilized were, 232 RAPD, 77 AFLP and 33 microsatellites markers in the total of 342 markers. The χ^2 was utilized to test the 3:1 segregation. Regression analysis were utilized to see molecular markers that is correlated to self-incompatible characteristic. From 68 plant evaluations, 25 were selfcompatible and 43 selfincompatible. The χ^2 test indicated 3:1 segregation distortion at 5% level. For the regression analysis using 342 molecular markers, 19 were significative at 5 %. Two of the most significative were sAU17960 and mTcCIR46 , respectively 40% and 17,6% of R^2 .This results showed that probably more than one locus is involved for incompatibility reaction in the case of ICS 1. It is interesting to test these 19 potential candidate in larger populations and also in other populations.

Key words: Cacao, molecular markers, genetic mapping, incompatibility.

Relação entre marcadores moleculares e incompatibilidade em T*heobroma cacao* L. O objetivo do presente trabalho foi verificar a relação entre marcadores moleculares e incompatibilidade e tentar elucidar a herança e o mecanismo de incompatibilidade. O clone Sca 6 é auto-incompatível e ICS 1 autocompatível e é esperado que na F2 as progênies segreguem para plantas autocompatíveis. Foram avaliadas auto polinizações em 68 plantas F2 obtidas por autofecundação de TSH 516 (SCA 6 x ICS 1). As polinizações que alcançaram 5% de vingamento foram consideradas autocompatíveis. Os marcadores utilizados foram, 232 RAPD, 77 AFLP e 33 marcadores de microssatélites no total de 342 marcadores. O χ^2 foi utilizado para testar a segregação 3:1. Análise de regressão foi utilizada para verificar marcadores moleculares que são correlacionados a característica auto-incompatível. Das 68 avaliações, 25 eram autocompatíveis e 43 autoincompatíveis. O teste de χ^2 indicou distorção da segregação 3:1 a nível de 5%. Para a análise de regressão com 342 marcadores moleculares, 19 foram significativos a 5%.Os dois mais significativos foram sAU17960 e mTcCIR46 com respectivamente 40% e 17,6% de R^2. Este resultado mostra que provavelmente mais que um locos é envolvido para reação de incompatibilidade, no caso de ICS 1. Seria interessante testar esses 19 potenciais candidatos em populações maiores e também em outras populações.

Palavras-chave: Cacau, marcadores moleculares, mapeamento genético, incompatibilidade.

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Introduction

The incompatibility in cacao was first verified by Harland in 1925 (Hardy, 1961) and confirmed by Pound (1932) that verified the change of self-incompatibility for selfcompatibility could happen in certain periods of year. The occurrence of cross-incompatibility was demonstrated by Pound (1933) in Trinitarian genotypes and later by Posnette (1945) in Upper Amazon genetic material.

The genetic explanation of the selfcompatibility was proposed for the first time by Knight and Rogers (1955). It was explained as sporophytic system of incompatibility controlled by a simple locus with 5 alleles in the following dominance order S1>S2=S3>S4>S5, being the same order in male or female side. The alleles S2 and S3 are independent. Later, Cope (1962) using some of the same trees of Knight and Rogers and many of the ICS clones found results that it could not be explained by the theory presented. The clones ICS 1 and ICS 45 were selfcompatible and when the two are crossed originated progenies that were selfincompatible. This results was only possible to be explained by 3 independent loci , A and B ,and the other, S locus. The loci A and B affect the expression of self-incompatibility and together they produce one precursor substance of incompatibility. In the absence of that precursor , as in the case of genotype aa or bb the trees become selfcompatible. All the possible combinations of those 3 loci are presented in Bartley and Cope (1973).

The two systems of incompatibility sporophytic and gametophytic involves the inhibition of the pollen in the stigma or in the style. The incompatibility system that happens in the ovary, before or after hybridization is rare in angiosperma and the system of cacao was considered anomalous by de Nettancourt (1977). Seavey and Bawa (1986) describe a system as late-acting but the genetic basis is unknown .Poligenic control has been proposed and it is sometimes confused with endogamic depression.

