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Identification of quantitative trait loci linked to Ceratocystis wilt resistance in cacao

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Abstract Ceratocystis wilt (CW) in cacao (*Theobroma cacao* L.), caused by *Ceratocystis cacaofunesta*, is a drastic disease that results in plant death. The pathogen was recently identified in the major cacao-producing region of Brazil–Bahia. The identification of genetic markers tightly linked to disease resistance loci is a valuable tool for the development of resistant cultivars using marker-assisted selection (MAS). Branches of 143 six-year-old individuals of an F2 Sca 6 × ICS 1 population were wounded by making a 3-mm deep cut with a sterile scalpel, and inoculated with a 20-µl drop of a spore suspension of 3×10^4 CFU/ml. The inoculation method used allowed the population to be quantitatively phenotyped. The length of the xylem discoloration followed a continuous distribution. These results imply that the resistance was quantitatively inherited. Quantitative trait loci (QTL) analysis revealed two genomic regions (in linkage groups 3 and 9) associated with CW resistance. The QTL explained individually from 6.9 to 8.6 % of the phenotypic variation. The QTL identified are crucial for identifying genes for resistance and can be applied in the genetic breeding of cacao using MAS.

Keywords *Ceratocystis cacaofunesta* · Marker-assisted selection · Quantitative resistance · *Theobroma cacao*

Introduction

Ceratocystis wilt (CW) is caused by the fungus *Ceratocystis cacaofunesta* (Engelbrecht and Harrington 2005), a specialized pathogen in cacao (*Theobroma cacao* L.). The fungus generally enters cacao plants through fresh wounds, such as those caused by pruning, pod harvesting (Malaguti 1952) or insect wounds, and moves through the host in the secondary xylem, causing wilting and death. The dissemination of the disease is carried out via galleries produced by beetles of the genus *Xyleborus* (Saunders 1965) or injuries caused by cultural practices; in

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the latter case, machete blades are considered an efficient means by which the pathogen spreads (Malaguti 1952).

Ceratocystis wilt (CW) was first reported in western Ecuador in 1918. It caused extensive damage in Colombia after 1940 and in Venezuela in 1958 (Thorold 1975). In Brazil, the disease was first reported in Rondônia (southwestern Amazon) in 1978 (Bastos and Evans 1978) and in the southern region of Bahia (Bezerra 1997). Thereafter, it has emerged as an important disease of cacao in plantations where phytosanitary control measurements, such as machete blade disinfection, are not applied. Although management practices can reduce the severity of the disease, the use of resistant cultivars would be the most effective way to control this disease (Swan et al. 2000). There are still many unanswered questions regarding the sources and mode of inheritance of CW resistance. Phenotypic evaluation of CW resistance has been based on qualitative measures of the disease, therefore hindering the determination of the type of inheritance involved in this pathosystem.

The identification of genetic markers that are tightly linked to disease resistance loci is a valuable tool for the development of resistant cultivars through marker-assisted selection (MAS) that could be applied in the CW resistance breeding program. Several research groups have developed linkage maps for cacao using different mapping populations and different molecular markers (Faleiro et al. 2006; Lanaud et al. 1995; Pugh et al. 2004). The first F2 genetic map in cacao was created in Brazil by CEPLAC by self-pollination of the hybrid-clone TSH 516 [selected in Trinidad and Tobago for witches' broom disease (WBD) resistance] resulting from a cross between Sca 6 (Amazon Forastero) and ICS 1 (Trinitario). Several genomic studies have been carried out with this population (Argout et al. 2011), including quantitative trait loci (QTL) analysis (Lanaud et al. 2009), but, to our knowledge, no QTL studies for CW resistance have been carried out yet.

The objectives of this study were to: (a) test the F2 Sca 6 × ICS 1 population for CW segregation; (b) understand the genetic control for CW resistance and (c) detect the cacao regions of the genome involved with the expressions of CW resistance using segregating F2 Sca 6 × ICS 1 individuals.

Materials and methods

Plant material

One hundred and forty-three F2 Sca 6 × ICS 1 individuals (8 years old), randomly distributed in three blocks in an experimental field of the Cacao Research Center (CEPEC) of CEPLAC in Bahia, Brazil, were used in this study.

