

TILLING, high-resolution melting (HRM), and next-generation sequencing (NGS) techniques in plant mutation breeding

ABSTRACT

Induced mutations have been used effectively for plant improvement. Physical and chemical mutagens induce a high frequency of genome variation. Recently, developed screening methods have allowed the detection of single nucleotide polymorphisms (SNPs) and the identification of traits that are difficult to identify at the molecular level by conventional breeding. With the assistance of reverse genetic techniques, sequence variation information can be linked to traits to investigate gene function. Targeting induced local lesions in genomes (TILLING) is a high-throughput technique to identify single nucleotide mutations in a specific region of a gene of interest with a powerful detection method resulted from chemical-induced mutagenesis. The main advantage of TILLING as a reverse genetics strategy is that it can be applied to any species, regardless of genome size and ploidy level. However, TILLING requires laborious and time-consuming steps, and a lack of complete genome sequence information for many crop species has slowed the development of suitable TILLING targets. Another method, high-resolution melting (HRM), which has assisted TILLING in mutation detection, is faster, simpler and less expensive with non-enzymatic screening system. Currently, the sequencing of crop genomes has completely changed our vision and interpretation of genome organization and evolution. Impressive progress in next-generation sequencing (NGS) technologies has paved the way for the detection and exploitation of genetic variation in a given DNA or RNA molecule. This review discusses the applications of TILLING in combination with HRM and NGS technologies for screening of induced mutations and discovering SNPs in mutation breeding programs.

Keyword: Mutation breeding; Mutagenesis; EMS; TILLING; High resolution melting; Next-generation sequencing; SNPs