Establishment of Detection Techniques for the Seedlings of Genetically Modified Papayas by the Polymerase Chain Reaction

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ABSTRACT

To detect seeds and seedlings of genetically modified (GM) papaya, we had established a PCR (polymerase chain reaction) detection technique in the first year. In the second year, the Seed Improvement and Propagation Station (SIPS) designed and supplied several steps of blind samples. Primers CPMO, 35S829, 35S463, PAPA215, PRSV322, and so on, for inserted genes and crop endogenous genes in GM papayas were designed according to the gene sequence in the NCBI database. The results revealed that GM and non-GM papaya could be distinguished by the PCR assays. The fragments from coat protein gene of papaya ringspot virus, CaMV 35S promoter, and papain gene could be amplified from the GM samples, and only the papain gene was amplified from the Non-GM samples. The fragments of CaMV 35S promoter and PRSV coat protein gene were amplified by PCR assays even from the 1% GM powder samples by the 35829, 35463 and PRSV322 primers. Primers PAPA215, CPMO, 35S463 , PRSV322, and P4441/P4831 were designed and used in the PCR assays to amplify the papain , CaMV 35S, PRSV coat protein, and NOS genes. No matter which primers were used, the results were the same. It has proved that the detection technique was very reliable.

MATERIALS AND METHODS

1.Dry powder of papaya mixed with 0%, 1%, 3%, 10%, 25%, 50%, 75% and 100% (w/w) GM content were prepared by Seed Improvement and Propagation Station (SIPS)and the DNA was extracted by the DNeasy Plant kit (Qiagen). Primers CPMO and 35S829 specific to the PRSV coat protein gene and CaMV 35S promoter sequence were used in PCR assay.

2. The blind samples were also made by SIPS. Primers PAPAIN215, CPMO and 35S829, which are specific to papain, PRSV coat protein and CaMV 35S genes, were used in PCR assay.

3. Papaya seedling samples obtained from L1, L2 and U1 seedling propagation stations. Primers PAPA215, 35S463 , PRSV322, P4441/P4831 and P4371 /P4780 were designed and used in PCR assays for amplifiing the papain , CaMV 35S, PRSV coat protein, NOS and vector genes.

RESULTS

1.The PCR products were amplified from the DNA of 1-100% GM powder samples by primers CPMO and 35829 in PCR assay(Fig. 1). The PCR products were then eluted and sequenced. Sequence of the fragment was identified to the CaMV 35S promoter and PRSV coat protein genes.

2.Blind sample tests：According to PCR assay, the 3rd-6th、11th-14th samples are normal papaya (Fig. 2a) and the 4th, 5th, 12th, 13th lane are GM papaya(Fig 2b). These results were the same as the answers. The 4th and 12th samples had just 1% GM leaves powder of papaya.

3. The L1 and L2 samples are GM papaya and the U1 samples are Non-GM papaya via double check by Hualien DARES and SIPS. In our PCR assays, no matter which primers were used, the results were the same. When primers PRSV322 were used, the PCR products were clearer in the non-GM samples than other primers in PCR assay (Fig. 3).

Fig.1. PCR products amplified from PRSV coat protein gene of 1-100% GM papaya samples with primers CPMO. Lane M：100bp DNA ladder
Lane 1：100%GM 2：75%GM 3：50%GM 4：25%GM 5：10%GM 6：5%GM 7：3%GM 8：1%GM 9：0%GM 10:H_2O

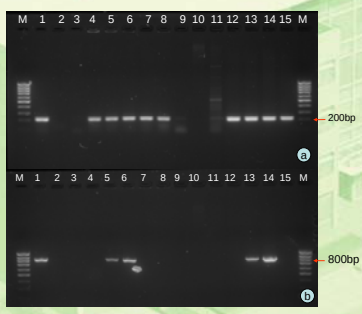

Fig. 2. PCR products amplified from papain and CaMV 35S sequence of blind samples of papaya with primers PAPA215(a)、35829(b)
Lane M: 100bp DNA ladder
Lane 1：100%GM papaya lane 2-7：blind samples 1-6 lane 8：NON-GM papaya lane 9：H_2O Lane 10-15： blind samples 1-6

Fig. 3. PCR products amplified from papain, CaMV 35S, PRSV coat protein, NOS and inserted Vector genes of papaya samples with primers PAPA215(a), 35S463 (b), PRSV322 (c) ,P4441/P4831(d), P4371/P4780(e) respectively
Lane M: 100bp DNA ladder,
L1 ,L2 and U1: papaya samples.
PCK: positive control (GM papaya)
CK: negative control (Non-GM papaya)
H: H_2O
1,2 and 3:different papaya seedlings from the sampling population.

MAIN REFERENCES

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