Inoculation and phenotyping

Inoculum was prepared from self-fertile, single-peritecia progeny of the isolate Cf 20 grown on PDA medium (2 % potato, 2 % agar and 0.1 % dextrose) at room temperature for 7 days. Each culture was flooded with 10 ml of sterile deionized water and scraped with a sterile spatula. The resulting spore suspension was filtered through four layers of sterile cheesecloth, and was rinsed with an additional 5 ml of water. The spore concentration was estimated with a hemacytometer (Orbeco Inc., Farmingdale, NY, USA), and diluted to 3.0×10^4 CFU/ml. Spore suspensions consisted of cylindrical endoconidia, ascospores, aleurioconidia and hyphal fragments.

Stem inoculations were performed in the field. Stem segments of 1.5–2 cm in diameter were wounded by a sterile dissection scalpel used to make an incision in the middle of the branches (Fig. 1a). Ten microliters of a spore suspension (3.0×10^4 CFU/ml) were deposited in each incision (inoculation site; Fig. 1b). Moistened cotton was placed in the inoculation site (Fig. 1c) and wrapped in parafilm (Fig. 1d). Plants were observed weekly for development of symptoms. Fifteen days after the inoculation (DAI), stems were collected and transported to the laboratory for evaluation. Stems were sliced open vertically above and below the point of inoculation (Fig. 2, arrows), and the length of the xylem discoloration (LXD) recorded (Fig. 2, squared area). Controls were inoculated with distilled water.

The experiment was established under a completely randomized block design, with 149 genotypes (2 parents and 143 F2 progeny plants), with three replications of single-three plots. For each plant, four branches were inoculated and the values averaged out for the analysis. The analysis of variance, to test the effect of genotype, was done

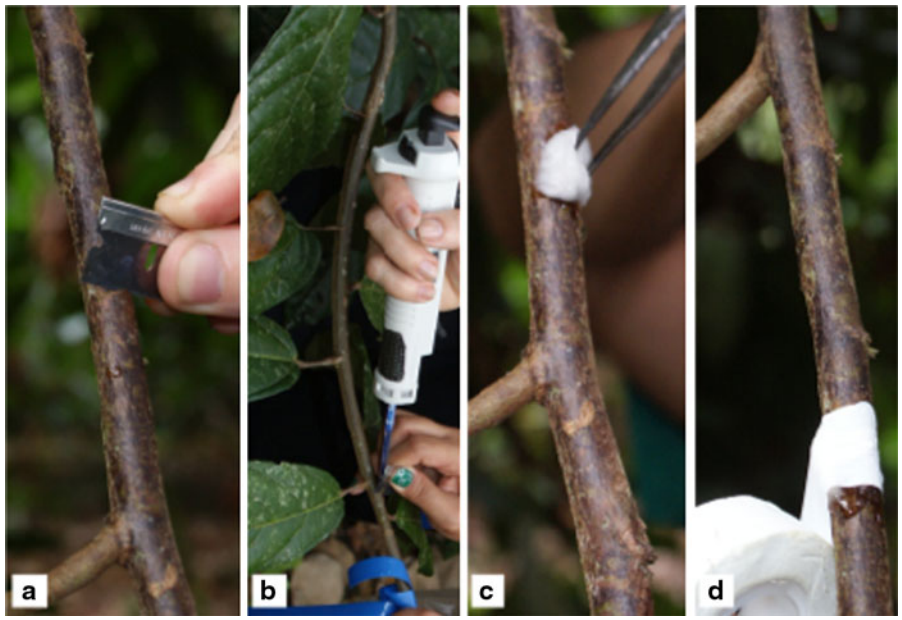

Fig. 1 Detail of inoculation in cacao stems in the field: **a** wounding by scalpel, **b** deposition in the incision, **c** insertion of moistened cotton, **d** wrapping in parafilm

