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Targeting Induced Local Lesions in Genomes and its Relevance to Polymorphism Assessment and Plant Breeding

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SUMMARY

By accumulation of beneficial alleles from huge plant genetic resources existing worldwide, development of superior and high yielding varieties of agricultural crops is feasible But as these alleles are left behind during the process of evolution and domestication, a major portion of these superior alleles cannot be used. For causing variations in the existing allele and allelic combinations, generally mutations will occur in the genic regions of the genome either as Single Nucleotide Polymorphism (SNP) or as Insertion and Deletion. The mutations in regulatory regions may have great effect on the phenotype by changing the encoded protein structure and function. Mutations that occur in noncoding regions of a gene has been silent without any effect on the phenotype. Even though most of the mutations are deleterious, yet 0.0001 % of the mutations are important leading to changes in gene function which may be highly necessary for the survival of the plant. The process of recognizing alleles of a known gene/locus that are involved in a particular function for any given trait and their variants within other genotypes or identifying novel, superior and beneficial alleles from the natural population is known as allele mining.

INTRODUCTION

The genomic information of many plant species have interpreted with the development of rapid and inexpensive sequence technologies. The emphasis on genomics has been changing from complete sequenced genomes to the study of the functional genomics. Many approaches like RNAi, gene knockout, site-directed mutagenesis, transposon tagging etc. have been exploited for many years to understand the function of genes. The use of transgenic material is being used in all these techniques which is not always possible in many commercially important crops. So it is not only impeding the functional analysis of genes but also retards the improvement of existing as well as the development of improved cultivars. A non transgenic technique called Targeting Induced Local Lesions IN Genomes (TILLING) was emphasized that determines the allelic sequence of induced point mutations in genes of concern (Rashid *et al*., 2011). TILLING is a valuable and non-transgenic reverse genetic strategy to study gene function that allows screening for mutations in genes with known sequences in a plant mutant population or allows rapid mutational screening to obtain induced lesions in a gene of interest. TILLING helps in the direct identification of induced point mutations in a gene by heteroduplex evaluation. It is suitable for most plants and enables the identification of single-base-pair (bp) allelic variation in a target gene in a high-throughput manner. It has several benefits over other techniques used to detect single-bp polymorphisms. Eco-TILLING is the first modification of the TILLING technique and was proposed by Comai *et al*. (2004). This technology is used to survey natural mutation throughout genes/ and or in germplasm. Although the process is generally the same (both techniques use mismatches produced by heteroduplexes of alleles of a gene) Eco-TILLING is ideal for the identification of natural variance within populations or even natural mutations within germplasm without using mutagenesis. DNA polymorphisms that can be discovered via Eco-TILLING include SNPs, small insertions and deletions (InDels).

Procedure of Tilling

- For TILLING, DNA is extracted from a mutagenized population (each individual mutant). DNA from up to eight individuals (mutagenized lines) is pooled.
- After extraction and pooling, samples are typically arrayed into a 96-well format. The target region (~1.5 kb in a gene of interest) is amplified by PCR with gene-specific primers that are end labeled with fluorescent dyes.
- The amplified products are denatured by heating and then allowed to cool slowly so that they randomly re-anneal.

- Heteroduplex molecules form, when a pool includes at least one plant that has a mutation in the amplified region, and become the substrate for enzymatic mismatch cleavage.
- Heteroduplexes are cleaved using a crude protein extract from celery containing the nuclease CEL I.
- The cleaved pieces (resultant products) are generally visualized on polyacrylamide denaturing gels electrophoresis and a gel readout platform such asthe LI-COR DNA analyser, to identify individuals that may have a mutation or polymorphisms in the gene of interest.

Reviews of previous studies:

Slade *et al*., 2005 reported the use of TILLING in polyploidy crop plant by identifying 246 alleles of the waxy genes by TILLING of each homoeolog in 1,920 allohexaploid and allotetraploid wheat individuals. These alleles encode waxy enzymes ranging in activity from near wild type to null, and they represent more genetic diversity than had been described in the preceding 25 years.

Minoia *et al*., 2010 generated a new mutant collection in the genetic background of the processing tomato cultivar Red Setter by treating seeds with two different ethylemethane sulfonate doses (0.7% and 1%). 9.5 kb of tomato genome was screened and 66 nucleotide substitutions were identified. The overall mutation density was estimated and it resulted to be 1/322 kb and 1/574 kb for the 1% EMS and 0.7% EMS treatment respectively.

Richand *et al*., 2018 showed that altering the expression of LCYE genes increases the flux towards the β-β branch, accumulating higher β-carotene levels. They produced durum wheat LCYE mutants through EMS to potentially increase β-carotene content. The LCYE point mutations created with EMS were identified using a Kronos TILLING (Targeting Induced Local Lesion IN Genomes) mutant population.

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