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ORIGIN OF THE INCOMPATIBILITY ALLELE CARRIED BY THE CACAO CLONE TSH-1188

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The sexual incompatibility is a limiting factor for the expression of the potential yield in cacao. However, because of other genes of interest present in self-incompatible clones (as yield and disease resistance) breeders have frequently used them in their programs. One of these clones is the TSH-1188 which, besides being recommended for planting in Bahia, Brazil, it was widely used in Ceplac´s (Executive Comission of the Cacao Farming Plan) breeding program in the recent past. However, this clone has a rare incompatibility allele of unknown origin. The objective of this paper was to clarify the origin of this allele, by crossing that clone with others in its pedigree, aiming to support studies of gene mapping, as well as planning crosses and establishing this clone in farms. It was shown that the incompatibility allele present in TSH-1188 comes from the clone P-18.

Key words: sexual incompatibility, inheritance, *Theobroma cacao* L.

Origem do alelo de incompatibilidade presente no clone de cacau TSH-1188. A incompatibilidade sexual é um fator limitante da expressão da produção potencial do cacaueiro. Entretanto, por causa de outros genes de interesse presentes em clones autoincompatíveis (como produtividade e resistência a doenças), melhoristas têm frequentemente usado os mesmos em seus programas. Um destes clones é o TSH-1188, que além de ser recomendado para plantio na Bahia, Brasil, foi amplamente usado no programa de melhoramento da Ceplac (Comissão Executiva do Plano da Lavoura Cacaueira) no passado recente. Entretanto, este clone tem um alelo raro de incompatibilidade, de origem desconhecida. O objetivo deste artigo foi esclarecer a origem deste alelo, através de cruzamentos daquele clone com outros em seu pedigree, visando dar suporte a estudos de mapeamento de genes, bem como ao planejamento de cruzamentos e estabelecimento deste clone em fazendas. Mostrou-se que o alelo de incompatibilidade presente no TSH-1188 vem do clone P-18.

Palavras-chave: incompatibilidade sexual, herança, *Theobroma cacao* L.

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Introduction

The cacao clone TSH-1188 (acronym for Trinidad Selected Hybrid) was generated by the breeding program at the Ministry of Agriculture, in Trinidad and Tobago in late 1960s (Gonsalves, 1984). Besides breeding for high yield and other traits, this program focused on the development of varieties resistant to witches´ broom (*Moniliophthora perniciosa*) (reviewed by Maharaj et al., 2011). Considering its high resistance to witches´ broom and good performance at the time, TSH-1188, together with other clones (CEPEC-42, TSH-516, TSH-565, EET-397), were the first to be recommended for planting in Bahia, Brazil, in 1995 (Lopes et al., 2011), few years after the disease arrival in this State, in 1989 (Pereira et al., 1989). Despite the resistance of those clones, all of them are self-incompatible, but compatible among themselves, except TSH-516 and TSH-565 that are cross-incompatible among themselves.

Parallel to the recommendation of those clones, many selections were made by farmers and Ceplac (Executive Comission of the Plan of the Cacao Farming) personnel in plantations, also with emphasis on resistance to witches´ broom (Lopes et al., 2003). Some of them, probably derived from the resistance sources Scavina-6 and 12, largely used as parents of hybrids recommended by Ceplac in the past, considering some paternity analysis (Yamada and Lopes, 1999) and their level of resistance. As the clones Scavina-6 and 12 and others used in Ceplac´s hybrids recommended for planting (IMC-67, Parinari-150, UF-613, among others) were self-incompatible, many of those selections (their descendants) were also self-incompatible. Some of those farmer selections were also widely planted in the region by farmers, as clones. However, considering the high frequency of incompatibility at the time, among the recommended clones and among the farmer selections being planted, Ceplac recommended planting them as a mixture or in alternate rows to increase the chances of successful pollination.

Surprisingly, despite TSH-1188 being self-incompatible, it was shown to be cross-compatible with all tested clones at Ceplac (results not published) as well as with farmers selections. Therefore, this clone was immediately adopted as a pollenizer clone, by many farmers in 1990s in Bahia. It was also observed that consistently this clone was cross-compatible with all farmer selections tested (many of them likely descendent of Scavina-6) and with other clones recommended by CEPEC, indicating that this clone carried out an incompatibility allele of rare occurrence in the Brazilian breeding population. It was also observed in Cepec´s breeding program that, selfed progenies of TSH-1188 segregate for self-compatibility. In those progenies, for example, the later author selected some self-compatible clones (CP-39, CP-40 and CP-50); indicating that TSH-1188 carries one allele of compatibility and another of incompatibility from unknown origin. However, it persisted the doubt about the origin of the incompatibility allele carried by TSH-1188.

Considering the importance of TSH-1188 for studies aiming to localize genes involved in cacao incompatibility (Phillips-Mora et al., 1995; Yamada et al., 2010; Royaert et al., 2011) and for breeding programs, it is important to clarify the origin of the incompatibility allele carried by that clone. This information can also help to plan planting layouts in farmer fields. Considering these aspects, the objective of this study was to clarify the origin of the incompatibility allele present in the clone TSH-1188.