Fig. 2 Stems of cacao plants inoculated with *Ceratocystis cacaofunesta* under field conditions: **a** F2 Sca 6 × ICS 1 progeny inoculated with distilled water, **b** Sca 6, **c** ICS1, **d** F2 Sca 6 × ICS 1. The *arrow* indicates the inoculation site and the *squared area* lesion extension

using Proc GLM in SAS version 9.1.3 [SAS Institute, Cary, NC, USA (2000)]. In order to group the genotypes, a cluster analysis using the Scott–Knott method (Scott and Knott 1974) was performed with the software SASM-Agri version 8.0 (Canteri et al. 2001). The Scott–Knott method was used instead regular multiple comparison methods because of the large number of genotypes involved in this study and the low rate of type I error for large numbers of treatments (Silva et al. 1999).

Genetic linkage map

We used a genotyping matrix used by Brown et al. (2005), with addition of markers from Faleiro et al. (2006), Lima et al. (2010) and Santos et al. (2011, submitted). Briefly, the map was constructed with the Joinmap 4.0 program (Van Ooijen 2004) using 190 molecular markers as follows: 172 microsatellites (Lanaud et al. 1999; Pugh et al. 2004; Risterucci et al. 2000), eight resistance gene homologues (Kuhn et al. 2003), four WRKY genes (Borrone et al. 2004), two expressed sequence tag–simple sequence repeat (EST–SSR) markers from expressed cacao–*Moniliophthora perniciosa* interaction (Lima et al. 2010), and four EST–SSR markers from cacao–*Ceratocystis cacaofunesta* interaction (Santos et al. 2011, submitted). All markers were first screened for polymorphism on the founding parents (Sca 6 and ICS 1) and the F1 population (TSH 516). The average distance between markers is 4.6 cM.

QTL mapping

Associations between molecular markers and resistance to CW were initially evaluated by simple interval mapping (SIM) using the software MapQTL v. 5.0 (Van Ooijen 2006). Significance thresholds were determined by data permutation (Churchill and Doerge 1994) of 10,000 permutations including 5 % chromosome-wide level (LODc, $P < 0.05$), 7 % genome-wide level (LODg, $P < 0.07$) and 5 % genome-wide (LODg, $P < 0.05$). The 5 % chromosome-wide threshold corresponds approximately to the "suggestive linkage" threshold proposed by Lander and Kruglyak (1995) with a 2.5 threshold for different chromosomes. In the second step, a composite interval mapping (CIM) referred to as MQM (Multiple QTL Models, Jansen and Stam 1994) was used after an automatic cofactor selection. A set of two cofactors, mTcCIR135 and mTcCIR108, was retained for the QTL detected on linkage groups 3 and 9; respectively. Kruskal–Wallis (KW) analysis, a single marker analysis, was also employed based on one-way ANOVA. The confidence interval was determined by the LOD-2 method for each QTL. The markers that showed a LOD score higher than the chromosome-wide threshold with $P < 0.05$ were identified as QTL. The estimation of the position, genetic effects and percentage of phenotypic variation explained (PVE) of the QTL were performed at the significant LOD peak in the region under consideration. The proportion of phenotypic variation explained by each QTL was calculated as the R^2 value, and the degree of dominance of a QTL was estimated as the ratio of the dominance to the additive effects.

Results

Phenotypic distribution of cacao resistance to CW

There was significant variation among the genotypes (parents and progenies) in the analysis of variance ($P < 0.0001$, not shown). The phenotypic distribution of LXD was continuous, suggesting that resistance was quantitatively inherited (Fig. 3). Mean parental disease scores were 12.14 ± 1.40 cm for Sca 6 and 29.57 ± 1.83 cm for ICS 1. The mean disease score for the entire population was 14.22 ± 3.5 cm. Individual progenies varied from 4.28 to 33.75 cm (Online Resource 1). Application of the Scott–Knott test resulted in the formation of six groups for CW resistance. The parents were assigned to the second (Sca 6) and fifth (ICS 1) groups (Online Resource 1).

