Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8025

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2020) *8*(2), 219-223

Review Article

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

TILLING - Reverse Genetics Technique for Identification of Mutation in Plants

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Received: 8.03.2020 | Revised: 13.04.2020 | Accepted: 17.04.2020

ABSTRACT

TILLING is widely used in plant functional genomics. Mutagenesis and SNP detection is combined to allow for the isolation of mutations in genes of interest. It can also be used as a plant breeding tool, whereby variation in known or candidate genes of interest to breeding programs is generated. Here we describe a simple low-cost TILLING procedure.

Tilling is a reverse genetic strategy which mutation is induced and followed by high throughput screening of point mutation in gene of interest. TILLING provides a powerful approach for gene discovery, DNA polymorphism assessment, and plant improvement. It is a rapid and low-cost method. Coupled with other genomic resources, TILLING and EcoTILLING can be used immediately as a haplotyping tool in crop improvement for identifying allelic variation in genes exhibiting expression correlating with phenotypes.

Keywords: *Reverse genetics, TILLING, Mutation, EMS,*

INTRODUCTION

Forward genetics is the molecular genetics approach of determining the genetic basis responsible for a phenotype. Forward genetics methods begin with the phenotype and leads to identification of mutation in the specific gene and to link this mutation to the phenotypic. Whereas, Reverse genetics strategy is used to help understand the function of a gene by analysing the change in phenotypic effects of specific gene after alteration of gene structure or its activity. Several reverse genetics technologies, such as insertional mutagenesis with T-DNA, transposon/ retrotransposon tagging or gene silencing using RNA interference, have been proposed for plant functional genomics. TILLING (Targeting Induced Local Lesions in Genomes), is one of the reverse genetic tool which combined traditional chemical mutagenesis with high-throughput screening for identification of single nucleotide polymorphism. Mutagens induced series of allelic variation followed by High-throughput screening allows the rapid and cost-effective detection of induced point mutations in populations of chemically mutagenized individuals.

Cite this article: Chittora, K. (2020). TILLING - Reverse Genetics Technique for Identification of Mutation in Plants, *Ind. J. Pure App. Biosci. 8*(2), 219-223. doi: http://dx.doi.org/10.18782/2582-2845.8025

TILLING method potentially applicable to any organism that can be mutagenized. Also, species which transgenic methods are limited or not applicable can be used in TILLING applications.

McCallum et al. (2000) first developed protocol of TILLING to screen point mutation in *Arabidopsis thaliana*.

Protocol of TILLING procedure in plant

1. Developing a Mutagenized Population

- Seeds are treated with chemical mutagen such as EMS.
- Treated seed are grown out to produce M1 plants, which are subsequently self-fertilized to produce the M2 generation.

2. DNA Pooling

- Leaf tissues from M2 plants are collected for DNA extraction and then used for mutational screening.
- DNA is extracted from the mutant population, the DNA is normalized and pooled together.

3. Detection of mutations in a targeted sequence

- The targeted gene is amplified using a forward primer with 700 nm dye label and a reverse primer with an 800 nm dye label attached to the 5' ends.
- The PCR products are heated and cooled to form heteroduplexes between the accessions in the pool.
- The resulting pool will contain a mixture of homoduplexes and heteroduplexes. Any mismatches (SNPs or small INDELS) will be detected by a mismatch endonuclease (CEL I) and cleaved into two separate products, which will be detected in the 700 and 800 dye channel of a LI-COR DNA Analyzer.

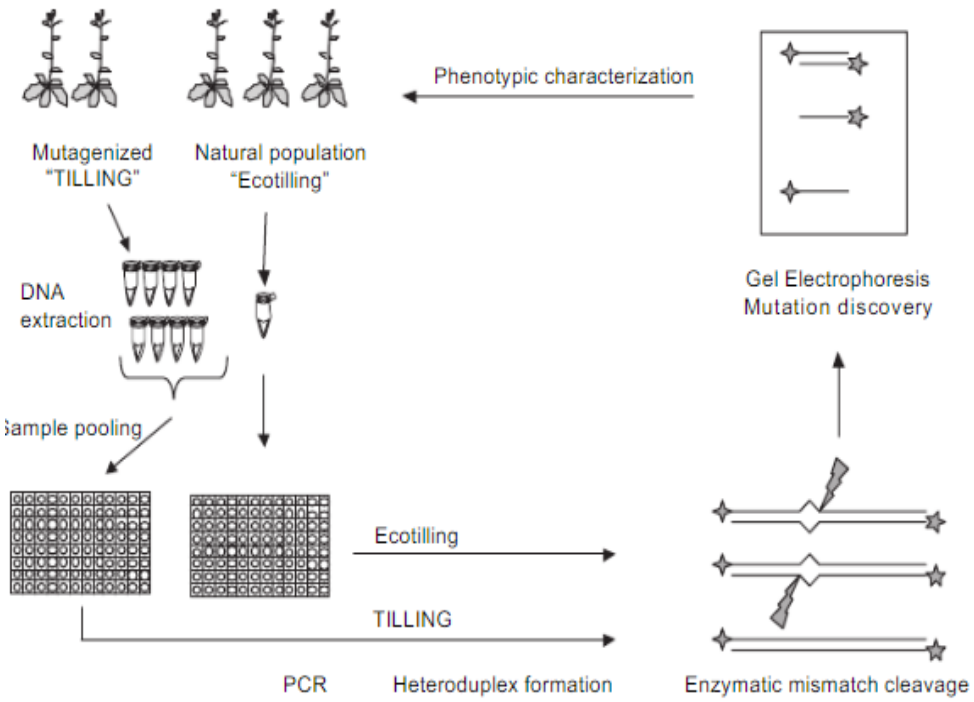

Fig. 1:

