Short Communication

Genetic diversity of native cultivated cacao accessions (*Theobroma cacao* L.) in Nicaragua

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Abstract

A total of 60 farmers' cacao accessions (*Theobroma cacao* L.) from Nicaragua were investigated using microsatellite markers to reveal their genetic composition and to identify potentially resistant genotypes against the black pod disease caused by *Phytophthora palmivora*. These accessions were compared with 21 breeders' accessions maintained locally, two Criollo accessions from Costa Rica and two accessions from Ecuador. The analyses showed a low level of differentiation among groups of farmers' accessions ($F_{ST} = 0.06$) and that six Nicaraguan accessions were genetically closely related to the two Criollo accessions used as a reference. In addition, seven distinct genotypes were found to have allelic composition that may indicate linkage to resistance alleles, thus being potential parental lines in future breeding programmes.

Keywords: farmers' and breeders' accessions; genetic differentiation; genetic diversity; microsatellite; *Phytophthora palmivora*; resistance

Experimental

Cacao (*Theobroma cacao* L.) is native to the rainforest from the Amazon basin to southern Mexico (Cuatrecasas, 1964). The traditional classification recognizes three types of grown cacao: Criollo, Forastero and Trinitario. Forastero is the most common, probably from the Amazon basin, while highly valued Criollo presumably originates from Central America. Trinitario found in Trinidad represents hybrids between Forastero and Criollo. However, Motamayor *et al.* (2008) proposed a classification

including ten groups: Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional and Guiana. This study aims to use microsatellite markers for the characterization of Nicaraguan cacao accessions to reveal genetic diversity and relationships among accessions, discover genotypes putatively resistant to the black pod disease caused by *Phytophthora palmivora* (Brown *et al.*, 2007), and identify promising landraces for cacao breeding conducted at the National Institute of Agriculture and Technology (INTA) of Nicaragua.

Sixty trees morphologically resembling Criollo were sampled from Nicaraguan farms to create a national Criollo collection (Fig. 1; see Supplementary Table S1, available online only at http://journals.cambridge.org). Additionally, 14 hybrid and seven other clones (breeders'

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Fig. 1. Sampling regions in Nicaragua. Masaya, Granada and Rivas belong to the region Pacifico Sur. Accession information is given in Supplementary Table S1 (available online only at http://journals.cambridge.org). (A colour version of this figure can be found online at http://www.journals.cambridge.org/pgr)

accessions; see Supplementary Table S1, available online only at http://journals.cambridge.org), conserved *ex situ* at the research station El Recreo, were included in the study. Two samples (Criollo 13 and Yucatan) representing Criollo germplasm obtained from CATIE, Costa Rica, and two samples (ER-011 and ER-012) introduced from Ecuador in 2003 and presumably closely related to Criollo were investigated for comparison (international accessions; see Supplementary Table S1, available online only at http://journals.cambridge.org). Breeders' accession SCA 6 is known to exhibit resistance to black pod (Spence, 1961). All samples are maintained at El Recreo.

Air-dried and fresh leaves were used for DNA extraction (Doyle and Doyle, 1990). Nine microsatellite loci (*mTcCIR1*, *mTcCIR7*, *mTcCIR8*, *mTcCIR10*, *mTcCIR11*, *mTcCIR12*, *mTcCIR15*, *mTcCIR18* and *mTcCIR21*; Lanaud *et al.*, 1999) were used to reveal genetic diversity and relationships among genotypes, and five microsatellites (*mTcCIR61*, *mTcCIR168*, *mTcCIR200*, *mTcCIR225* and *mTcCIR236*; Lanaud *et al.*, 2004; Pugh *et al.*, 2004) to reveal associations with resistance to black pod. PCR products were analysed using the Applied Biosystems 3730 DNA Analyzer, and fragment sizes were determined using Peak Scanner Software 1.0 (Applied Biosystems).

Following diversity parameters were estimated using POPGENE 3.2. (http://www.ualberta.ca/~fyeh/popgene.html): mean numbers of alleles per locus, and mean observed and expected heterozygosities (H_{obs} and H_{exp}). The calculation of the Shannon index (I), the quantification of genetic differentiation by the analysis of molecular variance (AMOVA), the determination of the F-statistics estimator F_{ST} and the detection of Hardy–Weinberg equilibrium (HWE) for all loci with χ^2 tests were conducted using Arlequin 3.11 (http://cmpg.unibe.ch/software/arlequin3/). To reveal relationships between genotypes, the principal coordinate analysis (PCA) was conducted using GENALEX 6.2 (http://www.anu.edu.au/BoZo/GenAlEx).