QTL analysis

QTL analysis using SIM revealed two genomic regions located in linkage groups 3 (LXD-LG3) and 9 (LXD-LG9) associated with the expression of the CW resistance (Table 1; Fig. 4) with a LODmax of

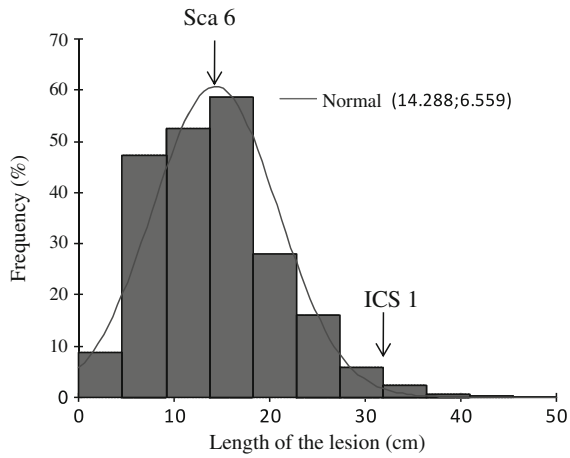

Fig. 3 Histogram of frequency of the F2 Sca 6 × ICS 1 population for ceratocystis wilt resistance

2.73 ($P \leq 0.01$ by KW analysis) and 3.27 ($P \leq 0.005$ by KW analysis), respectively. The QTL in LG3 and LG9 explained 6.9–8.6 % of the phenotypic variation of the LXD, respectively. In regard to the LXD-LG9, the estimated phenotypic means associated with each of the three genotypic classes, homozygous-ICS 1 (mu-A), heterozygous ICS 1/Sca 6 (mu-H) and homozygous-Sca 6 (mu-B), were 16.06, 13.52 and 13.12, respectively. In the LXD-LG3, they were 15.03, 16.55 and 14.15, respectively (data not shown).

For the LXD-LG9, the phenotypic means of genotype classes ICS 1 were greater than the phenotypic means of the Sca 6 genotype, therefore suggesting that Sca 6 alleles are associated with CW resistance. On the other hand, the LXD-LG3 phenotypic means for the heterozygous genotypes were less than the phenotypic means for the ICS 1 genotype, implying that the CW resistance has an association between the alleles. The allelic effect of Sca 6 is favorable to CW resistance and the additive effect was 0.43 (LXD-LG3) and 1.46 (LXD-LG9; Table 1).

Discussion

The dissemination of *Ceratocystis cacaofunesta* in the tropical cacao-producing region of southeastern Bahia is currently a major concern, requiring efforts for breeding elite cultivars resistant to this plant pathogen. Due to the characteristics of CW of cacao, genetically-based resistance is the most appropriate strategy for adequate control of this disease. Therefore, breeding programs need to assess and develop cacao genotypes that are resistant to CW as fast and reliably as possible.

We observed that the population segregated for resistance to CW. The segregation pattern of the trait in the F2 population presented a continuous distribution, suggesting that the CW resistance is controlled by multiple genes rather than a single major gene. Soria and Salazar (1978) reported that the resistance of cacao to CW is probably polygenic and recessive. These results are corroborated by our study regarding the nature of the polygenic trait, but the average LXD of the F2 progeny (14.22 ± 3.5 cm) is closer to the resistant parent (Sca 6; 12.14 ± 3.5 cm), suggesting that CW resistance is probably dominant. Transgressive segregation was also observed in this study, whereas individuals outside the range of parents regarding the trait and statistically different from

Table 1 QTL for cacao resistance to *Ceratocystis cacaofunesta* detected using simple interval mapping (SIM) and composite interval mapping (CIM)

Trait	LG	SIM							CIM						
		LODmax[a]	Flanking markers	Marker	Position	R^{2b}	AE	D	LODmax[a]	Marker	Flanking markers	Position	R^{2b}	AE	D
LXD-LG3*** [c]	3	2.57	mTcCIR254–mTcCIR128	mTcCIR135	78.63	8.1	0.48	2.10	2.73	mTcCIR135	mTcCIR128–SHRSTc7	81.33	7.7	0.43	1.95
LXD-LG9****	9	3.10	mTcCIR266–mTcCIR126	mTcCIR108	8.22	9.6	1.56	−1.08	3.27	mTcCIR108	mTcIR266–mTcCIR126	8.22	9.3	1.46	−1.06

