

Utility of the PCR methodology in detection of crop alleles in weedy species has the potential for improvement through techniques that improve efficiency and minimize the cost and time required. This study evaluated the multiplex PCR procedure in concurrent detection of multiple crop alleles in wild sorghum populations and interspecific hybridization. Crop loci were amplified from seed and leaf samples of *S. halepense*, *S. bicolor*, *S. sudanense*, *S. bicolor* ssp. *verticilliflorum* open pollinated populations. Simultaneous amplification of combinations of loci SB1764, SB3420, SB5058 and SB5458 in the accessions gave the expected DNA banding profile. Loci combinations involving SB1764/SB5058, SB3250/SB1764 and SB1764/SB3008/SB5058 were important in determining polymorphism and interspecific hybridization within these species. Multiplex PCR reduced 2 loci and 3 loci assay time from 31-15.5 and 46.5-15.5 h, respectively. Multiplex PCR was useful in evaluation of the parental, F1, F2 and BC1. Densitometric analysis of PCR fragments showed that amplifications from 35-50 ng of template had the best yield. *Sorghum sudanense* had higher affinity towards hybridization with the crop (45-76%) as well as the weedy materials (59-61%). Therefore, *S. sudanense* and its interspecific progeny seem to be an important bridge species in the sorghum genus.