Application of high resolution melting for detection of induced DNA polymorphism in Curcuma alismatifolia

Abstract

In order to reveal the induced variations in genomic DNA of mutants, simple sequence repeats (SSR) are used in plant genetics and breeding programs, which are commonly analyzed by fragment size separation using gel electrophoresis. However, post-PCR handling processes are laborious and costly. In addition, gel electrophoresis based methods cannot detect SNPs present in the sequences flanking repeat motif. High resolution melting curve (HRM) analysis is a new technique, which is efficient, accurate, and cost-effective in detecting the sequence differences in polymerase chain reaction (PCR) amplicons, even single nucleotide polymorphisms (SNPs) or insertions or deletions (INDELs). In present study, we used the HRM followed by DNA sequencing to discriminate induced microsatellite polymorphism among irradiated and non-irradiated individuals of Curcuma alismatifolia in third generation (M1V1). The results showed that the combination of HRM with sequence confirmation is a powerful high-throughput, accurate, and reproducible approach to detect induced SSR polymorphism among mutants of C. alismatifolia. For the mutants with SNPs polymorphism present in the flanking region, HRM also gave distinct melting curves, which the gel electrophoresis was not able to detect. In conclusion, it has been approved that HRM as pre-sequencing, efficient, and cost-effective screening method enables rapid SSR polymorphism detection in mutant population of C. alismatifolia.

Keyword: Curcuma alismatifolia; DNA polymorphism; Gamma rays; High resolution melting curve; SSR