AE additive effect of the QTL, *D* dominance, *LG* linkage group

[a] LOD peak value of the logarithm of the likelihood ratio observed for the QTL

[b] R^2 proportion of the phenotypic variation explained by the QTL

[c] Kruskal–Wallis analysis: ***$P \leq 0.01$, ****$P \leq 0.005$

them were observed (Fig. 4). The transgressive segregation for resistance and susceptibility is also an indication that resistance to CW is governed by several genes of small effect (Hautea et al. 1987). The transgressive segregation is of interest to the breeder because it allows the selection of individuals which segregate with a number of favorable genes higher than the parents (Ramalho et al. 2000).

We attributed this result mainly to the inoculation method used herein which allowed us to quantitatively phenotype the population. Previously, the selection of genotypes resistant to CW (Silva 2005) has been based on a qualitative binary trait based on the mortality and evaluation of young cacao plants, therefore not allowing quantitative evaluation of the disease. We showed that the inoculation of adult tree branches in the field (Fig. 1) plus the evaluation of the LXD (Fig. 2) as proposed by Baker et al. (2003) reduced the variation due to plant age, size of the portion of the inoculated plant and number of replications. Sanches et al. (2008) compared several inoculation methods on young plants and concluded that evaluation of the LXD resulted in the same pattern of response observed with the mortality parameter, regardless of the age of the plants under test. Zauza et al. (2004) studying the pathosystem *C. fimbriata*–eucalyptus found that young plants die more rapidly than adult plants. Therefore, our method in adult plants simulates the actual conditions that occur in the field. This suggests that the method utilized in our study can be employed as an alternative for genotypic comparison. Furthermore, the method can be used in routine breeding, testing and mapping populations without killing valuable phenotypes.

Using 143 genotypes in three replicates and with four inoculated branches for each individual (Fig. 1), we were able to obtain robust phenotypic data to carried out a QTL analysis under favorable conditions. QTL studies aiming to understand the genetic bases of resistance to diseases of cacao have been carried out in recent years. These studies aim to identify and manage more efficiently, through MAS, the accumulation of resistance factors in new varieties. Besides their use in MAS, QTL studies are crucial for identifying genes that underlie trait variation, after cloning them and characterizing their allelic diversity and their effects in the genetic resources.

The results of QTL mapping analyses are indicative of two suggestive regions of the genome, LXD-LG3 (LODmax = 2.73, $R^2 = 7.7$) and LXD-LG9 (LODmax = 3.27, $R^2 = 9.3$), with a positive additive effect of 0.48 and 1.56, respectively, increasing resistance to CW (Fig. 4; Table 1). Lander and Kruglyak (1995) propose the term "suggestive linkage" to allow for the publication of results that are not significant but point to a certain level of association between markers and trait. This term has been adopted to consider suggestive QTL based on chromosome-wide significance, and although the LOD scores of the QTL were lower than the genome-wide significance threshold (3.5 at $P < 0.05$) we believe that the identified QTL are suggestive of significant QTL, considering the following.

1. Chromosome-wide thresholds were already used in several published works. As pointed out by Whankaew et al. (2011), Aslam et al. (2011), Le Roux et al. (2010) and Vuylsteke et al. (2006), QTL that exceed the chromosome-wide *F*-critical threshold at $P < 0.05$ could be considered as significant QTL
2. Using the genome-wide method, the LOD score of LXD-LG9 is 3.27 at $P < 0.08$, which is very close to the critical threshold limit
3. According to Van Ooijen (1999), in the case of F2 segregation data, the threshold for significant QTL is fixed at 2.7. According to this criterion, both QTL LG9 and LG3 could be considered as significant;
4. We also applied to the data a Kruskal–Wallis (KW) test, which is a single marker analysis based on ANOVA (Table 1). Using this method we observed that the identified QTL LG9 showed significance by KW analyses at $P \leq 0.005$ and
5. The phenotype means indicated that one allele combination was markedly better at each location. The genotype classes ICS 1 were greater than the phenotypic means of the Sca 6 genotype, therefore suggesting that Sca 6 alleles are associated with CW resistance. These results are reinforced by the phenotypic disease resistance response. Mean parental disease scores were 12.14 ± 1.40 cm for Sca 6 and 29.57 ± 1.83 cm for ICS 1. Other studies assessing CW resistance have also shown that some Sca descendents are resistant to CW (Silva 2005).