Among the farmers' accessions, mean allele numbers per locus ranged from 2.8 in Matagalpa to 8.4 in RAAS, and I varied from 0.82 in Matagalpa to 1.81 in RAAS (Table 1). Similarly, H_{exp} was lowest in Matagalpa (0.57) and highest in RAAS (0.82). H_{obs} was lowest in Pacifico Sur (0.34) and highest in Chinandega (0.55). All groups showed heterozygote deficiencies. The international and breeders' accessions had the lowest (0.26) and highest (0.66) H_{obs} values, respectively, although their H_{exp} values (0.75 and 0.78) were similar (Table 1). Altogether, 43% of the loci deviated ($P < 0.05$) from HWE. AMOVA showed that 6.1 and 93.9% of genetic

Table 1. Mean number of alleles per locus (N_a), observed (H_{obs}) and expected (H_{exp}) heterozygosities, and Shannon index (I) in the Nicaraguan farmers' accessions from different regions, and in the international and breeders' accessions of *Theobroma cacao* based on nine microsatellite loci[a]

Groups	Sample size	N_a	H_{obs}	H_{exp}	I
RAAS	14	8.44	0.42 ± 0.08	0.82 ± 0.08	1.81
RAAN	3	3.44	0.44 ± 0.16	0.78 ± 0.10	1.14
Rio San Juan	10	6.22	0.51 ± 0.16	0.72 ± 0.13	1.47
Matagalpa	3	2.78	0.48 ± 0.28	0.57 ± 0.26	0.82
Chinandega	12	5.56	0.55 ± 0.25	0.67 ± 0.17	1.32
Pacifico Sur	18	7.78	0.34 ± 0.09	0.78 ± 0.06	1.66
International accessions	4	4.11	0.26 ± 0.06	0.75 ± 0.10	1.26
Breeders' accessions	21	9.00	0.66 ± 0.08	0.78 ± 0.32	1.66
Mean		5.92 ± 2.35	0.46 ± 0.12	0.73 ± 0.08	1.39 ± 0.32

[a]Standard deviations are shown. Accession information is given in Supplementary Table S1 (available online only at http://journals.cambridge.org).

variation were due to variance among and within the groups of the farmers' accessions, respectively. The mean F_{ST} (0.06, $P < 0.05$) indicated a fairly low level of differentiation.

The PCA results (see Supplementary Fig. S1, available online only at http://journals.cambridge.org) revealed three pairs of the farmers' accessions with similar genotypes (RAAN 0404 and RAAS 0409, MAT 0402 and CHI 0301A, and RSJ 0404 and RSJ 0504). However, based on resistance-associated loci, these genotypes were different. The international Criollo accessions Criollo 13 and Yucatan were very closely related to the farmers' accessions MAS 0402, SJC 0108 and Catarina 0108, and closely related to the farmers' accessions RAAS 0406, RAAS 0410 and RAAS 0414. Furthermore, RAAN 0403 resembled ER-012 from Ecuador. Several breeders' accessions gathered together (SCA 6, SCA 12, EET 62, UF 296, UF 650, IMC 67, UF 654 × Pound 12 and UF 676 × Pound 12), and three farmers' accessions (RSJ 0405, RSJ 0406 and H-3) were closely related to them. Also, accessions RAAS 0403 and UF 296 × Pound 12 were quite similar. The breeders' accession EET 62 was very different when compared with the other accessions.

To identify putative sources of resistance to black pod, genotypes were assessed using five loci linked to resistance and located in three linkage groups (Brown *et al.*, 2007). Based on the comparison, seven genotypes contained at least 80% identical allelic composition with resistant SCA 6, namely the breeders' accessions EET 95 × SCA 6 and EET 399 × Pound 12, and the farmers' accessions RAAN 0401, Mat 0401, EC 0108, Mombacho 0108 and Mombacho 0308 (see Supplementary Table S2 and Fig. S1, available online only at http://journals.cambridge.org).

Discussion

Heterozygote deficiencies detected in the Nicaraguan farmers' accession groups indicate the presence of inbreeding and/or the Wahlund effect, which coincides with previous results on cacao (e.g. Sereno *et al.*, 2006; Lachenaud and Zhang, 2008). Manually pollinated breeders' accessions possess heterozygosities only slightly lower than expected. Furthermore, AMOVA showed little differentiation among the groups of the farmers' accessions. Thus, human influence combined with inbreeding may have mixed the global gene pool of farmers' accessions while causing local homozygosity. However, Trognitz *et al.* (2011) discovered considerable differentiation within a young cacao production area in Nicaragua (Waslala), possibly caused by management and selection driven by varying environmental conditions.

The PCA results indicate that samples most closely related to the Criollo accessions Criollo 13 and Yucatan

are those from Pacifico Sur. Also, some samples from RAAS showed quite close relationships with Criollo. Otherwise, farmers' accessions were dispersed without appreciable grouping by region and with low Criollo background. Additionally, based on comparison with allele sizes found in resistant SCA 6, seven (9%) of the 82 genotypes tested contain ≥80% alleles possibly linked to resistance to black pod that causes great yield losses in cacao production (Brown *et al.*, 2007).

Breeding for disease resistance should be an important component in breeding programmes to enhance the utilization of valuable Criollo germplasm. Our results contribute to this goal by discovering novel germplasm and characterizing Nicaraguan cacao collections for genetic diversity and relationships among genotypes, and for loci presumably linked to quantitative trait loci providing resistance to black pod.

Acknowledgements

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