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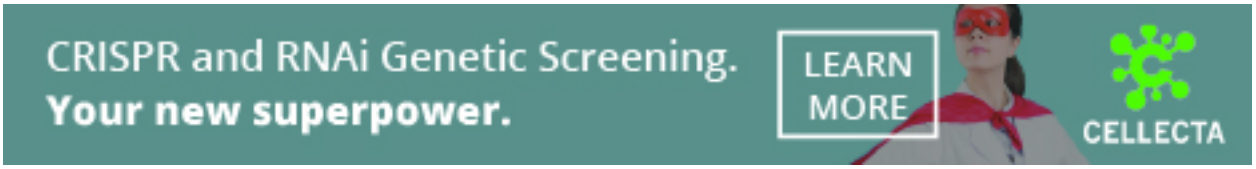

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The Sequence within Two Primers Influences the Optimum Concentration of Dimethyl Sulfoxide in the PCR

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We used the polymerase chain reaction (PCR) to examine extracts of transgenic tobacco plants for the presence of foreign DNA sequences. During this work, information regarding the effect of dimethyl sulfoxide (DMSO) in the PCR was obtained.

RESULTS AND DISCUSSION

The amplification reactions used the same two primers to detect two proteinase inhibitor cDNA clones [rice oryzacystatin-1 (OC-1) and 12K-corn inhibitor (12K-CI)], each cloned between the cauliflower mosaic virus (CaMV) 35S promoter and *nos* 3′ regions. Neither DNA sequence was detected in transgenic plant DNA in the absence of DMSO. The use of DMSO has been proposed in certain PCR applications.[1–3] However, we found that the optimum DMSO concentration required depended on the template. Increasing concentrations of DMSO were tested in the amplification reactions. Figure 1 shows that amplification of the two specific DNA sequences had different DMSO optima (4% for OC-1, 8–10% for 12K-CI). Amplification of OC-1 occurred at a lower concentration of DMSO and with a wider range of DMSO concentrations than amplification of 12K-CI. The apparent inhibitory effect of increasing DMSO concentrations in the PCR (Fig. 1) is presumably due to the inactivation of *Taq* DNA polymerase.[1] Variable concentrations of DMSO in the PCR also affect product specificity. A mixture of equal amounts of total DNA from a plant transformed with OC-1 and a plant transformed with 12K-CI was used as a PCR template. Only the PCR product of OC-1 was detected at 4% DMSO but products of both OC-1 and 12K-CI were detected at 9% DMSO (data not shown).

Several mechanisms of PCR enhancement with DMSO have been suggested. DMSO may affect the T_m of the primers, the thermal activity profile of *Taq* DNA polymerase, and the degree of product strand separation achieved at a given denaturation temperature.[1] The first two mechanisms seem unlikely with our materials because different DMSO effects were observed with two DNA sequences, even though the same temperature profile and primers were used. The effects of DMSO that we observed may be related in part to the destabilizing influence of DMSO on dsDNA. The presence of a high GC ratio would stabilize dsDNA in both PCR products and intramolecular secondary structures, and could inhibit the PCR. Both of the cDNA clones in this study contain relatively long GC-stretches.[4,5] The GC ratio of the 12K-CI protein coding sequence (0.465 kb) ex-

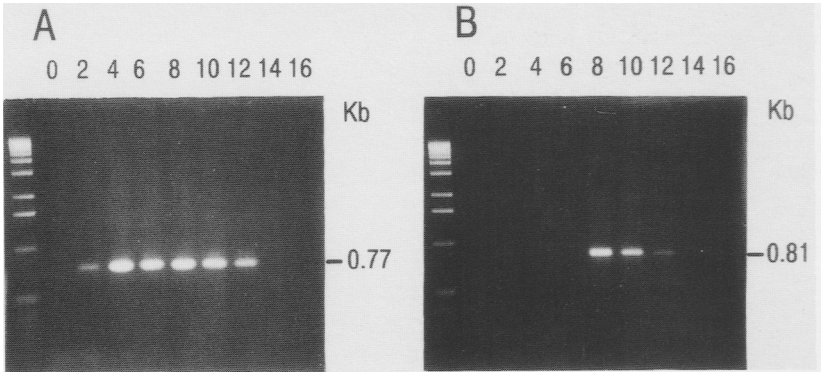

FIGURE 1 Effect of DMSO concentration on detection of two foreign chimeric genes by PCR. DNA of transgenic tobacco plants was extracted as described by Rogers and Bendich.[7] Template DNAs (200 ng) from two plants, transformed with either OC-1 (*A*) or 12K-CI (*B*), were used. DMSO concentrations (vol/vol) as indicated above each lane were added to 30-μl total reaction volumes containing KCl (50 mM), Tris-HCl (pH 8.5) at 25°C (10 mM), $MgCl_2$ (1.5 mM), 0.1% Triton X-100, dNTP (each 200 μM), and primers (each 1 μM). Oligonucleotide primers used (5′ CGC TGA AAT CAC CAG TCT CTC-3′ and 5′-CAT CGC AAG ACC GGC AAC AGG-3′) were from the transcribed sequences of the CaMV 35S promoter and *nos* 3′ regions flanking the two genes.[8] After denaturation at 94°C for 5 min and cooling at 65°C, 0.5 units *Taq* DNA polymerase (Promega, Madison, WI) was added. Thirty repetitive cycles for denaturation (94°C for 30 sec), annealing (58°C for 30 sec), and extension (72°C for 1 min) of DNA were employed. Reaction products were separated in 1.4% agarose and stained with ethidium bromide. The size markers (left lanes) are from a 1-kb ladder (GIBCO BRL, Gaithersburg, MD).

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ceeds 70%,[5] as does the 5′-untranslated region of the OC-1 gene.[4] The inclusion of 10% DMSO during sequencing dsDNA may decrease the stability of intramolecular secondary structures. [6]

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