The exact number of loci involved in the expression of this quantitative characteristic is still unknown. The assumption that quantitative inheritance is involved in

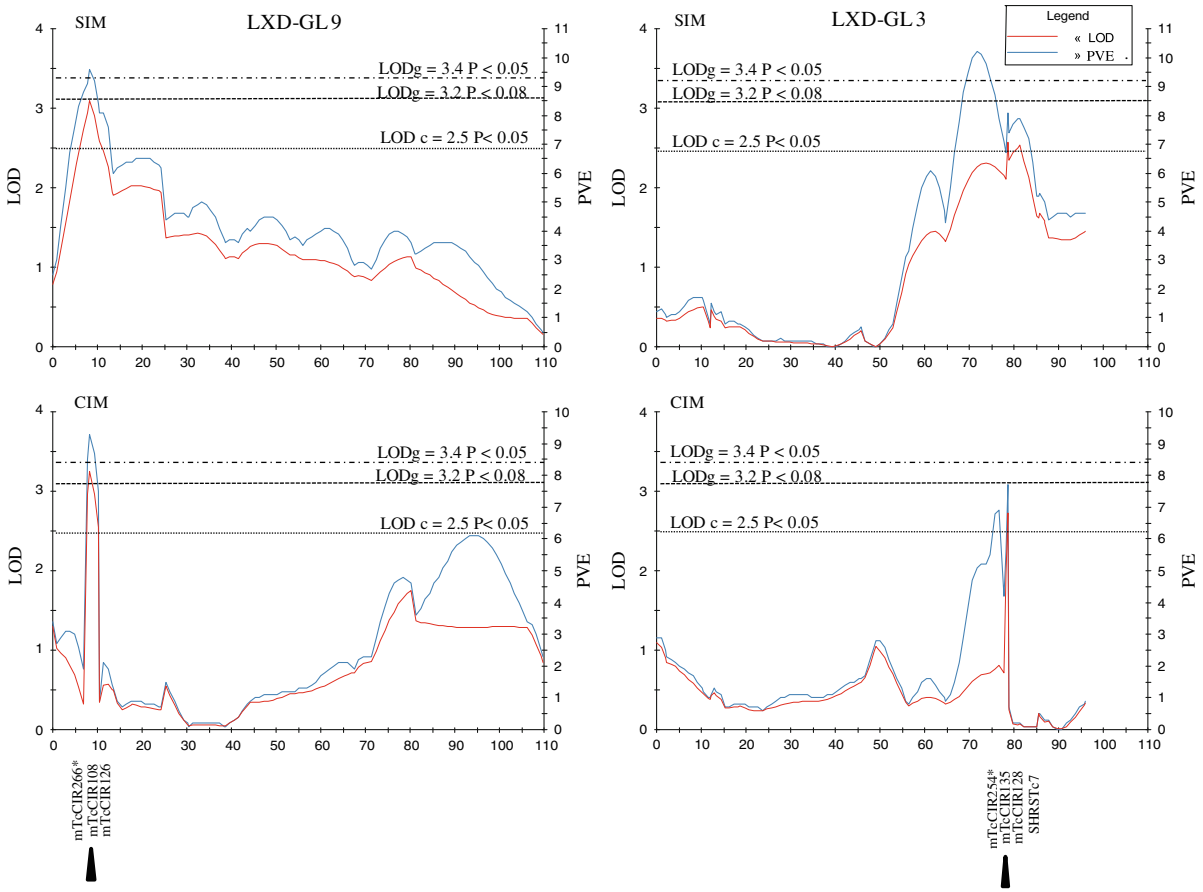

Fig. 4 Graphic display of QTL related to resistance to ceratocystis wilt in cacao by MapQTL 5.0 based on the F2 population of Sca 6 × ICS1. The *x*-axis indicates the relative position in the linkage map. *Red line* indicates the LOD and *blue line* indicates the percentage of the phenotypic variation explained (*PVE*). *LODg* genome-wide significance threshold; *LODc* chromosome-wide significance threshold

the control of resistance to CW is also supported by the identification of QTL explaining a low portion of the phenotypic variation (7.7 and 9.3 %). The variance not explained by the QTL in this study may be due to an undetected small effect QTL or to epistatic interactions between QTL.