The clone Sca 6 is selfincompatible and ICS 1 selfcompatible and it is expected that F2 of this progenies segregate for selfcompatible plants. The objective of the present work was to see the relationship of molecular markers and incompatibility and try to elucidate the mechanism and inheritance of incompatibility.

Materials and Methods

The self pollinations were accomplished in 80 F2 plants obtained by selfing TSH 516 (SCA 6 x ICS 1) using technique of Herrania that were used to genetic mapping (Faleiro et al.2006). In fact, the number of plants evaluated was 68 because the difficult of self pollination in some trees. The protection of flowers were made in the previous day performing 40 pollinations per each tree. The pollinations that reached 5% of setting were considered selfcompatible. The technique utilized for pollination and assessment of incompatibility is described in Yamada et al. (1982).

The markers utilized were, 232 RAPD, 77 AFLP and 33 microsatellites markers in the total of 342 markers. The details to the obtaining of these markers are described in Faleiro et al. (2006).

The χ^2 was utilized to test the 3:1 segregation probability. Simple linear regression analysis (Draper and Smith, 1981) using GQMOL packagee were utilized to see molecular markers that is correlated to self-incompatible characteristic.

Results and Discussion

From 68 plant evaluations, 25 were selfcompatible and 43 selfincompatible. The χ^2 test indicated 3:1 segregation distortion at 5% level (Table 1). For the regression analysis using 342 molecular markers, 19 were significative at 5 % being 5 microsatellites, 7 AFLP and 7 RAPD (Table 2). Two of the most significative were sAU17960 and mTcCIR46, respectively 40% and 17,6% of R^2.

The RAPD marker sAU17960 is located at the end of group 4 (Faleiro et al. 2006, Table 2) and most of selfincompatible has Scavina alleles (Figure 1). Also RAPD SAE 18.1095, St12.1320 and AFLP sacacac.311 are located in group 4. At the end of other side of group 4 is located AFLP marker iagccag.177. Philips-Mora and Fritz (1995) mapped incompatibility genes at the end of chromosome 4.

The microsatellites CIRAD 46 indicated that the Scavina-6 allele was correlated with selfincompatible and that heterozygous conditions is correlated with selfcompatible trees (Figure 2) . This marker is located in group 7 (Faleiro et al. 2006, Pugh 2004) and next to

Table 1 - Segregation analyses of cocoa self-incompatibility for selfcompatibility in the F2 population derived from a cross Scavina-6 and ICS-1.

Characteristic tested	Observed ratio	Expected ratio	χ^2	Prob. (%)
self-incompatibility:selfcompatibility	43:25	51:17 (3:1)	5.02	2,5

Table 2. Regression analysis of molecular markers and cocoa self-incompatibility for selfcompatibility in the F2 populations derived from a cross Scavina-6 and ICS-1.

Marker	Type	Prob (F)	BO	B1	B2	R^2 (%)	Linkage group
mTcCIR46	Microsatellite	0.005**	3.54	0.14	-0.76	17.6	7
mTcCIR56	Microsatellite	0.015*	3.49	0.20	-0.64	13.9	7
mTcCIR3	Microsatellite	0.043*	3.34	-0.34	-0.34	11.0	1
mTcCIR6	Microsatellite	0.024*	3.00	-0.33	0.50	12.7	5/6
mTcCIR22	Microsatellite	0.044*	3.00	-0.33	0.42	10.8	
iaagctc.198	AFLP	0.006**	3.16	0.71		12.0	1?
iacacta.283	AFLP	0.002**	3.34	0.58		14.3	D
iagccag.177	AFLP	0.018*	3.21	0.55		8.6	4
saaccag.106	AFLP	0.020*	3.26	-0.49		8.4	B
sacacac.234	AFLP	0.035*	3.25	0.45		6.9	3
sacacac.311	AFLP	0.014*	3.26	0.51		9.2	4
sacccta.287	AFLP	0.037*	3.25	-0.42		6.7	8 A
iAL02.1100	RAPD	0.031*	3.27	-0.46		7.2	1
iY20.800	RAPD	0.023*	3.24	-0.45		7.9	7
iAC01.695	RAPD	0.009**	3.32	0.54		12.4	D
sAE18.1095	RAPD	0.007**	3.28	0.68		15.2	4
sAU17.960	RAPD	0.000**	3.28	1.04		40.0	4
sT12.1320	RAPD	0.001**	3.21	0.75		17.7	4
sZ1.2070	RAPD	0.027*	3.32	-0.51		7.6	B

