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Optimizing the efficiency of the touchdown technique for detecting inter-simple sequence repeat markers in corn (*Zea mays*)

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ABSTRACT. We evaluated the efficiency of the touchdown method to determine the ideal PCR conditions for distinct inter-simple sequence repeat primers for processing DNA from common corn, popcorn, sweet corn, and a *Tripsacum*-maize hybrid. Genomic DNA was extracted from eight accessions of corn: two of the dent type, one *Tripsacum*-maize hybrid, one sweet corn, one flint-type corn, and three popcorn. Fifteen inter-simple sequence repeat primers were used: $(CT)_8RC$, $(CT)_8TG$, $(GA)_8T$, $(GA)_8YC$, $(CTC)_5RC$, $(GTC)_6$, $(GA)_6CC$, $(GT)_6CC$, $(CAC)_3GC$, $(AG)_8YT$, $(AC)_8CT$, $(AC)_8YG$, $(CT)_8RG$, $(GGAT)_3GA$, and $(GAA)_6AA$. The annealing temperature and the melting temperature for each primer were estimated using a formula for RW Genes products, or we used the temperatures indicated by the manufacturer (Invitrogen). The touchdown method was then applied to each primer, varying the number of final cycles (10 or 12) and the decrease in temperature (0.5º or 1.0ºC intervals). The gels were compared, considering the revelation quality, band sharpness and the number of bands visualized. The touchdown-PCR method was more efficient for band amplification for most of the primers, especially at higher annealing temperatures.

This type of system is useful for reducing the resources, time and effort needed for optimizing temperature conditions for a group of representative primers.

Key words: PCR; Method evaluation; Molecular marker; Laboratory optimization; ISSR

