

Molecular Characterisation of the Cultivar Bourbon L. C.

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SUMMARY

Three different strategies for the characterisation of the cultivar Bourbon LC, a natural somatic variant from variety Laurina, are reported: 1) Random Amplified Polymorphic DNA (RAPD) analysis. From 33 RAPD primers (Operon Technologies Inc.), 292 bands were obtained, 186 of which were either present or absent in different *C. a.* varieties. The multivariate analysis of the electrophoretic profiles allowed for the construction of a dendrogram showing high similarity but distinctness between Bourbon LC and Laurina and adequate distance of the Bourbon LC/Laurina group from the other varieties; 2) subtractive PCR. Bourbon LC DNA was digested by *Bam*HI and following the addition of adapters and competitive amplification (driver DNA: Caturra and Mundo Novo), two specific clones were obtained. Both clones are delimited by *Bam*HI sites, specific for Bourbon LC. 3) Sequence variations of the Internally Transcribed Spacers of the ribosomal genes, ITS1 and ITS2. Bourbon LC ITS1 was sequenced and the spanning of the region defined by comparison to other vegetal species. Bourbon LC and Caturra ITS 2 were cloned and 50 clones sequenced. About 20% of the Bourbon LC clones showed a sequence variation in position 5 in which the normal C was substituted by an A.

The RAPD approach allowed for a general characterisation of Bourbon LC, while the two other experimental approaches allowed for a very specific characterisation of this new coffee cultivar.