VALIDATION OF MOLECULAR MARKER FOR DETECTION OF BARLEY IN COMMERCIAL COFFEE

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The use of DNA markers to identify species is an effective way of ensuring the product quality and to avoid tampering with illegal materials. Coffee is a commodity liable to adulteration with cheap materials such as barley. Given this reality was sought in the online databases GENEBANK an endogenous gene to barley. The aim of this work was to found a DNA sequence as molecular marker. The first step was search for barley genome at GENEBANK, as a taxon gene for barley. The selected gene was submitted to the BLAST program to check the homologyes with other recorded organisms. The genes that showed homologous regions were discriminated in the free online program ClaustalW2 to compare phylogenetic caracteristics. After choosing the gene region, 3 primers pairs were designed using the online program GeneFisher2. For selectivity evaluation, the primers were tested by real-time PCR runs with SYBR GREEN detection system. The primer pairs that presented better selectivity was cevada3, which was assayed for specificity determination by running new reactions with genomic DNA from rice, corn, wheat, coffee, soybeans and barley. The dissociation curve for cevada3 demonstrated that this primer pairs is specific for barley detection. Commercial coffee samples were analyzed for barley presence with spiking samples for matrix effects evaluation. No matrix effects was observed and barley was detected in all samples, presenting limit of detection (LOD) 7,25 µg barley/g coffee. These results showed that barley detection in commercial coffee was feasible, sensitive and specific, being a potential alternative for quality assurance.