this markers is located CIRAD 56 that also showed significance in lower level.

This results showed that probably more than one locus is involved for incompatibility reaction in the case of ICS 1. This can be the reason why Cope(1962) could not explain incompatibility results based on Knight and Rogers theory of incompatibility. It is interesting to test these 19 potential candidate in larger populations and also in other populations.

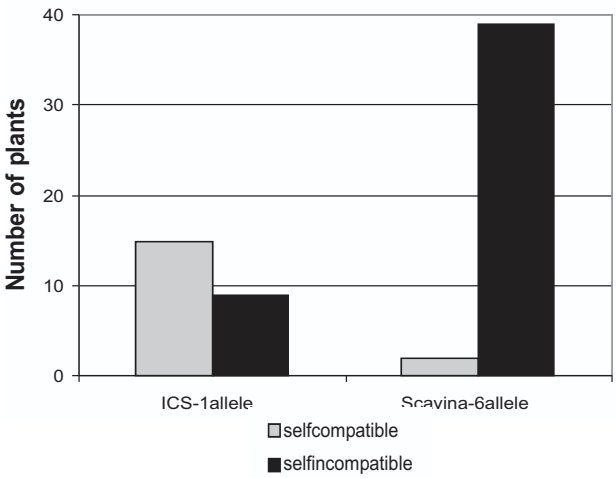

Figure 1. Number of F2 selfcompatible and selfincompatible plants (Scavina-6 x ICS-1) with the presence of ICS-1 and Scavina-6 allele generated by the sAU17960 RAPD marker.

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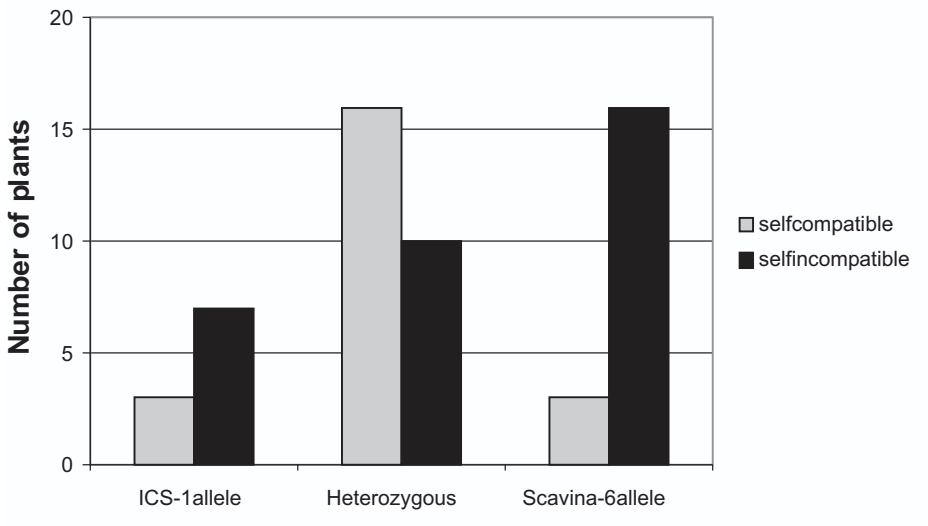

Figure 2. Number of selfcompatible and selfincompatible F2 plants (ISC-1 x Scavina-6) with the presence of ICS-1 allele, heterozygous and with Scavina-6 allele generated by mTcCIR46 microsatellite marker.

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