Materials and Methods

The first step in this study was clarifying the pedigree of the clone TSH-1188. Initially, in the absence of published information on the pedigree of TSH-1188 and considering that some other TSH clones introduced in Brazil are progenies of Sca-6 with ICS-1, it was thought these were also the parents of that clone. Other authors have cited TSH-1188 being a descendant of IMC-67, ICS-1, Sca-6 and P-18 (ex., Andrade, 2009). However, checking the unpublished records of the breeding program in Trinidad (Gonsalves, 1984) and a recent publication (Maharaj et al., 2011), it was observed the pedigree of that clone is as shown in Figure 1. Therefore, in the present study, TSH-1188 was pollinated with all the self-incompatible clones involved in its pedigree

Pollinations were made between 22/07/1999 and 19/08/1999 and repeated again in 26/02/2014. At least 20 flowers per cross were protected 24 hours before

the pollination and unprotected 24 hours after the pollination. The number of flowers set was recorded 15 and 30 days after the pollination, and the percent setting estimated, as done in other studies in this area (Terreros et al., 1983; Yamada et al., 1982; 1996).

Results and Discussion

The results of the pollinations are presented in Table 1. Overall, for the pollinations from 1999, the flower set was high when TSH-1188 was crossed with Sca-6 and IMC-67, but no setting was observed when it was crossed with P-18. The same trend was observed in the 2014 pollinations. This suggests that the allele of incompatibility present in TSH-1188 came from the clone P-18. Furthermore, when TSH-1188 was crossed with the self-incompatible clone TSA-641 (=Sca-6xIMC-67) and with TSH-774 (=TSA-641 x Unknown), a 77.5% of flower setting was observed. Also, TSH-774 although not directly involved in TSH-1188 pedigree, but being a sibling of TSH-753 (not present in Ceplac´s germplasm collection) (Figure 1) was also crossed with TSH-1188, resulting in 75,0% of flower setting (Table 1). Therefore, TSH-1188 does not have alleles of incompatibility present in either Sca-6 or IMC-67, further confirming that the incompatibility allele present in TSH-1188 came from the clone P-18.

Many authors have tried to identify incompatibility alleles in several cacao clones (e.g., Arevalo et al., 1972; Yamada et al., 1982; Terreros et al., 1983; Yamada et al. 1996) aiming mainly to predict the performance when crossing them in breeding programs or planning variety mixtures in farmer fields and seed gardens. A new perspective of identifying these alleles, in the genome era, is tagging them (marker-phenotype association) to track them in breeding populations. TSH-1188, despite being self-incompatible, is among the most resistant clones to witches´ broom in Bahia and presents other desirable attributes (Sanches et al., 2008; Lopes et al., 2011; Maharaj et al, 2011). Based on these, TSH-1188 has been one of first clones to be considered in mapping genes of incompatibility in cacao (Andrade, 2009; Royaert et al., 2011), although others have been also being considered (Phillips-Mora et al., 1995; Crouzillat et al., 1996; Yamada et al., 2010). Understanding the origin of that allele can help molecular biologists tagging it in other genetic backgrounds.

Table 1. Number of flowers pollinated and set, and percent of setting when clones in the pedigree of the clone TSH-1188 were pollinated using pollen of that clone, 15 and 30 days after controlled pollination (DAP), per year of pollination

Year	Clone	Flowers pollinated	Flowers Set		Setting (%)	
			15 DAP	30 DAP	15 DAP	30 DAP
1999	IMC-67	20	16	12	80.0	60.0
	P-18	20	0	0	0.0	0.0
	SCA-12	40	20	18	50.0	45.0
	SCA-6	28	10	10	35.7	35.7
2014	IMC-67	40	35	20	87.5	50.0
	P-18	40	0	0	0.0	0.0
	SCA-6	40	32	25	80.0	62.5
	TSA-641	40	31	22	77.5	55.0
	TSH-774	40	30	23	75.0	57.5

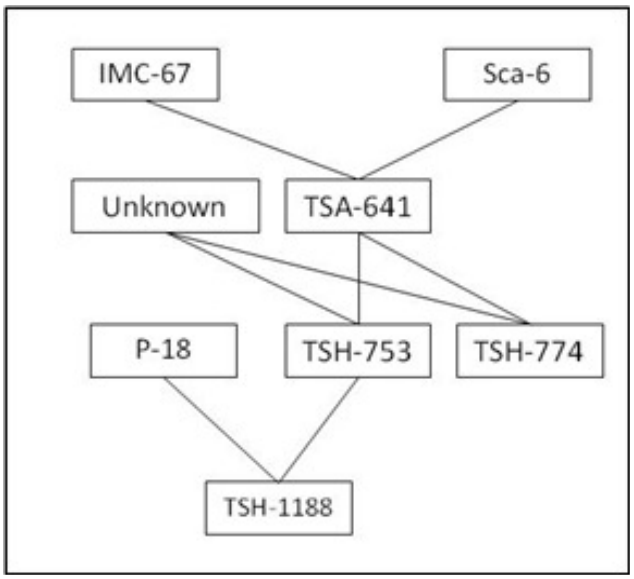

Figure 1. Pedigree of the clone TSH-1188, adapted from Maharaj et al. (2011).

The identification of the origin of the allele as coming from the clone P-18, and not from Sca-6 or IMC-67, explains why TSH-1188 was cross compatible with farmer selections and other Scavina descendents. While Sca-6 and IMC-67 were frequently involved in hybrid (full-sib families) varieties released by Ceplac to farmers before the witches´ broom arrival (Yamada and Lopes, 1999; Faleiro et al., 2004), P-18 was never used. Therefore, it is not expected finding, in farmer

fields, plants with the incompatibility allele of the clone P-18, unless this allele was present in other clones involved in the production of those hybrids.

Conclusions

The incompatibility allele carried by the clone TSH-1188 comes from its parental clone P-18.

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