In conclusion, the present study presents novel and valuable approaches to studying the cacao–*Ceratocystis cacaofunesta* interaction, which allowed us to: (a) determine the quantitative nature of CW inheritance, (b) identify suggestive regions linked to CW resistance with the aid of functional markers developed by Santos et al. (2011), and (c) develop a methodology that allowed the identification of resistant genotypes in field conditions without killing valuable phenotypes. The identification of two QTL involved in resistance to CW offers the possibility of improving the durability of resistance in cacao by an accumulation of many different resistance genes located in different chromosome regions using MAS. The marker alleles used for the introgression survey on MAS can be also used for characterization of unrelated germplasm and for finding new sources of resistance.

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References

Aslam ML, Bastiaansen JWM, Crooijmans RPMA, Vereijken A, Groenen MAM (2011) Whole genome QTL mapping for growth, meat quality and breast meat yield traits in turkey. BMC Genet 12:61

Baker CJ, Harrington TC, Kraus U, Alfenas AC (2003) Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. Phytopathology 93:1274–1284

Bastos CN, Evans HC (1978) Ocorrência de *Ceratocystis fimbriata* Ell & Halst. na Amazônia Brasileira. Acta Amazonica 8:534–543

Bezerra JL (1997) *Ceratocystis fimbriata* causing death of budded cocoa seedlings in Bahia, Brazil. Incoped Newsl 1:6

Borrone JW, Kuhn DN, Schnell RJ (2004) Isolation, characterization, and development of WRKY genes as useful genetic markers in *Theobroma cacao*. Theor Appl Genet 109:495–507

Canteri MG, Althaus RA, Virgens Filho JS, Gigliot EA, Godoy CV (2001) SASM—agri: sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scott-Knott, Tukey e Duncan. Revista Brasileira de Agrocomputação 1:18–24

Engelbrecht CJB, Harrington TC (2005) Intersterility, morphology, and taxonomy *of Ceratocystis fimbriata* on sweet potato, cacao, and sycamore. Mycologia 97:57–69

Faleiro FG, Queiroz VT, Lopes UV, Guimarães CT, Pires JL, Yamada MM, Araujo IS, Pereira MG, Souza Filho GA, Brown JS, Schnell R, Ferreira CF, Barros EG, Moreira MA (2006) Mapeamento genético molecular do cacaueiro (*Theobroma cacao* L.) e QTLs associados a resistência a vassoura-de-bruxa. Euphytica 149:227–235. doi:10.1007/s10681-005-9070-7

Hautea RA, Coffman WR, Sorrells ME, Bergstron JC (1987) Inheritance of partial resistance to powdery mildew in spring wheat. Theor Appl Genet 73:609–615

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136: 1447–1455

Kuhn DN, Heath M, Wisser RJ, Meerow A, Brown JS, Lopes U, Schnell RJ (2003) Resistance gene homologues in *Theobroma cacao* as useful genetic markers. Theor Appl Genet 107:191–202

Lanaud C, Risterucci AM, N'Goran AKJ, Clement D, Flament MH (1995) A genetic linkage map of *Theobroma cacao* L. Theor Appl Genet 91:987–993

Lanaud C, Risterucci AM, Pieretti I, Falque M, Bouet A, Lagoda PJL (1999) Isolation and characterization of microsatellites in *Theobroma cacao* L. Mol Ecol 8:2141–2152

Lanaud C, Fouet O, Clément D, Boccara M, Risterucci AM, Surujdeo Maharaj S, Legavre T, Argout X (2009) A meta-QTL analysis of disease resistance traits of *Theobroma cacao* L. Mol Breed 24:361–374

