Development of a PCR method for the detection of genetically modified constructs in tobacco (*Nicotiana tabacum L.*)

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Abstract

A PCR-based method was developed to detect the presence of regulatory elements in six tobacco samples, consisting of positive control (T_2 KE1CH1 18-4-5), negative control (KE1), two genetically modified samples (T_2 KM10 CH1 17-4-2, T_2 KE1 CH1 18-4-3 from Kutsaga Research Station and two commercial samples (TIMB18 and TIMB 19) from Tobacco Industrial Marketing Board. Plant samples were homogenized and deoxyribonucleic acid (DNA) was extracted using an adapted Cetyltrimethylammonium bromide (CTAB) method. DNA samples were screened for Cauliflower MosaicVirus (CaMV) 35S promoter, Nopaline Synthatase (NOS) terminator and Neomycin Phosphotransferase (NPTII) marker gene under specific reaction conditions of denaturation, annealing, extension for a total of 35 reaction cycles and a post-PCR cycle hold temperature at 72 °C for 7 minutes. Amplification products were electrophoresed in 1.5 % agarose gel for one and half hours along with DNA molecular weight marker IX and stained with ethidium bromide for 25 minutes. The PCR products of 180 bp, 195 bp and 785 bp were obtained for CaMV 35S, NOS and NPTII genes respectively in T_2 KM10 CH1 17-4-2 and T_2 KE1CH1 18-4-3 confirmed the samples were genetically modified as the same size of bands were obtained in a positive control. In commercial samples, TIMB18 and TIMB19 no products were obtained as in negative control, which was not transformed confirming the absence of the above gene, constructs in these samples. These results were consistent with the primer pairs 1-35S/ 2-35S for 35S promoter, 1-NOS/ 2-NOS for NOS terminator and 3-NPTII/ 4-NPTII for marker gene used. The method enabled the detection of GMOs to be carried out and can be used routinely for screening for the presence or absence of genetically modified DNA in tobacco or other agricultural products.