Figure 1. Outline of the basic steps for typical TILLING and EcoTILLING assays. DNA is collected from a mutagenized population (TILLING), or a natural population (EcoTILLING). For TILLING,DNAs from up to eight individuals are pooled. Typical EcoTILLING used to discover rare natural single-nucleotide changes (Till et al., 2006). After extraction and pooling, samples are typically arrayed into a 96-well format. The target region is amplifiedby PCR with gene-specific primers that are end-labeled with fluorescent dyes. Following PCR, samplesare denatured and annealed to form

heteroduplexes that become the substrate for enzymatic mismatchcleavage. Cleaved bands representing mutations or polymorphisms are visualized using denaturingpolyacrylamide gel electrophoresis. Plants with mutations predicted to affect protein function can becarefully analyzed for phenotypic abnormalitiesin

DNA samples and seeds collected from a large M_2 population are archived and put into databases to create TILLING platforms. Usually, platforms of 3,000–5,000 M_2 individuals are created, although larger populations that include 10,000 plants have also been reported. Once established, the TILLING platform can serve as a permanent source of mutations for both forward and reverse genetics. To facilitate the systematic gathering of information about the developed platform, including molecular and phenotypic data, TILLING databases are created and often made publicly available.

Mutagens used to create mutation in different tilling population: Mutagens such as ethyl methane-sulphonate (EMS), cause stable point mutations and thus produce an allelic series of truncation and missense changes that can provide a range of phenotypes. (Till et al., (2007). Chemical mutagenesis causes both point mutations, which are irreversible and produced in relatively high densities, and also chromosome breaks that cause various chromosomal rearrangements, which can reduce fertility and affect lethality. In the majority of TILLING experiments EMS has been applied as a mutagen. Used of other mutagens as *N*-methyl-*N*-nitrosourea (MNU, MNH), *Sodium Azide*, physical mutagens as, gamma-ray radiation and fast neutrons reported in several crop species.

Mutagens	**Type of Mutation**	**Reference**
EMS (Ethyl methane sulfonate)	Transitions and Transversions (G/C to C/G and A/T to C/G).	Gilchrist and Haughn (2005); Martín et al. (2009)
N-methyl-*N*-nitrosourea (MNU)	G/C to A/T transitions only	Cooper et al. 2008 and Suzuki et al. 2008.
Sodium Azide	A/T to G/C transitions	Sadiq and Owais. 2000
NaN_3	*Transitions G/C to A/T*	Talamè et al. 2008
Physical mutagens (Gamma-ray radiation and fast neutrons)	Tranversions (C/G to A/T and C/G to G/C)	Sato *et al*., 2006.
De-TILLING	*Broad range of deletion , Knockout of tandem repeats*	Li et al. 2001,
N-ethyl-N-nitrosourea (ENU)	*Mispairing, base pair substitution, and base pair loss*	Guenet, 2004

Tilling in various crop species:

TILLING was first applied to *Arabidopsis thaliana* (McCallum *et al*. 2000). Perry and colleagues adapted the TILLING method for the model legume *Lotus japonicus* (Perry et al. 2003). The feasibility of TILLING in a polyploid species was shown for wheat by Slade and colleagues (Slade et al. 2005). Caldwell and colleagues used TILLING to identify mutations in barley (Caldwell et al. 2004). In collaboration with Tom Tai, the STP has developed TILLING for rice (Till et al. 2007).

EcoTILLING: TILLING identifies induced mutations in mutagenized populations. EcoTILLING detects naturally occurring SNPs, especially in landraces and wild accessions (Comai et al. 2004; Haughn & Gilchrist 2006). Ecotilling could also be used in animal species as well as in any plant species including heterozygous as well as polyploid. Wild relatives of cultivar which having limited genetic diversity, could also be

explored using this method .In the future the genetic resources discovered using EcoTilling can also be developed as new variety.

Advantages of TILLING:

1. Technology relies on traditional methods of mutation to create mutations.
2. Induced mutations are stable.
3. TILLING is appropriate for both small- and large-scale screening, because the high density of mutations requires relatively few individual plants.
4. Most steps of TILLING are suitable for automation. The choice of PCR amplicon can be automated (for high-throughput) and streamlined for interactive use (by users requesting genes for TILLING).
5. Using TILLING, make possible to induce a series of alleles in a targeted locus.
6. TILLING does not require transformation and, thus, is the only reverse genetics strategy applicable for species that are not transformable.
7. It is recommended as non-GMO technology, so in TILLING, GMO procedures and controversies are avoided.
8. TILLING is not technically demanding and can be performed at a relatively low cost.
9. The feasibility of TILLING has already been demonstrated for a large number of agronomically important crops, including rice, barley, wheat, maize, sorghum, soybean, rapeseed and tomato plants.

CONCLUSION

TILLING is high-throughput mutation detection systems that exploit non-transgenic allelic variation. The technique has proven highly efficient in obtaining desirable mutant lines and is also extremely versatile. The flexibility of the system has allowed the technology to become widely adopted in many crop plants. The development of TILLING requires support from local agencies, so that the populations generated, and downstream techniques and supplies, can be used efficiently by the research community.

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