Gilchrist E.J., C.H.D. Sidebottom, C.S. Koh, T. MacInnes, A.G. Sharpe, G.W. Haughn. 2013. A Mutant *Brassica napus* (Canola) Population for the Identification of New Genetic Diversity via TILLING and Next Generation Sequencing.,DOI: 10.1371/journal.pone.0084303

Abstract

We have generated a *Brassica napus* (canola) population of 3,158 EMS-mutagenised lines and used TILLING to demonstrate that the population has a high enough mutation density that it will be useful for identification of mutations in genes of interest in this important crop species. TILLING is a reverse genetics technique that has been successfully used in many plant and animal species. Classical TILLING involves the generation of a mutagenised population, followed by screening of DNA samples using a mismatch-specific endonuclease that cleaves only those PCR products that carry a mutation. Polyacrylamide gel detection is then used to visualise the mutations in any gene of interest. We have used this TILLING technique to identify 432 unique mutations in 26 different genes in *B. napus* (canola cv. *DH12075*). This reflects a mutation density ranging from 1/56 kb to 1/308 kb (depending on the locus) with an average of 1/109 kb. We have also successfully verified the utility of next generation sequencing technology as a powerful approach for the identification of rare mutations in a population of plants, even in polyploid species such as *B. napus*. Most of the mutants we have identified are publically available.