Lander ES (1995) Genetic dissection of complex traits guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Le Roux PMF, Khan MA, Broggini GAL, Duffy B, Gessler C, Patocci A (2010) Mapping of quantitative trait loci for fire blight resistance in the apple cultivars Florina and Nova Easygro. Genome 53:710–722

Lima LS, Gramacho KP, Pires JL, Clement D, Lopes UV, Carels N, Gesteira AS, Gaiotto FA, Cascardo JCM, Micheli F (2010) Development, characterization, validation, and mapping of SSRs derived from *Theobroma cacao* L.–*Moniliophthora perniciosa* interaction ESTs. Tree Genet Genomics 6:663–676

Malaguti G (1952) *Ceratostomella fimbriata* en el cacao de Venezuela. Acta Científica Venezolana 3:94–97

Pugh T, Fouet O, Risterucci AM, Brottier P, Abouladze M et al (2004) A new cacao linkage map based on codominant markers: development and integration of 201 new microsatellite markers. Theor Appl Genet 108:1151–1161

Ramalho MAP, Santos JB, Pinto CABP (2000) Genética na agropecuaria. Lavras (ed) UFLA, p 472

Risterucci AM, Grivet L, Ngoran JA, Pieretti I, Flamen MH et al (2000) A high-density linkage map of *Theobroma cacao* L. Theor Appl Genet 101:948–955

Sanches CLG, Pinto LRM, Pomella AWV, Silva SDVM, Loguercio LL (2008) Assessment of resistance to *Ceratocystis cacaofunesta* in cocoa genotypes. Eur J Plant Pathol 122:517–528

Santos RMF, Clement D, Lemos LSL, Legrave T, Lanaud C, Schnell RJ, Pires JL, Lopes UV, Micheli F, Gramacho KP (2011) Identification, characterization and mapping of EST-derived SSRs from the cacao Ceratocystis cacaofunesta interaction. Tree Genet Genomics (submitted)

Saunders JL (1965) The Xyleborus–Ceratocystis complex of cacao. Cacao 10:7–13

Scott AJ, Knott M (1974) A cluster analysis method for grouping means in the analysis of variance. Biometrics 30:507–512

Silva SDVM (2005) Ensaios para avaliação do cacaueiro à murcha-de-Ceratocystis na Bahia, Brasil. In: Proceedings of the 14th international cocoa conference, 13–18 Oct, Cocoa Producers, Alliance, Accra, Ghana

Silva EC, Ferreira DF, Bearzoti E (1999) Avaliação do poder e taxas de erro tipo i do teste de Scott-Knott por meio do método de Monte Carlo. Ciência e Agrotecnologia 23:687–696

Soria J, Salazar G (1978) Pruebas preliminares de resistência a *Ceratocystis fimbriata* em clones e híbridos de cação. Turrialba (Costa Rica) 15:290–295

Statistical Analysis System (2000) SAS user's guide: statistics. SAS Institute, Cary, NC

Swan LJ, Backhouse D, Burgess LW (2000) Surface soil moisture and stubble management practice effects on the progress of infection of wheat by *Fusarium pseudograminearum*. Aust J Exp Agric 40:693–698

Thorold CA (1975) Diseases of cocoa. Oxford University Press, Oxford

Van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624

Van Ooijen JW (2004) JoinMap V4, software for the calculation of genetic maps in experimental populations. Kyazma BV, Wageningen

Van Ooijen JW (2006) MapQTL V5, software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen

Vuylsteke M, Van Den Daele H, Vercauteren A, Zabeau M, Kuiper M (2006) Genetic dissection of transcriptional regulation by cDNA-AFLP. Plant J 45:439–446

Whankaew S, Poopear S, Kanjanawattanawong S, Tangphatsornruang S, Boonseng O, Lightfoot DA, Triwitayakorn K (2011) A genome scan for quantitative trait loci affecting cyanogenic potential of cassava root in an outbred population. BMC Genomics 12:266

Zauza EAV, Alfenas AC, Harrington TC, Mizubuti ES, Silva JF (2004) Resistance of Eucalyptus clones to *Ceratocystis fimbriata*. Plant Dis 88:758–760